

Targeting Polo-like Kinase in Cancer Therapy

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Abstract

Polo-like kinases (Plk) function in mitosis and maintaining DNA integrity. There are four family members, of which Plk1 represents a target for anticancer therapy. Plk1 is only expressed in dividing cells with peak expression during G2/M. Plk1 functions in multiple steps of mitosis, and is overexpressed in many tumor types. Mitotic arrest and inhibition of proliferation, apoptosis, and tumor growth inhibition have been observed in preclinical studies using small interfering RNAs (siRNA) or small molecules that inhibit Plk1. Preclinical studies also show that Plk1 inhibitors may be active against tumors with RAS mutations and that tumor cells with mutations in TP53 are more sensitive to inhibition of Plk1. Several Plk inhibitors are in phase I or II clinical studies. As expected, hematologic toxicity is the primary dose-limiting toxicity. Some patients have achieved clinical response, although in some studies only at doses above the maximum tolerated dose defined in the study. Further evaluation is necessary to discern the clinical utility of Plk1 inhibitors. *Clin Cancer Res*; 16(2); 384–9. ©2010 AACR.

Background

Plk1 as a cancer target. Antimitotics form the basis of therapy for patients with multiple types of solid tumors and hematologic malignancies. However, by stabilizing (taxanes) or depolymerizing (vinca alkaloids) microtubules, current antimitotics affect both dividing and non-dividing cells. An ideal next generation antimitotic should possess the following characteristics: target function(s) required in dividing cells but not nondividing cells; overexpression of the target in tumor with minimal expression in normal tissue; proven role for the target in inducing oncogenesis; robust pharmacodynamic (PD) marker(s) to monitor the inhibition of the target; and predictive marker(s) allowing enrichment of potentially responsive patient populations.

One of the emerging next generation antimitotic targets is Polo-like kinase 1 (Plk1). Plks are a family of serine-threonine kinases with a kinase domain at the N terminus and one or two polo-box domains involved in phosphopeptide-binding at the C terminus (1).

Among the four members of Plks, Plk1 is best characterized. As an antimitotic target, Plk1 is only expressed in dividing cells. Its expression and activity oscillates during cell cycle progression, with peak levels occurring at M phase (2). Plk1 is essential for mitosis, is required for al-

most every step of mitosis through its different protein-binding and phosphorylation activities, and its cellular localization changes accordingly during mitotic progression (Fig. 1). Plk1 directly promotes mitotic entry by phosphorylating cyclin B1 (3) and Cdc25C (4). It also contributes to centrosome maturation, drives microtubule nucleation by recruiting γ -tubulin ring complexes to the centrosomes (5), and phosphorylating proteins that are involved in microtubule dynamics such as ninein-like protein (Nlp) and translationally controlled tumor protein (Tctp; refs. 6, 7). It is located at the centrosomes during interphase and prophase. Then in prometaphase and metaphase it localizes to kinetochores and spindle poles, and regulates kinetochore assembly and contributes to spindle assembly checkpoint (8). It is further required for cytokinesis (9–11) and initiates mitotic exit by activating the anaphase promoting complex (APC) through phosphorylating inhibitors like early mitotic inhibitor 1 (Emi1; ref. 12), which leads to its destruction. These activities coincide with Plk1's relocation to central spindles during anaphase and telophase.

Besides promoting proliferation, Plk1 overexpression contributes to oncogenesis by promoting chromosome instability and aneuploidy through the check point functions. To ensure genomic stability, cell cycle progression is monitored by multiple checkpoints, including the G2/M, kinetochore, and spindle checkpoints (13). Overriding these mitotic checkpoints by overexpression of mitotic kinases like Plk1 can lead to immature cell division without proper chromosome alignment and segregation resulting in aneuploidy, a hallmark of cancer (14). Plk1 is essential for the recovery from DNA damage-induced G2/M arrest by activating Cdk1 (15). Interestingly, besides its well understood role in mitosis, and the fact that its expression and activity peak at G2/M phase, it was recently shown that Plk1 accumulates in the nucleus

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doi: 10.1158/1078-0432.CCR-09-1380

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during S-phase and plays an important role in maintaining genomic stability during DNA replication (16, 17).

Consistent with its role in promoting proliferation, overexpression of Plk1 has been observed in many tumor types, including breast cancer (18), colorectal cancer (19), esophagus and stomach cancer (20), endometrial carcinomas (21), head and neck squamous cell carcinomas (22), non-small cell lung cancer (23), ovarian cancer (24), pancreatic cancer (25), and skin cancer (26) among others. In some tumor types overexpression of Plk1 correlates with a worse prognosis.

One could argue that Plk1 overexpression is simply a reflection of fast proliferating cancer cells and is not directly responsible for driving tumorigenesis. Evidence supporting Plk1 as a valid cancer target includes a study showing that overexpression of Plk1 in NIH3T3 fibroblasts transformed the cells, enabling the cells to grow in soft agar and form tumors when injected into nude mice (27). It was also substantiated by numerous studies using small interfering RNAs (siRNA) or small molecule inhibitors to show that when Plk1 is inhibited, cells would go through mitotic arrest, proliferation inhibition, apoptosis, and tumor growth inhibition in mice (28–34). Importantly, these studies also revealed that Plk1 inhibition preferably kills cancer cells compared with normal cells, providing a potential therapeutic window (28, 30, 35).

However, with all the evidence pointing to Plk1 as a worthwhile cancer target, it should be noted that the genetic evidence of *PLK1* being an oncogene is not very strong. The *PLK1* gene is rarely amplified with the only report of its gene amplification being a study of esophageal squamous cell carcinoma (36). Several missense mutations of *PLK1* have been identified in cancer cell lines, however these mutations seem to reduce Plk1 stability by suppressing the interaction between Plk1 and Hsp90 (37) and have not been reported in primary tumors.

Potential importance of selectivity for Plk1 inhibition.

Whereas only one polo kinase is found in yeast and *Drosophila*, four Plks have been identified in vertebrates: Plk1, Plk2 (Snk), Plk3 (Prk, Fnk), and Plk4 (Sak). Plk4 is structurally most divergent from other Plks (38). There are multiple Plk inhibitors that inhibit other Plks besides Plk1. The question remains; is a selective Plk1 inhibitor more desirable than an inhibitor of multiple Plks?

The different Plk proteins seem to have different expression patterns as well as nonoverlapping functions. During the cell cycle, Plk1 is expressed at the highest level at G2/M (39); *PLK2* is an immediate-early response gene and is expressed predominantly in G1 (40); Plk3 protein is expressed throughout cell cycle and seems to be primarily involved in cellular response to DNA damage (41). Plk4 seems to play a role in M phase-progression (42). The nonoverlapping functions of the Plks raise the concern that a lack of selectivity may result in undesired effects.

Another concern is that in multiple ways, Plk2 and Plk3 seem to have opposing functions to Plk1; thus inhibiting them along with inhibiting Plk1 might reduce the efficacy of inhibiting Plk1 alone. Furthermore, both Plk2 and Plk3

exhibit signs of tumor suppressor activity. In contrast to the prevalent overexpression of Plk1 in cancer, epigenetic inactivation of *PLK2* via aberrant CpG methylation in the transcriptional regulatory elements of the gene is a common event in B-cell malignancies (43). *PLK3* was also reported to be downregulated in lung cancer (44), head and neck cancer (45), and cancers of the uterus and bladder (46). However increased expression of *PLK3* was also reported in some hepatoblastomas (46), ovarian tumors (47), and breast cancer samples (48).

Plk2 and Plk3 also have an opposite relationship with p53 and response to DNA damage compared with Plk1. Plk1 is inhibited by DNA damage in G2 and mitosis (49), whereas Plk2 and Plk3 are both activated (50, 51). Plk1 negatively regulates the activity as well as stability of p53 (46), whereas Plk2 is a direct transcriptional target of p53 (50), and Plk3 mediated phosphorylation of p53 at Ser-20 is reported to stabilize p53 (51).

In contrast to Plk1 overexpression, which transforms NIH3T3 cells to a cancerous state (27), overexpression of wild-type Plk3 suppressed growth of human cancer cell lines and RAS transformed fibroblasts (52, 53).

Biomarkers for Plk1 inhibitor. Both PD biomarkers and predictive biomarkers play an important role in drug development. A PD marker is able to confirm an on-target effect and, ideally, show a dose-response relationship. It has been shown in preclinical studies that inhibition of Plk1 causes G2/M arrest, and consequently increases the level of the mitosis marker phospho-histone H3 (pHH3; ref. 54). In preclinical studies, the increase of pHH3 signal is very strong and correlates with the concentration of the Plk inhibitor. Because baseline pHH3 levels are low, background staining is usually clean, making the signal easily detected by immunohistochemistry. All these features make pHH3 a robust PD marker to monitor the effects of a Plk inhibitor.

Predictive biomarkers can improve the benefit-to-risk for patients by aiding in the identification of tumors that are more likely to respond to the agent. For antimetabolites like Plk inhibitors, identifying predictive biomarkers is challenging because Plk1 interacts with multiple downstream elements and forms multiple different protein complexes. Another complication is that identification of a predictive biomarker requires access to both sensitive and nonsensitive tumors. Using an *in vitro* 2-D proliferation assay, most of the fast proliferating cancer cell lines have been considered sensitive to multiple Plk inhibitors (29, 34, 54). To further differentiate these cell lines, we stratified them on the basis of the response to a washout and outgrowth assay intended to mimic the clinical dosing schedule for the Plk1 specific inhibitor GSK461364A.¹ By comparing specific genetic and global genomic alterations of the cell lines, we found that cell lines harboring *TP53*

¹ Y. Degenhardt, J. Greshock, S. Laquerre, et al. Sensitivity of cancer cells to Plk1 inhibitor GSK461364A is associated with loss of p53 function and chromosome instability. Submitted for publication 2009.

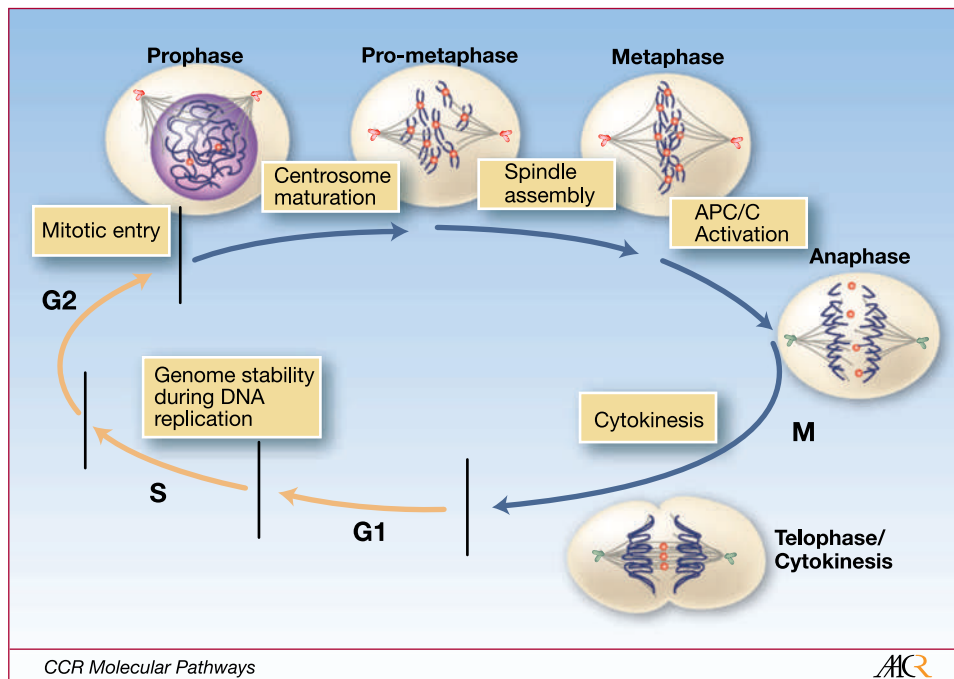


Fig. 1. Plk1 is required for almost every step of mitosis through its different protein-binding and phosphorylation activities, and its cellular localization changes accordingly during mitotic progression. Plk1 directly promotes mitotic entry. It also contributes to centrosome maturation and drives microtubule nucleation. Plk1 is located at the centrosomes during interphase and prophase. Then in prometaphase and metaphase it localizes to kinetochores and spindle poles, functioning to regulate kinetochore assembly and contribute to spindle assembly checkpoint. It is further required for cytokinesis and initiates mitotic exit by activating the APC complex. These activities coincide with Plk1's relocation to central spindles during anaphase and telophase. In addition, it was recently shown that Plk1 accumulates in the nucleus during S-phase and plays an important role in maintaining genomic stability during DNA replication.

mutations tended to be more sensitive to GSK461364A.¹ Furthermore, sensitive cell lines also had increased levels of chromosome instability, a characteristic associated with *TP53* mutations. A recent report using *TP53* isogenic colon cancer cell lines (55) demonstrated that under stress of ionizing radiation, *TP53* null cells also showed greater sensitivity to loss of Plk1 function.

It has been known that p53 function and Plk1 function are closely related to each other. Plk1 can physically bind to p53 and negatively regulate its stability and function (46). Conversely, p53 can negatively regulate Plk1 expression (56), and is a key player in the precise restriction of Plk1 gene expression during the G2/M phase (57). siRNA studies against Plk1 have also shown that knocking down Plk1 preferentially reduced survival of cells with mutant *TP53* (30, 58).

Interestingly, in a synthetic lethal siRNA library screen, inhibition of a mitotic pathway involving Plk1 resulted in prometaphase accumulation and subsequent death of *Ras* mutant cells, raising the possibility that a Plk1 inhibitor can be used to treat tumors with *RAS* mutations (59).

Clinical-Translational Advances

Several Plk inhibitors have been studied in clinical trials, with other compounds in preclinical development.

A complete list of Plk inhibitors in development is summarized elsewhere (60). Results from phase I or II studies have been reported for four Plk inhibitors; BI2536, GSK461364, ON-01910, and HMN-214 (summarized below). As expected for antimitotic compounds, hematologic toxicity has consistently been the primary dose-limiting toxicity. Various dosing schedules have been studied with these compounds and clinical activity and PD effects have also been observed.

BI2536 has been studied in patients with solid tumors or hematologic malignancies. During phase I trials, intravenously (IV) administered BI2536 showed dose proportional pharmacokinetics when administered using various dosing schedules. Hematologic toxicity (i.e., neutropenia, anemia, thrombocytopenia, or neutropenic infection) was dose limiting, establishing maximum tolerated doses of 175 mg or 200 mg when administered every 21 days to patients with relapsed or refractory non-Hodgkin lymphoma (NHL), or advanced solid tumors, respectively (61, 62). Stable disease (SD) lasting at least 3 months was observed in 9 of 40 patients (23%) with advanced solid tumors at the maximum tolerated dose (MTD) or above (62). Among 41 patients with NHL, three complete responses (CR), one partial response (PR), and 12 patients with SD was observed (61). In patients with AML, dose-dependent effects on

the number of cells in G2/M and undergoing apoptosis provide evidence of the intended antimitotic effect. No objective responses were observed in a phase I/II study of 23 patients with relapsed, small cell lung cancer (63). Phase II studies in patients with non-small cell lung cancer and hormone refractory prostate cancer have shown modest activity, but BI2536 is unlikely to be further evaluated as a single agent in patients with these tumor types (64, 65).

A phase I study of GSK461364A in 40 patients with advanced solid tumors established MTDs for two dosing schedules and showed on-target effects, dose-proportional pharmacokinetics, and anticancer activity (66). An MTD of 225 mg was established when GSK461364A was administered IV weekly for 3 weeks of a 4-week cycle. When administered by IV twice a week for 3 weeks out of a 4-week cycle (days 1, 2, 8, 9, 15, 16, every 28 days), the MTD was 75 mg. The observed dose-limiting toxicities were neutropenia, thrombocytopenia, and bone marrow suppression. Additional side effects included thrombotic events reported in eight patients, six of whom were on the twice a week schedule. In addition to the expected hematologic toxicity for an antimitotic, an increase in pHH3 in circulating tumor cells provided evidence of antimitotic effects. Six patients had prolonged stable disease lasting at least 3 months at doses of 150 and 225 mg on the once a week schedule and 75 and 100 mg on the twice a week schedule. The half-life of GSK461364A was 7 to 11 hours with a clearance rate of 76 to 83 L/h.

A phase I study of ON 01910.Na in 20 patients with advanced solid tumors established an MTD of 3,120 mg when administered intravenously on days 1, 4, 8, 11, 15, and 18 of a 28-day cycle (67). One patient experienced a dose-limiting toxicity of grade 3 abdominal pain and seven patients experienced grade 2 pain. A patient with ovarian cancer experienced a PR lasting 24 months after receiving doses of 4,370 mg ON 01910.Na. Terminal half-life was 20 to 27 hours with a clearance rate of 13 L/hr. In a phase I/II study of patients with trisomy 8 myelodysplastic syndrome, significant decreases in peripheral blast counts were observed in four of the first five patients enrolled (68).

In a HMN-214 phase I study in 29 patients with advanced solid tumors, an MTD of 8 mg/m²/d was established when administered orally, daily for 21 days of a

28-day cycle (69). Dose-limiting toxicities included severe myalgia and/or bone pain syndrome. Hematologic toxicity was not observed on this dosing schedule, but was on a daily × 5 schedule in a separate study. HMN-214 is a pro-drug that is rapidly converted to HMN-176. No accumulation of HMN-176 was observed with this administration schedule. Stable disease was observed in 24% of patients, including one patient with breast cancer who had SD for 6 months. Future development will potentially focus on patients with high expression of Plk1 (e.g., patients with pancreatic or prostate cancer).

Conclusion and Outlook

Preclinical data provide support for pursuing Plk1 as a target for cancer therapy, suggest the potential advantage of Plk1 selective compounds, and provide early evidence for potential biomarkers for patient selection (*TP53* and/or *RAS* mutations). Both preclinical and clinical data provide support for the use of pHH3 as a PD biomarker for Plk1 inhibition. As expected for a compound with antimitotic activity, hematologic toxicities were observed for most Plk inhibitors evaluated in clinical studies. Clinical activity has been noted following treatment with Plk1 inhibitors, however, a narrow therapeutic index has been observed with activity occurring at or above the MTD. In summary, Plk1 seems to be an appealing target for cancer therapy, however, further evaluation is necessary to discern the utility of Plk1 inhibitors in a clinical setting and determine how they will compare with currently available antimitotic agents.

Disclosure of Potential Conflicts of Interest

Both authors are employees of and stock-holders in GlaxoSmithKline.

Acknowledgments

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Received 11/3/09; accepted 11/6/09; published OnlineFirst 1/12/10.

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