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CARDIOVASCULAR TOXICITY OF BRUTON TYROSINE KINASE INHIBITORS: FORGET ABOUT SELECTIVITY BUT WATCH THE CLOCK

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Commentary on

Moslehi et al., "Cardiovascular events reported in patients with B-cell malignancies treated with Zanubrutinib". *Blood Adv*. 2024 Mar 19:bloodadvances.2023011641. doi: 10.1182/bloodadvances.2023011641.

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CARDIOVASCULAR TOXICITY OF BRUTON TYROSINE KINASE INHIBITORS: FORGET SELECTIVITY BUT WATCH THE CLOCK

Bruton Tyrosine Kinase (BTK) is an important molecular driver of B cell malignancies such as chronic lymphatic leukemia (CLL), Waldenstroem macroglobulinemia (WM) and others. Ibrutinib, first generation BTK inhibitor (BTKI), and acalabrutinib and zanubrutinib, second generation BTKIs, therefore represent valuable opportunities for managing B cell malignancies but all of them also cause cardiovascular (CV) toxicity in the form of hypertension, atrial flutter/fibrillation, or less frequently, ventricular arrhythmias.[1] In this issue of the Journal Moslehi and colleagues[2] describe the results of a pooled analysis of ten clinical trials in which zanubrutinib was used as single agent, primarily in the settings of CLL/small lymphocytic leukemia (SSL), and focus on head-to-head comparisons between zanubrutinib and ibrutinib in the ALPINE trial of patients with relapsed/refractory CLL/SLL and in cohort 1 of the ASPEN trial of patients with WM.[3,4] The analysis illuminates zanubrutinib as a well tolerated BTKI that causes significantly lower exposure-adjusted incidence rates of atrial flutter/fibrillation, hypertension and ventricular arrhythmias in comparison with ibrutinib. The improved CV safety of zanubrutinib emerges even when spurious data from the ALPINE trial, which indicated similar rates of hypertension from zanubrutinib and ibrutinib, are taken in due account.[2] The thoughtful analysis by Moslehi et al. is well in agreement with data reported by other investigators who scrutinized the same data base albeit with different aims and approaches.[5]

CV toxicity of BTKIs does not come as a surprise. BTK is highly homologous to TEC and Src kinases that relay homeostatic signals in CV system;[6] moreover, ibrutinib, acalabrutinib and zanubrutinib inhibit BTK by covalent bond to Cys481 in the ATP-binding pocket of the kinase,[7] but Cys481 and other druggable cysteines are found in many other kinases, including PI3K and the kinase domains of Erbb2 and Erbb4 receptors, all being important CV regulators.[8-12] BTKIs can thus inhibit many more kinases than just BTK, paving the road to off target CV effects.

Why does zanubrutinib cause fewer CV events than ibrutinib? One intuitive explanation is that zanubrutinib is more selective than ibrutinib at inhibiting BTK,[2,5,13] but logical as it may sound this assumption does not come without limitations. Colourful renditions of the human kinome exposed to BTKIs do suggest that both zanubrutinib and acalabrutinib show improved selectivity towards BTK[13,14] but data

were generated under conditions that call for caution. The concept of selectivity embraces parameters such as affinity of the inhibitor for the intended target (measured as K*i* in binding experiments) and the potency with which the target is inhibited (measured as IC_{50} in enzymatic assays). For covalent inhibitors, as the BTKIs are, selectivity should be measured by setting the inhibitor at concentrations not too different to K*i* or IC_{50}, so as to avoid artefactual inhibition of kinases that BTKIs would bind at higher concentrations;[15] yet, one popular kinome assay, yielding a selectivity rank order of acalabrutinib ≥ ACP-5862 (acalabrutinib active metabolite) > zanubrutinib > ibrutinib, was generated with BTKIs at 1 μM, which was orders of magnitude higher than IC_{50} or K*i* values.[14] In a different kinome assay, yielding a selectivity rank order of zanubrutinib > acalabrutinib > ibrutinib > ACP-5862, BTKIs were used at 100xIC_{50};[13] this was partially appropriate for ibrutinib and zanubrutinib, whose concentrations remained lower than K*i*, but it was not so for acalabrutinib and ACP-5862, whose concentrations were overly higher than K*i* (**Table 1**). The bright side of this story is that zanubrutinib seems to be more selective than ibrutinib in both kinome assays, which in principle would be consistent with its improved CV liability; the dark side of the story is that the rank order of selectivity of the three BTKIs changes from one experimental setting to another, making a comprehensive picture difficult to obtain at this point in time.

Having considered all caveats and uncertainties around selectivity, I suggest that looking at the characteristics of patients candidate for BTKIs may help look at these drugs from different viewpoints. Advanced age and comorbidities fingerprint the vast majority of these patients. Moslehi and colleagues report that ~6% of them presented at treatment with history of prior atrial fibrillation,[2] a figure consistent with a Mayo Clinic retrospective analysis of 2444 patients diagnosed with CLL/SLL.[16] Perhaps more importantly, the Mayo Clinic analysis showed that patients without prior atrial fibrillation eventually developed it during follow-up at an apparent rate of 1%/year, in spite of that no more than one third received active treatment and no more than 3% received ibrutinib as part of treatment.[16] These facts illuminate atrial fibrillation (and hypertension) as inevitable pathophysiologic events in a population of older comorbid patients, and suggest that BTKIs might be viewed as "triggers" rather than etiological agents of CV events.

Possible differences between ibrutinib and second generation BTKIs as triggers of CV events were characterized, for the first time in the phase III trial of ibrutinib versus acalabrutinib in patients with previously treated CLL.[17] In that study median time to any grade atrial fibrillation was significantly longer

for acalabrutinib compared to ibrutinib. but the curves of cumulative incidence eventually aligned after ~50 months follow-up. Pointing on head-to-head comparisons in ASPEN cohort 1 or in ALPINE trial, Moslehi and colleagues show that atrial fibrillation occurs early during the first six months of treatment with ibrutinib but develops more slowly during treatment with zanubrutinib. Of note, and different to acalabrutinib, the curve of zanubrutinib-triggered atrial fibrillation did not align with ibrutinib at longer than 40 or 50 years follow-up in ALPINE[3] or in ASPEN cohort 1[4], respectively. Pending availability of new and more extensive data on hypertension in head-to-head comparisons of zanubrutinib versus ibrutinib, this focus on time to atrial fibrillation may help identify different means for differentiating BTKIs, with both acalabrutinib and zanubrutinib proving less potent than ibrutinib at accelerating the development of events that otherwise would occur at a later time point; moreover, zanubrutinib holds promise as a BTKI with both longer time to, and reduced overall incidence of CV events. Clinical management of patients treated with BTKIs should perhaps be tailored to such differentiation so as to intensify surveillance over the time window when the risk of triggered events increases. Watching the clock may thus become important (**Figure 1**).

Shifting the focus from selectivity towards time to event may also help refine research directions, Whereas better characterization of selectivity remains a must by definition, understanding the kinetics of off target effects over time emerges as an equally important goal. How do BTKIs approach unintended kinases in peripheral compartments and cell types that express such kinases? How does the irreversible inhibition of off target kinases compare with target resynthesis and steady state concentrations of BTKIs at that target? Questions like these should be answered soon, the clock is ticking.

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Authorship

Giorgio Minotti designed and wrote the article

COI Statement

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LEGEND TO FIGURE

Conceptualization of cardiovascular events in the at-risk patient with B cell malignancy. Absent BTKIs, CV events would occur spontaneously at a given time point but ibrutinib (IBR), acalabrutinib (ACA) and zanubrutinib (ZAN) act as triggers that precipitate CV events at earlier time points, with the potency of triggering being IBR > ACA > ZAN.

Table 1. Limited selectivity conditions in two human kinome assays:of BTKIs

Reference	Reported selectivity (from high to low)	IC_{50} (μM)	K*i** (μM)	BTKi in the assay (μM)	Selectivity conditions vs IC_{50}	Selectivity conditions vs K*i*
Podoll et al. (14)	acalabrutinib	0.005	0.181	1	-	-
	ACP-5862†	0.005	0.188	1	-	-
	zanubrutinib	0.0005	0.126	1	-	-
	ibrutinib	0.0015	0.054	1	-	-
Shadman et al. (13)	zanubrutinib	0.00071	0.126	0.071	-	√
	acalabrutinib	0.0240	0.181	2.400	-	-
	ibrutinib	0.00032	0.054	0.032	-	√
	ACP-5862†	0.0630	0.188	6.300	-	-

*All from Podoll et al. (14)

†Acalabrutinib active metabolite

Figure 1