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Review

Cardiovascular safety of protein kinase inhibitors: putting their "QT-phobia" in perspective

Rashmi R Shah

Pharmaceutical Consultant, 8 Birchdale, Gerrards Cross, Buckinghamshire, UK

E-mail: clinical.safety@hotmail.co.uk, tel . + (44) 1753 886348

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Abstract

Many potentially valuable drugs, including protein kinase inhibitors (PKI), risk being dropped from further development, without exploration of their clinical benefits, if early studies show these drugs to inhibit hERG channel and therefore, to have a potential for prolonging ventricular repolarisation (QT interval). This QT-phobia results from a perceived possibility of the clinical risks of QT-related ventricular proarrhythmia, further aggravated by uncertainties surrounding the regulatory evaluation of the risk and either approvability or restrictive labelling of the drug concerned. In reality, QT interval prolongation per se is only an imperfect surrogate of the proarrhythmia risk which is much smaller than perceived and compared to their other cardiovascular and non-cardiovascular risks. PKI-induced clinical hepatotoxicity, also evaluated on the basis of surrogate markers (serum transaminases and bilirubin) is another risk that far exceeds any risk arising from PKI-induced QT interval prolongation. This review of the currently approved 28 PKIs places the QT-phobia surrounding the development of PKIs in its perspective by juxta-positioning their potential to induce ventricular dysfunction, arterial thrombotic events and hepatotoxicity. Available evidence suggests that hERG channel may prove to be a valuable therapeutic target in oncology. Therefore, the development, approval and labelling of such vital oncology drugs requires careful assessment of their benefits and their risk/benefit generally, without being overtly consumed by their potential QT-liability, in terms of their more direct consequences on clinically relevant endpoints of morbidity, mortality and quality of life.

Keywords

Arterial thrombotic events; Cardiotoxicity; Hepatotoxicity; hERG channel; Left ventricular dysfunction; Proarrhythmias, QT interval; Transaminases; Torsade de pointes; Protein kinase inhibitors

Introduction

Treatment of various cancers has been significantly revolutionized by the development of small molecule protein kinase inhibitors (PKIs), a vast majority of them in current clinical use being tyrosine kinase inhibitors. The perceived benefits of these highly targeted agents have led regulatory authorities to approve a majority of them on an expedited or priority basis, often on limited preliminary data indicative of their safety, efficacy and a favourable risk/benefit ratio [1-3]. Often, such approvals are associated with requirements for appropriate post-approval studies to substantiate these early expectations.

As of 31 May 2016, 27 such agents have been approved by the US Food and Drug Administration (FDA) for use in oncology indications, beginning with the first approval of imatinib in May 2001. Two additional PKIs have also been approved by the FDA but for non-oncology indications – tofacitinib and nintedanib for

the treatments of rheumatoid arthritis and of idiopathic pulmonary fibrosis, respectively. In the European Union (EU), however, nintedanib is also approved for non-small cell lung cancer and at the time of writing, one of the 29 agents (alectinib) approved by the FDA was still under review, whereas tofacitinib had been rejected for approval, by the European Medicines Agency (EMA).

In addition to the development of new PKIs, the agents already approved for one cancer are also being tested for their potential therapeutic benefits in other cancers and the range of oncology indications for many of these previously approved agents is gradually expanding [4-7]. For example, imatinib, initially approved for use in chronic myeloid leukaemia, now enjoys no less than seven distinct indications. The approval dates and the broad cancer-specific indications of the agents approved by the FDA and/or the EMA are shown in Table 1.

Unfortunately, PKIs are also associated with a number of adverse side effects, many potentially fatal, on cardiovascular system (e.g. QT interval prolongation of the surface electrocardiogram (ECG), hypertension, left ventricular (LV) dysfunction, arterial thrombotic events (ATEs), venous thrombo-embolic events, bleeding and symptomatic bradycardia) and non-cardiovascular systems (such as hepatic, pulmonary, dermatological thyroid, ocular and renal) [8]. Many of these adverse effects are on-target effects which correlate with efficacy and therefore, difficult to avoid without compromising therapeutic benefit by simple dose reductions [8-17]. It is beyond the scope of this review to discuss the full safety profile of PKIs and the interested reader is referred to other reviews [2, 3, 18-39]. Despite this constellation of side effects associated with PKIs, the one that has engendered a phobia, often leading to early termination from further development, is the potential of a PKI to prolong the QTc interval. In rare cases, prolonged QT interval may predispose the patient to potentially fatal ventricular tachyarrhythmias. The FDA guidance on pre-marketing risk evaluation and assessment recommends the sponsor to address, as a part of the new drug application (NDA) for all new small molecule drugs, their potential for six serious adverse effects, one of them being drug-related QTc prolongation [40]. The others are drug-related liver toxicity, nephrotoxicity, myelotoxicity, interactions with other drugs and polymorphic drug metabolism.

This review aims to put in perspective the “QT-phobia” that surrounds the development of PKIs by highlighting just two of their other more serious and relatively more frequent cardiovascular effects, namely LV dysfunction and ATEs. It will focus on 28 oncology-related PKIs including nintedanib which enjoys an oncological indication in the EU. For the purpose of this review, these 28 PKIs approved during the last 15-years since 2001 are divided into two groups with reference to the dates of their first approval by the US FDA. The dates selected are the FDA approval date because typically, the FDA is also the authority that has led the way in first approvals of each agent [1]. One group includes 16 (57 %) agents approved over 11.5 years from May 2001 to September 2012 (henceforth referred to as Period 1) and the other includes 12 (43 %) agents approved more recently during the last 3.7 years from October 2012 to May 2016 (Period 2). The pace of development of these agents is self-evident; 1.39 agents per year in period 1 and 3.24 agents per year in period 2. The labels of approved PKIs continue to evolve as more data become available and therefore, the information reviewed herein is sourced from a variety of documents referred to in earlier reviews [1-3], the most current updated labels and the author’s overall assessment of the data available.

Table 1. Approval dates and current indications of protein kinase inhibitors (PKIs) as of 31 May 2016

PKI	FDA approval date	EU approval date	Broad-term indication(s) (for use in selected patients with)
Afatinib	12 July 2013	25 Sept. 2013	Non-small cell lung cancer
Alectinib	11 Dec. 2015	UNDER REVIEW	Non-small cell lung cancer
Axitinib	27 January 2012	13 Sept. 2012	Renal cell carcinoma
Bosutinib	4 Sept. 2012	27 March 2013	CML
Cabozantinib	29 Nov. 2012	21 March 2014	Thyroid cancer, Renal cell carcinoma
Ceritinib	29 April 2014	6 May 2015	Non-small cell lung cancer
Cobimetinib	10 Nov. 2015	20 Nov. 2015	Melanoma
Crizotinib	26 August 2011	23 Oct. 2012	Non-small cell lung cancer
Dabrafenib	29 May 2013	26 August 2013	Melanoma
Dasatinib	28 June 2006	20 Nov. 2006	CML, ALL
Erlotinib	18 Nov. 2004	19 Sept. 2005	Non-small cell lung cancer, Pancreatic cancer
Gefitinib	5 May 2003	20 June 2009	Non-small cell lung cancer
Ibrutinib	13 Nov. 2013	21 Oct. 2014	Mantle cell lymphoma, CLL, Waldenström's macroglobulinaemia
Imatinib	10 May 2001	11 Nov. 2001	CML, ALL, GIST, Mastocytosis, Hypereosinophilic syndrome, Myelodysplastic/myeloproliferative disease, Dermatofibrosarcoma protuberans
Lapatinib	13 March 2007	10 June 2008	Breast cancer
Lenvatinib	13 February 2015	28 May 2015	Thyroid cancer
Nilotinib	29 October 2007	19 Nov. 2007	CML
Nintedanib	17 October 2014 (Not applicable)	15 Jan. 2015 21 Nov. 2014	Idiopathic pulmonary fibrosis Non-small cell lung cancer
Osimertinib	13 Nov. 2015	2 Feb. 2016	Non-small cell lung cancer
Pazopanib	19 October 2009	14 June 2010	Renal cell carcinoma, Soft tissue sarcoma
Ponatinib	14 Dec. 2012	1 July 2013	CML, ALL
Regorafeniib	27 Sept. 2012	26 August 2013	Colorectal cancer, GIST
Ruxolitinib	16 Nov. 2011	23 August 2012	Myelofibrosis, Polycythaemia vera rubra
Sorafenib	20 Dec. 2005	19 July 2006	Hepatocellular carcinoma, Renal cell carcinoma, Thyroid cancer
Sunitinib	26 January 2006	19 July 2006	Renal cell carcinoma, GIST, Pancreatic neuroendocrine tumour
Trametinib	29 May 2013	30 June 2014	Melanoma
Vandetanib	6 April 2011	17 Feb. 2012	Thyroid cancer
Vemurafenib	17 August 2011	17 Feb. 2012	Melanoma
Tofacitinib	6 November 2012	REFUSED on 26 April 2013	Rheumatoid arthritis

ALL = Acute lymphoblastic leukaemia; CML = Chronic myeloid leukaemia; GIST = Gastrointestinal stromal tumours; CLL = Chronic lymphocytic leukaemia

Background to QT-phobia

The QT interval measured on the ECG varies with the heart rate and therefore requires correction to compute a heart rate-corrected QT interval (QTc interval) to determine the effect of an intervention. The correction formula most widely used for regulatory submissions and safety assessment is the Fridericia, study population-specific or individual subject-specific correction formula to compute QTcF, QTcP or QTcI interval, respectively. QTc interval prolongation per se is not harmful. However, at the level of an individual patient, prolonged QTc interval, when excessive (typically significantly greater than 500 ms) and/or in presence of certain risk factors, induces a potentially fatal form of ventricular tachyarrhythmia, known as torsade de pointes (TdP) [41,42]. Although typically of short duration, asymptomatic and self-terminating, TdP is infrequently sustained and symptomatic in a small proportion of cases and in 20 % or so of these patients, it degenerates into ventricular fibrillation, leading to death [43,44]. Historically, this proarrhythmia (or the potential thereof) has been responsible for about a third of the drug withdrawals from the market [45, 46]. The approval of many others has been either delayed or associated with prescribing restrictions in terms of narrow indication(s), contraindications and/or warnings and monitoring precautions [47].

Arising from this historical experience with a large number of drugs across a wide range of pharmacological and therapeutic classes including oncology, the potential of new drugs to prolong the QTc interval and induce TdP is one principal safety issue that has continued to worry the regulatory authorities over the last two decades, although QTc interval prolongation per se does not correlate well with torsadogenic potential. This imperfect correlation between QT prolongation and risk of TDP is explained by the fact that whereas QT prolongation results from blockade of human ether-a-go-go (hERG) channel and prolongation of action potential duration (APD), TdP is a triggered activity arising from early after-depolarizations (EADS) and the risk of EADs followed by TdP is modulated by other ancillary properties of the drug, such as blockade of calcium or late sodium currents or autonomic receptors [48-51]. Ranolazine (an antianginal drug) and sertindole (a neuroleptic agent) best illustrate the role of late sodium current and α-blocking activities, respectively, in modulating the risk of TdP following QT interval prolongation [52-56]. In essence, therefore, TdP and fatality following drug-induced QTc prolongation are events that are much rarer than would be anticipated. For example, Laksman et al. [57] have reported that, among 172 in-hospital patients with QTc interval > 550 ms, in-hospital mortality was 29 %, with only 4 % of patients experiencing arrhythmic deaths, all of which were attributed to secondary causes.

In a vast majority of cases, drug-induced prolongation of the QTc interval results from delayed ventricular repolarization due to inhibition of the (major) outward repolarizing potassium current mediated by hERG subunit of the rapid component of the delayed rectifier channel (IKr) [58]. Non-antiarrhythmic drugs can be characterised for this off-target undesirable pharmacological property in nonclinical in vitro and in vivo studies as well as clinical in vivo studies. Not surprisingly, therefore, regulatory authorities have reacted to this potential by promulgating guidelines for pre-approval characterisation of all new drugs with systemic bioavailability for their “QT-liability” [47, 59-62]. This is the only adverse drug effect that has called for specifically targeted and internationally harmonised detailed guidelines [60, 61]. Clinically, this QT-liability is determined in a formal and dedicated study, referred to as the thorough QT (TQT) study, that includes two dose levels of the investigational drug, a placebo and an active control. In order to avoid any false negatives and as a matter of precaution, the threshold of regulatory concern for a maximum mean increase in QTc interval from baseline in the TQT study has been (arbitrarily) set low at around 5 ms as evidenced by an upper bound of the 95 % confidence interval (CI) around the mean effect on QTc of 10 ms

[61]. However, QTc prolongation is an imperfect surrogate of torsadogenesis and therefore, it is acknowledged by all concerned (including the regulatory authorities) that a drug endowed with QT-liability does not necessarily mean that the drug is proarrhythmic; a breach of the above regulatory threshold simply calls for more diligent and intensive ECG monitoring in subsequent clinical trials. Nevertheless, the sponsors have frequently reacted by terminating further development of new agents, often on the basis of an early in vitro hERG study or first-in-human study, because of the fears and regulatory uncertainties surrounding the possible finding of a modest but often clinically irrelevant breach of the regulatory threshold by the drug concerned in what is a cost-ineffective and resource-intensive TQT study.

QTc-liability of protein kinase inhibitors

Overall, as shown in Table 2, 11 (39 %) of the 28 PKIs have the potential to prolong the QTc interval to a variable extent. These 11 agents are considered to have the QT-liability on the basis that their QTc effect had breached the above very conservative threshold of regulatory concern; as a result, the labels of two (nilotinib and vandetanib) carry a black box warning. Interestingly, QTc-liability is reported for eight (50 %) of the 16 agents approved in period 1 in contrast to only 3 (25 %) of the 12 approved in period 2, possibly suggesting early termination of PKIs from development during period 2 due to this pharmacological property.

However, experience with QTc-prolonging drugs that are known to be torsadogenic and non-torsadogenic has shown that drugs associated with maximum mean placebo-corrected increases of 6-10 ms are unlikely to be torsadogenic whereas drugs associated with increases of 16-20 ms are probably torsadogenic [63]. Lin and Kung have also summarized data which suggest that a mean QTc increase of 19.3 ms associated with strong torsadogens is significantly greater than the 8.0 ms for borderline torsadogens [64].

By comparison with known non-PKI torsadogens, the QTc effect of majority of the PKIs that prolong the QTc interval (often determined at supratherapeutic doses) appears to be relatively mild/modest with a maximum mean effect of around 10 ms (95 % upper bound around their mean effects being around 15 ms). Six PKIs (osimertinib, ceritinib, sunitinib, lapatinib, nilotinib and vandetanib) appear to have somewhat larger effects with a maximum mean effect ranging from 13.5 to 34.7 ms (95 % upper bound around the mean effect being 17.6, 22.2, 22.4, 23.4, 25.8 and 36.4 ms respectively) [2,3]. However, since some of these effects are not placebo-corrected, the true extent of the drug's QTc-prolonging effect remains uncertain. Therefore, it is interesting to note that the number of patients receiving any of these PKIs who developed a QTc interval prolongation > 500 ms is typically well below 0.5 % except lapatinib (6 %) and vandetanib (4.3 %) [2]. In pre-approval clinical trials, only pazopanib and vandetanib were each associated with two cases of TdP, although the causal association with pazopanib in one case appeared uncertain since the patient was also receiving amiodarone [2]. In a dedicated QT study as required by the regulatory guidance, the maximum mean increase in baseline-adjusted, time-matched QTcF interval in pazopanib-treated patients versus placebo was only 4.4 ms (95 % upper bound 11.2 ms) [65]. Mixed-effects modelling did not indicate a significant concentration-dependent effect of pazopanib or its metabolites on QTcF interval.

Table 2. Trend analysis of selected toxic effects of protein kinase inhibitors (PKIs) with reference to approval period

Protein kinase inhibitor	Period 1 (May 2001 – September 2012) Number of PKIs approved = 16				Period 2 (October 2012 – May 2016) Number of PKIs approved = 12			
	QT effect	Hepatotoxicity	LV dysfunction	Arterial effects	QT effect	Hepatotoxicity	LV dysfunction	Arterial effects
Afatinib **						√	√	
Alectinib **						√		
Axitinib *		√	√Δ	√				
Bosutinib *		√	√					
Cabozantinib**					√			√
Ceritinib **					√	√		
Cobimetinib**						√	√	
Crizotinib *	√	√	√Δ					
Dabrafenib **							√Δ	
Dasatinib *			√	√				
Erlotinib *		√		√				
Gefitinib *	√	√						
Ibrutinib **								
Imatinib *		√Δ		√Δ				
Lapatinib *	√	√□Δ	√					
Lenvatinib **						√	√	√
Nilotinib *	√□	√	√Δ	√Δ				
Nintedanib **						√		√
Osimertinib **					√		√	
Pazopanib *		√□	√Δ	√				
Ponatinib **						√□	√	√□
Regorafeniib *		√□		√				
Ruxolitinib *								
Sorafenib *	√Δ		√	√				
Sunitinib *	√	√□Δ	√	√Δ				
Trametinib **							√	
Vandetanib *	√□		√	√				
Vemurafenib *	√	√						
PKIs with the effect	**50 %**	**75 %**	**63 %**	**63 %**	**25 %**	**58 %**	**58 %**	**33 %**

* PKI approved in Period 1; ** PKI approved in Period 2; □ With black box warning; Δ Post-approval addition or revision

The rarity of QT-related proarrhythmia risk of PKIs is further corroborated by post-marketing data. During the post-marketing use of 16 PKIs approved in period 1 and for which some data are available, there were a total of 463 reports of QT interval prolongation but only 20 reports of TdP; only vandetanib was associated with significant risk of TdP [3]. Spontaneous reporting data must of course be interpreted with great caution but although crizotinib, dasatinib, vandetanib and nilotinib were associated with 14, 35, 57 and 246 reports of QTc interval prolongation during their post-marketing use, the corresponding number of reports of TdP were only 0, 1, 2 and 3, respectively, thus further emphasising that not all events

of QT interval prolongations degenerate into TdP and the rarity of this potentially fatal proarrhythmia. Although there were a total of 220 reports of sudden death, there was similar discordance between the QT-liability of a drug and the number of reports of sudden death associated with it. Kloth et al. [66] have also reported a post-marketing observational study of 363 patients who were eligible for the analyses of QTc interval before and during treatment with erlotinib, gefitinib, imatinib, lapatinib, pazopanib, sorafenib, sunitinib or vemurafenib. The median on-treatment time before the ECG was performed was 43 days. Mean (range) QTc intervals were 401 (388–415) ms at baseline and 415 (397–431) ms following therapy. A total of 33 patients (9.1 %) were characterised by an increased grade of Common Terminology Criteria for Adverse Events (CTCAE). Only two individuals passed from grade 1 to grade 2 or 3 and nine patients (2.5 %) had a decrease in their CTCAE grade for QTc interval. However, 321 (88.4 %) patients did not have a change in their CTCAE grade after the start of PKI treatment. Only five patients (1.4 %) developed QTc $\geq$ 500 ms after starting the therapy, with all of them experiencing an increase of $\geq$100 ms from baseline. Despite this marked increase from baseline, none was reported to have a proarrhythmia.

One potential explanation for the above discordance, also observed all too often with drugs in other pharmacological or therapeutic classes, is that the PKIs concerned may have a risk-mitigating inhibitory effect on other ion currents involved in APD, such as the calcium and/or late sodium currents as explained earlier [48-51]. Whether or not PKIs generally inhibit these ion channels is at present unclear since they are not routinely studied for these effects. Protein kinases activate downstream signalling via phosphoinositide 3-kinase (PI3K). The complexity of the interactions is demonstrated by one study reporting that chronic but not acute exposure to nilotinib prolonged the APD in canine cardiomyocytes, an effect which was reversed by intracellular dialysis with phosphatidylinositol 3,4,5-trisphosphate (which is a downstream effector of PI3K) [67, 68]. These investigators also observed that APD prolongation by nilotinib was mediated by decreases in both the rapid (IKr) and slow (IKs) components of the delayed rectifier potassium current, as well as by an increase in late sodium current (an effect which in theory ought to aggravate the proarrhythmia risk [69]); however, nilotinib also reduced the calcium current.

One obvious and hitherto unquantified by-product of the “QT-phobia” is that many new candidate compounds are abandoned very early in their development without fully exploring their potential for significant therapeutic benefit. The unfortunate reality, however, is that the “QT-liability” of a drug is only an imperfect predictor of the proarrhythmia risk, and just one component of its much wider cardiovascular and overall safety. Of equal concern with regard to determining the duration of QTc interval in routine clinical practice is the fact that the QTc interval value depends on the correction formula applied to the measured QT interval. The most common formula applied in routine clinical practice is the Bazett’s formula (that computes QTcB interval) which has been shown to be highly imprecise when there are significant changes in heart rate [70-73]. Although regulatory documents and drug labels provide guidance on dose adjustment in patients who develop, or are at risk of developing QTc prolongation, they provide no guidance on the formula to be used. Similarly, the US National Cancer Institute, which has recently modified the criteria for QTc interval in the latest version of CTCAE (v4.03) [74], provides no guidance on the choice of a formula for computing QTc interval duration.

It is therefore timely to contrast and put in perspective the foregoing rarity of adverse clinical outcomes from PKI-related effects on QTc interval with their effects on two specific cardiovascular effects, LV dysfunction and ATEs, which are of much greater concern and have a significant direct impact on morbidity, mortality and risk/benefit.

Left Ventricular Dysfunction due to Protein Kinase Inhibitors

Left ventricular (LV) dysfunction is now a well-recognised toxicity of a number of PKIs. It can range from asymptomatic ECG changes (in QRS and T-waves and ST-segment) through decrease in LV function (detectable noninvasively only by echocardiography or radionuclide techniques) to clinically manifest severe congestive heart failure (CHF) and generalised fluid retention [75-77]. Although not precisely known or understood, the potential mechanism underpinning this effect has been reviewed previously by others [2, 78-81].

Post-approval, the US labels of a number of PKIs have been revised well after their initial approval to include new warnings and precautions regarding the risks of LV dysfunction and/or fluid retention. These are axitinib, dabrafenib, imatinib, nilotinib and pazopanib, although there are some question marks concerning the potential of imatinib to induce cardiac dysfunction. During its post-marketing use as of 25 February 2015, about 14,700 patients worldwide had received crizotinib since its approval [82]. Forty cases of cardiac failure had been reported in the post-marketing setting. A review by European medicines regulators of data from clinical trials and reports from clinical practice concluded that this side effect is common, occurring in 1-10 % of the patients taking crizotinib [82]. In most cases, cardiac failure occurred within one month of starting the treatment and affected patients with or without pre-existing cardiac disorders. The reports included cases where the evidence of symptoms of cardiac failure resolved after stopping crizotinib and recurred when it was reintroduced. Some cases had a fatal outcome. It is noteworthy that in contrast to the revised European Union prescribing information, the most current FDA label of crizotinib (dated 29 April 2016) warns about symptomatic bradycardia but does not make any reference to cardiac failure [83].

Currently, 17 (60 %) of the 28 PKIs, approved as of 31 May 2016, are known to induce LV dysfunction, often loosely termed as “cardiomyopathy” or “cardiotoxicity”, and cardiac failure. It is evident that 10 (63 %) were among the 16 approved in period 1 and 7 (58 %) among the 12 approved in period 2. This trend is in contrast to the substantial reduction in the number of QT-prolonging drugs approved during period 2 compared to period 1 (from 50 % to 25 % for QTc-prolonging drugs versus from 63 % to 58 % for drugs affecting cardiac dysfunction). Not only that but also the total number of PKIs with an adverse effect on cardiac function (n=17; 60 %) is much higher than those with a QT-liability (n=11; 39 %).

The complexity surrounding the estimation of incidence and interval to onset has been reviewed before since these depend on the dose of the drug and the criteria used to define LV dysfunction as well as previous chemotherapy. In broad terms, asymptomatic decrease in LV ejection fraction is by far the most frequent. However, overt CHF is not uncommon with an incidence as high as 5-8 % with sunitinib and pazopanib. A number of recent meta-analyses of clinical trials have quantified the risk of PKI-induced symptomatic cardiac failure [21,22,84]. These events are typically categorised as all- and high-grade (grade 3 and higher) as defined by CTCAE. Grade 3 CHF events require intervention, and grade 4 CHF events usually include life-threatening dysfunction. These meta-analyses reveal that overall, the incidences of all-grade and high-grade CHF are about 2.8 % and 1.1 %, respectively, with a relative risk (RR) of 2.5 and 1.5, respectively. The risk appears independent of tumour type or the PKI used. The outcome has been fatal in a number of cases whereas others require anti-failure therapy and/or withholding the culprit PKI. In routine clinical oncology, the scale of the problem is likely much higher as patients with cardiac disease who are the most at risk of cardiotoxicity have traditionally been excluded from pre-approval clinical trials.

As stated above, four (23 %) of the 17 PKIs with a potential for inducing cardiac failure revealed this risk

during their post-marketing use. Currently, there are no regulatory guidelines for pre-approval characterisation of a drug for its effect on LV function. However, as Yang and Papoian from the FDA point out, these reports of cardiac toxicity following the clinical use of PKIs are unexpected and not well predicted by nonclinical studies [85] and clinical findings have exposed gaps in current nonclinical drug testing for predicting the development of cardiac toxicities in humans. They have suggested the use of cultured cardiomyocytes and application of isolated perfused heart methodology to chronic or sub-chronic rodent studies or including echocardiography in chronic large animal toxicity studies as potentially valuable tools to study the likelihood of PKI-mediated cardiotoxicity. Available data suggest that nonclinical studies, when adequately designed to address a specific issue, are capable of detecting these effects as was seen in studies with some PKIs approved more recently. For example, in vivo cardiac safety pharmacology studies with osimertinib did suggest equivocal findings of decreased contractility dogs and guinea pigs whereas increased findings of cardiomyopathy compared to findings in control animals were reported in the 4-week rat toxicology study with cobimetinib though no other cardiac effects were reported in animal studies [86, 87]. With regard to afatinib, a decrease in left ventricular function was noted at a dose of 30 mg/kg in a single continuous intravenous administration study in domestic pigs [88]. Use of echocardiography unequivocally identified sunitinib-induced cardiac LV dysfunction but failed to detect an effect of bosutinib [89, 90]. Although electrocardiography is widely used to study QTc effects, echocardiography has not been used as frequently to study LV function in nonclinical studies. There clearly lies a significant challenge in pre-approval characterisation of a drug for its effect on LV function. Recent studies have shown that a multi-parameter approach examining both cardiac cell health and function in human induced pluripotent stem cell-derived cardiomyocytes provides a comprehensive and robust assessment that can aid in determining potential cardiotoxic liability [91].

Arterial thrombotic events due to protein kinase inhibitors

Among the most serious adverse effects of the PKIs are their prothrombotic effects as illustrated by ponatinib [reviewed in 3]. Following the post-marketing experience with ponatinib (see below), ATEs have now emerged as a major safety concern with PKIs and the previously approved labels of a few have required revisions to reflect this risk.

Ten (63 %) of the 16 PKIs approved during period 1 have been reported to be associated with arterial thrombosis (axitinib, dasatinib, erlotinib, imatinib, nilotinib, pazopanib, regorafenib, sorafenib, sunitinib and vandetanib). Among the 12 newer PKIs approved in period 2, four (33 %) have been reported to be associated with ATEs (cabozantinib, lenvatinib, nintedanib, and ponatinib, with the last one carrying a black box warning) [3]. The rates of ATEs appear to be comparable for PKIs from both periods and the ATE events typically include cerebral infarction, cerebral ischaemia, cerebrovascular accidents (CVA), myocardial infarction and myocardial ischaemia. In pre-approval clinical trials, the incidences of ATEs were 5 % with lenvatinib, 2.5 % for nintedanib and 2 % for cabozantinib, the corresponding placebo rates being 2 %, 0.8 % and 0 % respectively. Thus, the mean drug-emergent effect was of the order of 2.2%. Myocardial infarction was the most common adverse reaction, occurring in 1.5 % of nintedanib-treated patients compared with 0.4 % of placebo-treated patients. The incidence of grade 3 or higher events was 3 % in lenvatinib-treated patients compared with 1 % in the placebo group.

One meta-analysis involving 10,255 patients receiving sunitinib and sorafenib revealed an incidence of ATEs to be 1.4 % (95 % CI: 1.2–1.6) [23]. Another meta-analysis involving a total of 9,711 patients from 19 trials concluded that the overall incidence of ATEs was 1.5 % (95 % CI: 1.0–2.3) following the use of VEGFR

PKIs [24]. The most common ATEs were myocardial ischaemia/infarction (67.4 %), central nervous system ischaemia (7.9 %) and CVA (6.7 %). The odds ratio (OR) was significantly increased when compared with controls (OR 2.26, 95 % CI: 1.38–3.68; p = 0.001) and this did not vary significantly with tumour types (p = 0.70), VEGFR PKIs (p = 0.32), treatment regimens (p = 0.76), phase of trials (p = 0.37) and sample size (p = 0.89). Thus, the overall incidence of ATEs with VEGFR inhibitors is about 1.50 % with an RR in the region of 2.6. Available limited evidence suggests that EGFR inhibitors may not be associated with this risk.

In a post-marketing observational study by Srikanthan et al. [92], three agents were studied: erlotinib, sorafenib and sunitinib. Patients treated with these agents were compared to 128,415 age and gender matched individuals without cancer who served as controls. Of the 1,642 PKI-treated patients followed up, 1.1 % developed a myocardial ischaemic event requiring hospitalization, 0.7 % developed a CVA requiring hospitalization and 1,184 (72.1 %) died. 61 % of the myocardial ischaemic event events and 73 % of the CVA events were associated with erlotinib, and these proportions closely mirrored the relative frequency of drug use in the population. Cardiovascular events predominantly occurred late in follow-up. When patients with and without baseline ischaemic heart disease were compared, 3.3 % versus 0.5 %, respectively, developed a myocardial ischaemic event and 1.2 % versus 0.5 %, respectively, developed CVA. However, the mortality rates were no different (72.5 % vs. 72.0 %, respectively). These investigators summarised that individuals treated with PKIs have a significantly higher hazard of death relative to the general population but cause-specific hazards of ischaemic heart disease and cerebrovascular accidents are not increased. Jang et al. [93] have recently reported cardiovascular events in 171 (26 %) of the 670 patients aged > 65 years treated with sunitinib or sorafenib. The incidence rates for CHF or cardiomyopathy, acute myocardial infarction and stroke were 0.87, 0.14, and 0.14 per 1000 person-days, respectively. The use of either agent was associated with an increased risk of cardiovascular events (hazard ratio (HR) 1.38; 95 % CI: 1.02-1.87) and stroke (HR 2.84; 95 % CI: 1.52-5.31) in comparison with 788 patients diagnosed with advanced renal cell carcinoma who did not receive either agent.

Case of ponatinib

When first approved in December 2012, ponatinib was already strongly associated with cardiovascular, cerebrovascular, and peripheral vascular thrombosis, including fatal myocardial infarctions and strokes [94]. Overall, fifty-one (11 %) of the 449 patients experienced an arterial thrombotic event of any grade. Myocardial infarction or worsening coronary artery disease was the most common event which occurred in 21 patients (5 %) of ponatinib-treated patients. The range of events included congestive heart failure (concurrent or subsequent to the myocardial ischaemia), cerebrovascular events, hemorrhagic conversion of the initial ischemic event, stenosis of large arterial vessels of the brain and peripheral arterial events with digital or distal extremity necrosis. Serious arterial thrombosis occurred in 34 (8 %) of the 449 of ponatinib-treated patients, as a consequence of which 21 patients required various revascularization procedures. Thirty of these 34 patients had one or more of the well recognised cardiovascular risk factors. Patients with cardiovascular risk factors appeared to be at increased risk for arterial thrombosis following treatment with ponatinib. As a condition of approval, the FDA had required the sponsor to characterize the safety of ponatinib and submit long-term safety data over a follow-up period of at least 12 months from all ongoing patients in a specific randomized controlled trial (referred to as AP24534-12-301) that adequately isolated the effect of the drug [95].

However, just over 10 months later as of 31 October 2013, approximately 24 % of patients in one phase II clinical trial (median treatment duration 1.3 years) and approximately 48 % of patients in a phase I clinical trial (median treatment duration 2.7 years) had experienced serious adverse vascular events [96]. A

number of these patients required urgent surgical procedures to restore blood flow. In some patients, fatal and serious adverse events occurred as early as 2 weeks after starting ponatinib therapy.

Since a safe dose or duration of exposure could not be identified, the sponsor agreed to the request from the FDA to suspend marketing and sales of ponatinib [96]. Following a thorough assessment of all available data, the FDA on 20 December 2013 required several new safety measures to be implemented, including restricted indication and additional warnings and precautions, before resumption of marketing to appropriate patients [97]. The FDA also required a risk evaluation and mitigation strategy (REMS) and the sponsor to conduct post-marketing investigations to further characterise the dose and the safety of the drug.

The most current label for ponatinib, dated 2 June 2016 [98], includes a more detailed account of vascular occlusion with age-related increase in incidence (18 %, 33 % and 56 % for those aged $\leq$ 49, 50-74 and $\geq$ 75 years, respectively and prior history of risk factors and 12 %, 18 % and 46 % respectively for those without prior risk factors. The overall incidence is computed to be 24 % for vascular occlusion and 20 % for arterial thrombosis and occlusion. It was also found to cause renal artery stenosis, associated with worsening, labile or treatment-resistant hypertension in some patients. However, the dose that was approved initially has remained unchanged over time at 45mg once daily [94, 98].

Leaving aside the rather atypical example of ponatinib, it is still sufficiently evident that PKI-induced ATEs, rather than their QT-liability, have a far greater impact on quality of life, morbidity and mortality of patients receiving PKI therapy.

Protein kinase inhibitors and fatalities due to non-QT related cardiovascular adverse events

From the foregoing, it is not surprising that non-QT related cardiovascular effects feature heavily in various studies of fatal adverse events (FAE) associated with PKIs, especially those agents that target angiogenesis (VEGFR inhibitors).

VEGFR inhibitors

Three large and independent meta-analyses [99-101], summarised in Table 3, involving 12,870 patients receiving VEGFR inhibiting PKI and 11,114 control patients have quantified the incidence of FAE to be in the range of 1.50-2.26 % with a RR in the range of 1.64-2.23. The PKIs studied were sunitinib, sorafenib, pazopanib, vandetanib, axitinib, cabozantinib, lapatinib and regorafenib. Adverse effects such as cardiac failure, myocardial infarction, stroke, thrombo-embolism, haemorrhage and cardiopulmonary insufficiency feature prominently in the list of these FAEs. Other often reported events included hepatic failure, respiratory events, intestinal perforation and renal failure as well as sudden death. Yang et al. [102] have recently reported that patients treated with sorafenib had a significantly greater risk of mortality than those in placebo/control groups, with an RR of 1.75. Among different VEGFR-PKIs, sorafenib and sunitinib were associated with a significant risk of death when compared with control arms, respectively. Combination of VEGFR-PKIs with other antineoplastic agents, but not VEGFR-PKI monotherapy, significantly increased the risk of treatment-related deaths.

Subgroup analyses have revealed some interesting results. Whereas Schutz et al. [100] reported no difference in the rate of FAEs found between different VEGFR PKIs or tumour types, Hong et al. [101] reported a significantly increased risk of death in patients with non-small cell lung cancer and colorectal cancer. This indication-related increased risk of death following treatment with sorafenib has also been reported by Yang et al. [102] who found an overall incidence of sorafenib-associated mortality to be 3.3 %

but patients with renal cell carcinoma and thyroid cancer had treatment-related mortality ≥5 %. Recently, Jean et al. [103] have also reported a distinct increase in the rate of occurrence of adverse effects of sorafenib when used in differentiated thyroid cancer compared with renal and hepatocellular cancer. While many theoretical explanations have been advanced to explain this indication-related difference in toxicity profile, the exact mechanism for this remains unclear.

Table 3. Summary of three meta-analyses of fatal adverse events (FAEs) associated with protein kinase inhibitors (PKIs)

Study	PKI group N =	Control group N =	PKIs	Risk (95% CI)	Incidence (%)	CV Events	Ref.
Sivendran *et al.*	2,762	2,401	Sunitinib Sorafenib Pazopanib Vandetanib	RR = 1.64 (1.16 – 2.32) p = 0.01	2.26 vs 1.26	Cardiac failure Pulmonary embolism	[99]
Schutz *et al.*	2,461	2,218	Sunitinib Sorafenib Pazopanib	RR = 2.23 (1.12 – 4.44) p = 0.023	1.50	Haemorrhage Myocardial Infarction Cardiac failure Stroke Pulmonary embolism Sudden death	[100]
Hong *et al.*	7,644	6,495	Axitinib Cabozantinib Lapatinib Pazopanib Regorafenib Sunitinib Sorafenib Vandetanib	OR = 1.85 (1.33 – 2.58) p = 0.01	1.90	Cardiopulmonary insufficiency Thrombo-embolism Haemorrhage Sudden death	[101]

EGFR inhibitors

In contrast to VEGFR-PKIs, Qi et al. [104] reported a meta-analysis of 7,508 patients treated with two widely used EGFR-PKIs (erlotinib and gefitinib) and compared them with 6,317 control patients to determine the incidence and risk of FAEs. The overall incidence of FAEs was 1.9 % (95 % CI: 1.2 - 2.9), and the RR was 0.99 (95 % CI: 0.70 - 1.41; p = 0.97). No increase in FAEs was detected in any pre-specified subgroup. This analysis suggests that the use of EGFR-PKIs does not increase the risk of FAEs in patients with advanced solid tumours, and EGFR-PKIs are safe and tolerable by cancer patients, especially for those previously treated patients. In the context of this finding, it is worth noting that typical EGFR-PKIs are not known to induce cardiovascular adverse events that are so typical of VEGFR-PKIs, thus emphasising the impact of these events on the risk/benefit of PKIs.

PKI in combination with cytotoxic agents

In a more comprehensive meta-analysis of 43 trials involving 16,011 patients (8,460 on PKIs and 7,551 controls) that balanced the risks versus the benefits of PKIs active at VEGFR and EGFR, Funakoshi et al. [105] evaluated the safety and efficacy of combining cytotoxic chemotherapy with PKIs, which were divided into two subgroups: VEGFR PKI (axitinib, cabozantinib, pazopanib, regorafenib, sorafenib, sunitinib and vandetanib), and EGFR-family PKI (erlotinib, gefitinib and lapatinib). They found that compared with chemotherapy alone, the addition of a PKI was associated with a significant improvement in progression free survival (hazard ratio 0.82; 9 5% CI: 0.76–0.89), but not overall survival (hazard ratio 0.99; 95 % CI: 0.95–1.03). However, the addition of a PKI significantly increased the risk of FAEs (RR = 1.63, 95 % CI: 1.32–2.01), treatment discontinuation (RR = 1.80, 95 % CI: 1.58–2.06), and any severe adverse event (RR = 1.25, 95 % CI: 1.16–1.36). Surprisingly, the RR associated with addition of a PKI was 1.49 (95 % CI: 1.16-1.90) with

VEGFR inhibitors compared to 2.04 (95 % CI: 1.38-3.01) with EGFR inhibitors. These findings serve to caution the physicians to weigh the risk of toxicity versus the modest benefit in terms of progression free survival associated with chemotherapy plus a PKI in patients with solid cancers.

Safety of protein kinase inhibitors in routine clinical practice

In routine clinical practice, however, the risk/benefit is likely to be inferior to that determined from highly controlled clinical trials. Whereas patients in pre-approval clinical trials are carefully selected, treatment of wider and less selected patient population in routine oncologic practice may increase the likelihood of toxicity and lower the probability of benefit [106]. The problem may be further compounded by off-label use of oncology drugs. One study from Switzerland reported a total of 985 consecutive patients receiving 1,737 anticancer drug treatments and found that overall, 32.4 % of all patients received at least one off-label drug, corresponding to 27.2 % of all anticancer drugs administered [107].

Following a detailed analysis of the adverse drug reactions of targeted anticancer agents from their reporting in pivotal randomized clinical trials and subsequently updated drug labels, Seruga et al. [108] concluded that many rare but serious and potentially fatal adverse drug reactions associated with these agents are not reported in clinical trials. One study on cancer drugs in Japan reported that of the 111 fatal adverse drug reactions detected in the eight post-marketing surveillances, only 28 (25.0 %) and 22 (19.6 %) were described on the initial global and the initial Japanese drug label, respectively, and 58 (52.3 %) fatal adverse drug reactions were first described in the all-case post-marketing surveillance reports [109].

hERG blockade in oncology – a friend or a foe?

Most of the 14 drugs withdrawn from the market due to their QT-related proarrhythmic proclivity were either old drugs with more effective newer alternatives or were indicated for relatively benign indications. As a result, their risk/benefit was considered unfavourable and it was prudent to have withdrawn them form the market. However, for the PKIs, the risk/benefit components are different. Not only are they indicated for life-threatening conditions with potentially no alternatives but also, their post-marketing performance suggests that the risk of QT-related proarrhythmia may have been over-estimated. Critically, as explained earlier, the risk assessment is based on a parameter (QT prolongation) that is known to be a poor predictor of the risk. If PKIs can be approved and continue to be marketed and used clinically despite clinically the most relevant risks of LV dysfunction and arterial thrombotic events, there seems no reason why they should be abandoned early during the course of their development because of the phobia about QT-related over-estimated risks of proarrhythmias.

Since both ICH S7B and ICH E14 are principally “hERG-centric” and “QT-centric”, respectively, a new paradigm, referred to as the Comprehensive In vitro Proarrhythmia Assay (CiPA), is now gathering momentum among all the stake holders, including the regulatory authorities [110]. This paradigm, aiming to characterise the risk of proarrhythmias as opposed to QTc prolongation, recognises the critical role of multiple ion channels blockade and among other recommendations, calls for all new drugs to be studied for their effects on multiple ion channels and incorporation of these effects in an in silico computer modelling of human ventricular electrophysiology to predict a drug’s proarrhythmic potential.

Of greater concern is the real possibility that discarding a PKI because it inhibits hERG channel may prove to be counter-productive since hERG is expressed in a variety of malignancies [111-113]. Early indications are that hERG channel blockers attenuate the progression of both hematologic malignancies and solid tumours [114-119]. Not surprisingly, hERG channel has been suggested as a potential target for

anticancer drugs [112, 120]. Among the interesting such drugs is astemizole, withdrawn from the market in 1999 for its torsadogenic potential [46], which is not only a hERG blocker but also an antihistamine [121] and histamine favours proliferation of normal and cancerous cells. If hERG channel were to prove to be a valuable therapeutic target in oncology, new paradigms will have to evolve regarding the management of PKI-induced QT interval prolongation.

Discussion and conclusions

In contrast to their QT-liability, the hepatotoxic potential of PKIs is far greater. Nineteen (68 %) of the 28 PKIs are labelled as potentially hepatotoxic with five them carrying a black box warning (Table 2). Twelve (75 %) of the 16 PKIs approved in period 1 and 7 (58 %) of the 12 approved in period 2 are deemed to be hepatotoxic. Four of the 12 from period 1 and one of the 7 from period 2 carry black box warning (lapatinib, pazopanib, regorafenib and sunitinib, and ponatinib, respectively). Although the potential for clinically relevant hepatic injury is typically evaluated on the basis of the magnitude of increases in serum aspartate and alanine transaminases (AST and ALT), alkaline phosphatase (ALP) and bilirubin, the risk assessment and labelling are based on the number of cases that meet “Hy’s rule”. According to this rule, a significant risk of severe hepatotoxicity is associated with medications which cause hepatic injury (elevation in ALT) together with reduction in hepatic function (the synthesis and transportation of bilirubin) in absence of any evidence of biliary obstruction (e.g. elevation of ALP) or of other causes that can reasonably explain these elevations in ALT and bilirubin. Hy’s rule has been validated and confirmed in two large studies of drug-induced liver injury in which approximately 10 percent of subjects with hyperbilirubinaemia or jaundice died or needed liver transplant [122, 123]. Finding one Hy’s rule case in the clinical trial database is worrisome; finding two is considered highly predictive of the drug having the potential to cause severe drug-induced liver injury when given to a larger population [124]. In pre-approval clinical trials of relevant PKIs, there were cases that met Hy’s rule or fatal cases of hepatic failure. Attribution of the risk of clinically relevant hepatotoxicity has been justified by numerous post-marketing reports of PKI-induced hepatotoxicity that required revisions to the labelling of several PKIs. Interestingly, however, although bosutinib is labelled as potentially hepatotoxic, a post-approval observational study in which 248 patients received bosutinib for a median duration of 27.5 months revealed no cases in the bosutinib arm that led to hospitalization, were associated with permanent hepatic injury or liver-related deaths, or met Hy’s rule criteria [125].

Thus, although the risks of proarrhythmia and hepatotoxicity are both determined on the basis of surrogate markers, the assessment of hepatotoxicity is based on a combination of clinically relevant and validated endpoints. There are also significant challenges to evaluating QT- and transaminase-based clinical risk. Whereas QT prolongation does not correlate well with the risk of proarrhythmias for the reasons already explained earlier, diagnosing drug-induced liver injury can be difficult in presence of metastasis. Development of newer PKIs should proceed on the basis of assessment of clinically meaningful risks and therapeutic benefits in terms of morbidity, mortality and quality of life if potentially valuable agents are not to be discarded early in their development programme without exploring their clinically meaningful benefits. Safe and effective use of these valuable drugs requires close collaboration between the oncologists and their colleagues in other specialties such as the collaboration which has been forged by oncologists and cardiologists [3].

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