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PLK1 Inhibitors: Setting the Mitotic Death Trap

Polo-like kinase 1 (Plk1) regulates mitotic progression in all eukaryotes and has been implicated in the transformation of human cells. Analysis of the cytological and anti-tumor activities of BI 2536, a novel, selective pharmacological inhibitor of Plk1, has connected chemistry and biology to the bedside.

Simon Plyte[1] and Andrea Musacchio[2]

In the time it takes you to read two recent papers by Lenart *et al.* [1] and Steegmaier *et al.* [2], several thousand cells in your body will have successfully undergone mitosis, during which a mother cell containing a replicated genome divides into two (usually identical) daughter cells. Mitosis involves a superbly choreographed dance whereby the mitotic spindle, one of nature's more beautiful macromolecular structures, seeks, captures, aligns, verifies and then separates the chromosome partners in a wonderfully elegant process, orchestrated by several protein kinases. The circumstances for mitotic entry are set by the ignition of a protein kinase called Cdk1 (Figure 1A). The Cdk1 engine keeps running until all chromosomes have established solid connections with the spindle. At that point, the key is removed from the engine (Cyclin B, the Cdk1 activator, is degraded), an event that promotes chromosome partition and mitotic exit, heralding the birth of two new cells [3].

A mitotic cell swarms with activity. Phenomena such as chromosome condensation, kinetochore and spindle assembly, and the formation of microtubule bundles stably connecting the chromosomes to the spindle, all occur within minutes and are essential for the error-free partition of the genetic material (Figure 1A). Accuracy in these processes is essential to maintain euploidy in normal cells, and accordingly, safety devices have developed to monitor these events and ensure their seamless execution [3].

The mitotic cell division process, however, is a phase of vulnerability not only for normal cells, but for cancer cells, too. The papers from Lenart *et al.* [1] and Steegmaier *et al.* [2] provide a stunning demonstration that as cancer cells navigate their way through mitosis, they are susceptible to an ambush. The work is the fruit of an academic-industrial collaboration and describes the use of BI 2536, a small-molecule inhibitor of polo-like kinase 1 (Plk1), as an analytical tool and potential cancer theraputic. The studies delineate some of the pleiotropic functions attributed to Plk1 in mitosis and demonstrate that inhibition of Plk1 function translates into significant anti-tumor activity *in vivo*.

Like Cdk1, Plk1 is a protein kinase [4], and as such utilizes ATP to add phosphate groups onto substrates, thereby potentially influencing the activity, stability, or subcellular localization of specific protein targets. In the last fifteen years, the pharmaceutical industry has developed an obsessive passion for the attenuation of protein kinases, as these enzymes are implicated in the control of almost every biological process. The ATP-binding pocket of protein kinases represents an ideal site for the binding of small-molecule inhibitors, and several classes of ATP-mimetic compounds, of which BI 2536 is an excellent example, are being investigated for their potential to inhibit a plethora of protein kinases [5]. Besides Cdk1 and Plk1, several other mitotic kinases display their regulatory influence on the mitotic scene – most notably the Aurora A and Aurora B kinases [6]. Both Plk1 and the Aurora kinases have long been recognized as potential targets for cancer therapy, and numerous small-molecule inhibitors of Aurora kinases are currently undergoing clinical development. It appears that Polo-like kinase inhibitors have now joined the race to become blockbuster anti-cancer drugs [7,8].

Understanding how these drugs work to kill cells is very important. For example, taxanes work by preventing the depolymerization of microtubules, and this, among other things, prevents the assembly of a functional mitotic spindle, leading to cell death [9]. However, inhibition of microtubule function is detrimental to several cellular processes, including axonal transport and cell movement, and as a result there are several mechanisms of cell death in response to taxane treatment, including apoptosis, mitotic catastrophe, lytic necrosis, and induced senescence. More recently, inhibitors of Eg5, a kinesin motor protein, have entered clinical trials. These agents should disrupt microtubule dynamics in a mitosis-specific manner. The mechanism of cell death here is predominantly apoptotic and can be induced rapidly from within mitosis or after slippage into an abnormal G1 phase by a p53-dependent mechanism [9]. The first-generation Aurora inhibitors are active against both

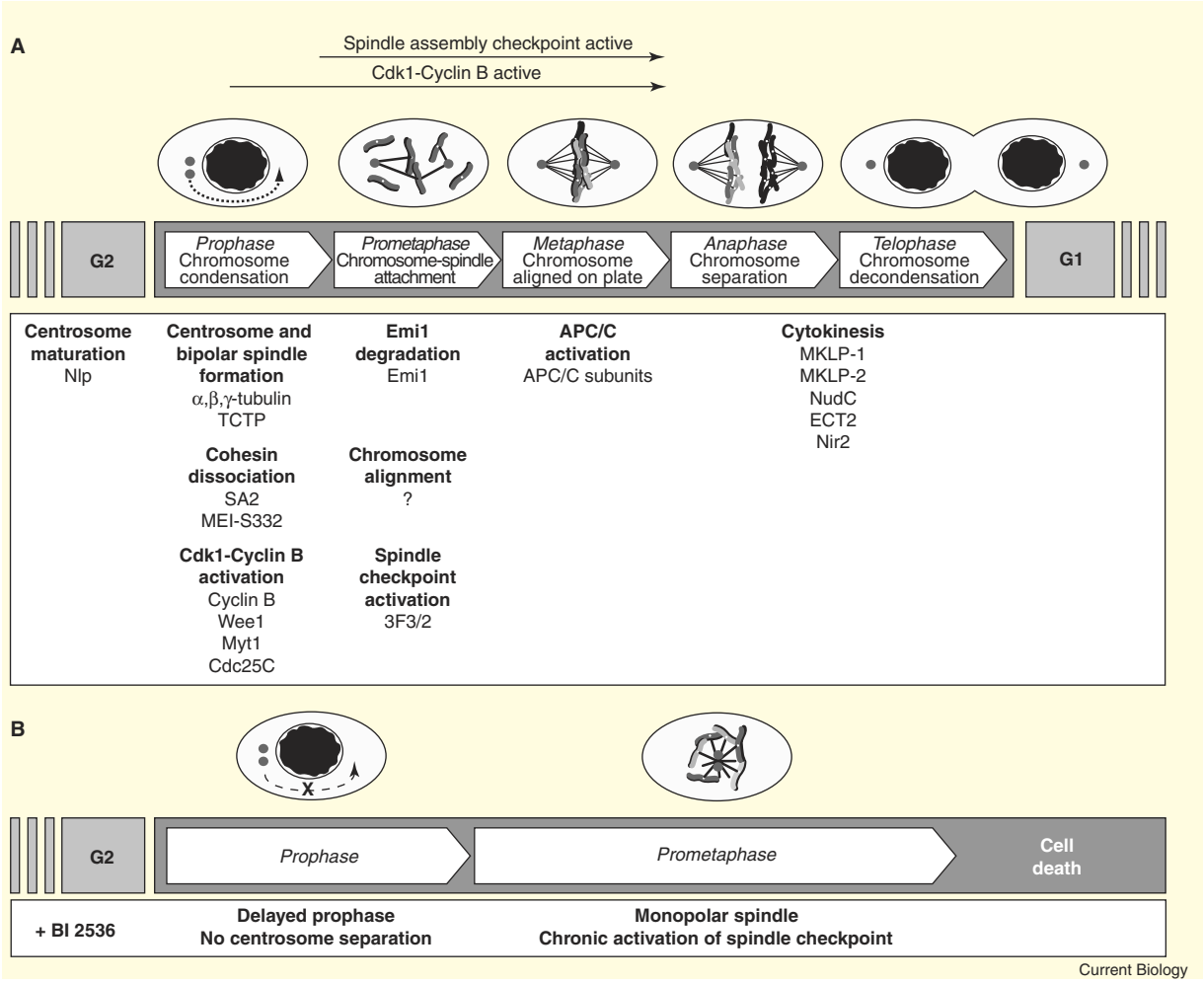

Figure 1. Pleiotropic functions of Plk1.

(A) Mitosis separates the G2 phase of a mother cell from the G1 phase of two daughter cells. Based on morphological criteria, the process has been traditionally divided into five phases (Prophase to Telophase) [1]. Centrosome separation is mandatory to form a bipolar spindle. Spindle microtubules bind chromosomes at kinetochores, complex protein disks that form on centromeric DNA. When chromosomes are aligned at the metaphase plate, the spindle assembly checkpoint is switched off, Cyclin B is degraded, and loss of cohesion between sister chromatids results in the segregation of chromosomes to the daughter cells. Plk1 controls several aspects of mitosis. In the white box, we report events in which Plk1 function (bold) has been implicated; under these are known Plk1 substrates involved in those events [4]. (B) Addition of BI 2536 perturbs the events shown in panel (A). Prophase is prolonged, possibly due to difficulties in activating Cdk1-Cyclin B. Centrosome maturation and separation are hampered, so that a monoastral spindle is formed rather than a bipolar one. This leads to a chronic activation of the spindle assembly checkpoint and to cell death without ever exiting mitosis. Other anti-mitotics result in different patterns of cell damage and cell death that might be important for toxicity and efficacy during anti-tumor therapy (see text for details).

Aurora A and Aurora B. Inhibition of Aurora A results in a mitotic arrest with monopolar spindles, while inhibition of Aurora B causes silencing of the SAC and mitotic exit without chromosome separation [10–12]. Precocious mitotic exit with defective cytokinesis and polyploidy is the predominant phenotype with these dual inhibitors. This raises some concern regarding the long-term implications of inhibiting Aurora B in normal cycling cells. Polyploidy has been shown to facilitate transformation [13], and the accumulation of polyploid cells during therapy might be expected to result in secondary tumors.

How does BI 2536 kill cells? Lenart and co-workers [1] analyzed the effects of BI 2536 on mitotic progression of HeLa cells, a widely used cellular model to study mitosis. As the succession of events is very rapid in mitosis, a small chemical like BI 2536 with specific inhibitory effects provides an invaluable tool to perform razor-sharp dissection experiments requiring fast temporal resolution. In a nutshell, BI 2536 caused a prometaphase arrest due to severe perturbation of normal mitotic progression, followed by mitotic catastrophe, a form of cell death taking place from within mitosis (Figure 1B). The precise causes of mitotic catastrophe (that is, the actual offences that trigger this type of cell death) are largely unclear. Its occurrence in the presence of BI 2536, however, is undoubtedly the basis for the efficacy of this

compound in animal models of cancer, as described by Steegmaier *et al.* [2]. Mitotic entrapment and subsequent cell death translated into significant growth inhibition in a wide range of tumor cell lines. More strikingly, BI 2536 demonstrated a remarkable efficacy *in vivo* in several tumor xenograft models and even caused regression of large tumors. How these *in vivo* efficacies will translate to the clinic, where tumors are more heterogeneous, remains to be seen. Given that cell death occurs predominantly from mitosis, with almost no polyploid progeny, the undesired effects of other anti-mitotic agents should be reduced with Plk-1 inhibitors.

The reason why different anti-mitotics result in so many different mechanisms of cell death remains unclear. Chemical biology analyses like the one reported by Lenart *et al.* [1] can help shed light on this mystery, and the reader is referred to this paper for a full description of the cellular phenotypes caused by BI 2536. In short, the investigation covers the roles of Plk1 in cell cycle progression and in mitosis. Several effects of inhibiting Plk1 have been described in previous papers, including a number of recent chemical biology analyses [4,14,15]. Lenart *et al.* [1] summarize and extend these previous observations, providing a beacon for those wishing to understand the cellular function of Plk1. A particular advantage revealed in the new work is the remarkable potency and selectivity of BI 2536 (0.8 nM) towards Plk1. Lenart *et al.* [1] report that HeLa cells treated with BI 2536 were first delayed in prophase (Figure 1B), consistent with a role for Plk1 in the activation of the CDK1-Cyclin B complex [4]. After reluctantly entering mitosis, cells became blocked in prometaphase with aberrant monopolar spindles that failed to attach stably to chromosomes [1]. The prometaphase arrest was maintained by the spindle assembly checkpoint (SAC), a safety device that monitors the attachment of microtubules to mitotic chromosomes [16]. SAC activity results in the stabilization of Cyclin B, which in turn prolongs CDK1 activity to maintain the mitotic state. Aficionados of mitotic affairs will be interested in reading that in agreement with previous results [17,18], BI 2536 blocked the degradation of the early mitotic inhibitor Emi1 [1]. Nonetheless, the degradation of Cyclin A took place normally, showing that the degradation of Emi1 is not a prerequisite for degrading Cyclin A. This also rules out the possibility that the prometaphase arrest caused by BI 2536 depends on failure to degrade Cyclin A, which has been previously shown to prevent mitotic exit [19]. Another remarkable observation made possible by the use of small-molecule inhibitors is that the activity of Plk1 is required not only to obtain, but also to maintain, chromosome bi-orientation on the mitotic spindle [1,15].

Recently, with the success of the broad-spectrum kinase inhibitor Sunitinib [20], the paradigm for receptor tyrosine kinase inhibitors has shifted toward the notion of "dirty is good for efficacy," because multi-kinase inhibition is probably required to successfully combat the adaptability and flexibility of mitogenic signal transduction in uncontrolled cancer cells. Further, in contrast to original fears, these compounds demonstrate that some 'broad spectrum' kinase inhibitors can have manageable toxicities in the clinic. But mitosis, compared to mitogenic signal transduction, is hard-wired and tightly controlled, and to guarantee fidelity of the process, has little or no redundancy afforded in its orchestration. With this in mind, it would seem that good potency and selectivity probably contribute significantly to the observed efficacy of BI 2536. High selectivity is probably required to prevent cancer cells from getting 'held up' in other phases of the cell cycle, thereby allowing them to go running headlong into the mitotic trap. Potent Plk1 inhibition ensures a prolonged arrest in prometaphase, effectively springing the trap and ultimately leading to cell death (Figure 1B). Possibly, Plk1 inhibitors are bringing the kinase-inhibitor paradigm back toward the idea that "clean is good" – at least for mitotic inhibitors.

In summary, BI 2536 defines a new anti-proliferation mechanism of action that will undoubtedly lead to a different spectrum of tumor efficacies and toxicities compared to the Aurora inhibitors and existing anti-mitotic agents. The authors report that BI 2536 was well tolerated in their mice studies, and early results from Phase I clinical trials have revealed neutropenia (loss of neutrophils) as the dose-limiting toxicity [2]. This is expected for a potent anti-mitotic agent. In contrast to Aurora inhibitors, which generated great excitement, the first Plk1 inhibitor has slid quietly into clinical trials, and early results look promising. Inhibitors of both kinase families, with their different mechanisms of action, hold much promise for the treatment of a wide range of human cancers, and we wait with great expectations for the results from clinical trials.

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Conservation Ecology: Area Trumps Mobility in Fragment Bird Extinctions

Tropical forest understory birds are highly sensitive to habitat fragmentation. Recent results from a monumental Amazonian fragmentation experiment show that habitat needs of these specialized birds make mobility a liability, leading to their extinctions from forest fragments.

Cagan H. Sekercioglu

Tropical forests are disappearing at a rapid rate while the remnants face an increasing number of threats [1]. Although a growing number of studies [2–6] have addressed the effects of forest fragmentation on tropical bird communities, most have had to draw inferences from species distribution patterns, rather than examine colonization and extinction dynamics directly [5,6]. Two recent mark-recapture studies [7,8] are welcome exceptions. Contrary to expectations [4,6], these new studies [7,8] show that, not only can tropical forest understory birds (Figure 1) disperse long distances, but their need to use large areas may make mobile species more susceptible to extinction in fragments.

The groundbreaking new findings [7,8] are based on nearly 50,000 bird captures from the Biological Dynamics of Forest Fragments Project (BDFFP) in the Brazilian Amazon. With 11 fragments (1–100 hectares) and 12 intact lowland forest sites (1–600 hectares) in a 41 kilometer-wide area sampled over two decades, the extent of this experiment is unequalled in the tropics [3,8]. Tom Lovejoy began this experiment in 1979 by convincing loggers to set aside fragments of 1, 10 and 100 hectares [2,9]. Unlike most fragmentation studies, pre-fragmentation sampling enabled natural abundance estimates to be made of all species. Fragment size and distance from intact forest were precisely controlled.

The BDFFP has produced many critical insights into avian responses to fragmentation [2,3,9,10]. For example, Ferraz *et al.* [9] showed that fragments less than 100 hectares lose half their forest-dependent bird species in under 15 years, too fast for forest regeneration to help, and that even isolated 10,000 hectare fragments could lose half their species in a century. Combined with another study [11], Ferraz *et al.* [9] concluded that even a

Figure 1. Ochre-breasted Antpitta (*Grallaricula flavirostris*), a typical tropical forest understory insectivore.

(Photo by Cagan H. Sekercioglu.)