Polo-like Kinase 1 Inhibitors and Their Potential Role in Anticancer Therapy, with a Focus on NSCLC

René H. Medema1, Chia-Chi Lin2, and James Chih-Hsin Yang2,3

Abstract

Cytotoxic platinum-doublet chemotherapy that includes antimitotic agents is a current standard of care in advanced non–small cell lung cancer (NSCLC). Microtubule-targeting antimitotics, taxanes, and Vinca alkaloids are effective anticancer therapeutics that affect both dividing and nondividing cells. A new generation of antimitotic agents that target regulatory proteins—mitotic kinases and kinesins—has the potential to overcome the limitations related to the role of tubulin in nondividing cells that are associated with traditional antimitotics. This review concentrates on Polo-like kinase 1, a key regulator of mitosis, outlines a rationale for its development as an anticancer target, and discusses data from preclinical and clinical studies of Plk1 inhibitors with a particular focus on NSCLC.

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Current Treatment Options

Lung cancer is the second most common malignancy in the United States (219,440 cases) and was the leading cause of cancer-related mortality (159,390 deaths) in 2009 (1). Non–small cell lung cancer (NSCLC) represents 85% of all lung cancers (2); approximately 40% are diagnosed with advanced or metastatic disease (3) with a 5-year survival rate of 14% (4).

Chemotherapy with platinum-based doublets (e.g. paclitaxel/carboplatin, docetaxel/cisplatin, gemcitabine/cisplatin, and pemetrexed/cisplatin) is the standard of care in first-line treatment of advanced NSCLC with median overall survival of approximately 8 months (5) and overall response rate of 19% to 30% (5, 6). Combination of bevacizumab with platinum doublets increases overall response rate and prolongs progression-free survival in selected NSCLC patients (7, 8). Second-line treatments comprise monotherapies, such as docetaxel (9, 10), pemetrexed (11), or erlotinib (12), and the benefits of these agents as maintenance therapy are currently debated (13). Erlotinib is recommended for patients who have failed more than 1 or 2 therapy regimens (12); cytotoxic chemotherapies in such patients are largely ineffective (14). Gefitinib is restricted to second- and third-line patients currently or previously benefiting from gefitinib or to certain conditions, such as the presence of epidermal growth factor receptor (EGFR)-activating mutations. Clinical development of erlotinib and gefitinib—tyrosine kinase inhibitors of EGFR—has opened up the possibility of these agents in targeted patients whose tumors harbor activating EGFR gene mutations (15, 16). Significant improvements in response and survival have been reported with erlotinib and gefitinib in such patients (17–21).

Novel Approaches Targeting Mitosis

The development of novel agents takes advantage of rapidly accumulating knowledge of the pathways altered in cancer cells. Targeting mitosis is a validated approach, and agents that affect the mitotic spindle are well-established components of many oncotherapeutic regimens, including NSCLC (22). Taxanes (paclitaxel and docetaxel), microtubule-stabilizing agents, Vinca alkaloids (vinorelbine, vinblastine, etc.), and microtubule-destabilizing agents impair normal mitotic spindle function and block cellular proliferation (23). New approaches to mitosis inhibition target regulatory proteins—mitotic kinases and kinesins—that control the process (Fig. 1) and have the potential to overcome limitations of traditional antimitotic agents related to the role of tubulin in normal cells (22). One such target is Polo-like kinase 1 (Plk1), a key regulator in several essential cell-cycle events, including mitotic entry, centrosome maturation, bipolar spindle formation, chromosome segregation, anaphase-promoting complex regulation, and cytokinesis (Fig. 2; ref. 24).

Plk1 in Cancer

Plk1 is the best characterized member of the Plk family (Plk1–5; ref. 25). It is expressed in dividing cells and
detectable only in proliferating adult tissues (e.g., spleen and testis; ref. 26). High Plk1 levels are a marker of cellular proliferation (27), observed in NSCLC (28) and other malignancies, including cancers of the breast, colon, endometrium, head and neck, esophagus, ovary, pancreas, prostate, and stomach, as well as glioblastoma, non-Hodgkin lymphoma, leukemia, and melanoma (26, 29–31). Plk1 levels in NSCLC tumors correlate inversely with survival, indicating that Plk1 may have prognostic value (28); this correlation has also been reported in other tumor types (26, 30, 31). In addition, high tumor mitotic rate and risk of metastatic disease have been associated with high Plk1 levels (32), implying a role for Plk1 in aggressive cancer.

Mechanistic studies using RNA interference (RNAi) have provided additional insight into the role of Plk1 in cancer cells and the molecular consequences of Plk1 inhibition. Plk1 depletion using siRNA in human cancer cell lines interrupts spindle assembly and maturation resulting in mitotic (Polo) arrest characterized by scattered chromosomes on a monopolar spindle and apoptosis (33, 34). This apoptosis is p53 independent; moreover, p53-null or -depleted cells are more sensitive to Plk1 depletion than wild-type cells (35, 36), suggesting that cancers such as NSCLC with p53 loss of function may be particularly sensitive to Plk1 inhibitors.

Genome-wide RNAi screening has identified Plk1 as an essential survival factor in Ras-driven cells that experience Ras-induced mitotic stress; such cells may be selectively sensitized to Plk-1 inhibition (37). K-ras activating mutations are common in NSCLC (38) and may serve as an indicator of better response to Plk1 inhibitors. K-ras mutations are found less frequently in the squamous cell carcinoma histologic subtype (~5%) and more commonly in the adenocarcinoma histologic
subtype (~15%–30%; ~25% in Western countries, but less common in Asian populations) of NSCLC.

Short hairpin RNAs against Plk1 reduced the growth of NSCLC A549 cells in mouse tumor xenografts and suppressed Plk1 tumor expression (39). Systemic treatment with siRNA against Plk1 inhibited the growth of A549 cells in the mouse liver, showing a role for Plk1 in lung cancer liver metastasis (40). These in vivo studies have confirmed the anticancer effects of Plk1 depletion in vitro and provide a rationale for pharmacologic exploration of Plk1 as a novel target.

Preclinical Studies of Plk1 Inhibitors

Plk1 contains a highly conserved N-terminal protein kinase domain and a C-terminal "Polo box domain" (PBD) that is required for Plk1 to dock to its intracellular targets (24, 41). These functionally critical sites have been explored in the development of drugs targeting Plk1 (42).

Inhibitors targeting the Polo box domain of Plk1

Given the large number of substrates and cellular functions of Plk1, attempts have been made to improve the selectivity of Plk1 inhibitory drugs by targeting the PBD of Plk1. This has the potential benefit of a higher selectivity for Plk1 over the related Plks and may allow for interference in some, but not all, cellular functions of Plk1. Selective small drug-like inhibitors of the PBD of Plk1 are purpurogallin (43), poloxin, and thymoquinone (44).

BI 2536. The compound is a dihydropyridinone derivative that shows more than 10,000-fold higher inhibitory potency (IC_{50} = 0.83 nmol/L) toward Plk1 than the majority of 63 other protein kinases tested (45). BI 2536 inhibited proliferation of all 32 examined human cancer cell lines (EC_{50} = 2–25 nmol/L; ref. 45). Comparable inhibition was also observed in nontransformed, immortalized cells, indicating that BI 2536 inhibition affects all proliferating cells. Cells treated with BI 2536 are blocked in G2–M and form monopolar mitotic spindles, a phenotype similar to that induced by siRNA against Plk1, suggesting that Plk1 is the main cellular target (45). BI 2536 (40–50 mg/kg) administered i.v. twice a week inhibited the growth of mouse tumor xenografts, including NSCLC (A549 and NCI-H460), and induced accumulation of "Polo-arrested" tumor cells followed by apoptosis, showing BI 2536–mediated mitotic arrest and tumor cell death in vivo (45).

Although cellular sensitivity to BI 2536 was independent of tumor suppressor or oncogene mutations (45), a recent report has shown that tumor cells and xenografts with K-ras mutations were more sensitive to BI 2536 than isogenic wild-type cells (37). These findings support the hypothesis that Ras-mutant cells may be more dependent on Plk1 for mitotic progression (37). Specific
pharmacologic agents targeting Plk1 may selectively kill Ras-mutant cells by exacerbating their mitotic stress and thus benefit patients who bear tumors with activated Ras oncogenes (37).

**Volasertib (BI 6727).** This dihydropteridinone derivative targets Plk1 with selectivity and efficiency (IC₅₀ = 0.87 nmol/L) similar to that of BI 2536 (46). It is also a potent inhibitor of cellular proliferation (EC₅₀ = 11–37 nmol/L) that induces cellular effects (G₂-M cell-cycle block, phospho-histone H3–positive staining, and monopolar mitotic spindles; ref. 46). Volasertib showed antitumor activity in mouse xenograft models of human cancers, including NSCLC (NCI-H460), using i.v. or oral administration schedules of 70 mg/kg/wk or 10 mg/kg/d (46). Notably, it also inhibited taxane-resistant xenograft tumors. The high volume of distribution of volasertib—indicative of good tissue penetration, a long terminal half-life in mice and rats, and sustained exposure in tumor tissue (46)—was an additional factor in the selection of volasertib for priority clinical development.

**GSK461364.** This imidazotriazine, ATP-competitive inhibitor, exhibits more than 1,000-fold higher potency toward Plk1 than the majority of 48 other protein kinases tested (47). Cellular treatment with GSK461364 showed not only signs typical of Plk1 inhibition (mitotic arrest with monopolar or apolar spindles) but also micronucleation indicative of mitotic slippage, resulting in tetraploidy (47). The extent of mitotic arrest was concentration dependent, and high concentrations (>300 nmol/L) caused a G₂ delay. GSK461364 inhibited the growth of a majority of 74 tested cancer cell lines with 89% of the EC₅₀ values lower than 100 nmol/L and induced cytotoxic as well as cytostatic effects that were cell line dependent. Intraperitoneal administration of GSK461364 at various doses induced partial regressions in 2 of 3 mouse tumor xenograft models of NSCLC (47).

**HMN-176.** This stilbene derivative is an active form of an oral prodrug, HMN-214. HMN-176 disrupts mitotic spindle assembly, inhibits centrosome-dependent microtubule nucleation, and is considered an “anticentrosome” agent (48). It does not directly inhibit the enzymatic activity of Plk1 but rather affects subcellular distribution of Plk1 (49). HMN-176 showed potent cytotoxicity against 22 human tumor cell lines (mean IC₅₀ = 118 nmol/L; ref. 50). HMN-214 reduced the growth of primary NSCLC tumors in human tumor cloning assay (51) and several xenografts in mice, including NSCLC (50).

**ON 01910.Na.** This benzyl styryl sulfone analogue is an ATP-noncompetitive, multitargeted inhibitor of several tyrosine kinases and cyclin-dependent kinase 1 (Cdk1; IC₅₀ = 18–260 nmol/L). It is reported to have a particularly strong potency (IC₅₀ = 9–10 nmol/L) toward Plk1 (52), although results from another report suggest that Plk1 may not be the primary target (45). ON 01910.Na inhibited proliferation of more than 100 cell lines, including those resistant to paclitaxel, with EC₅₀ in the range of 50 to 250 nmol/L. It also showed efficacy with minimal toxicity as a monotherapy or in combinations with cytotoxic drugs in xenograft models (52).

Preclinical studies with Plk1 inhibitors showed the feasibility and selectivity of a pharmacologic approach to targeting Plk1 as well as the anticipated mode of action of inhibitors and their potent antiproliferative effects in a majority of cancer cell lines and tumor xenografts. These results led to initiation of clinical studies and further development of Plk1 inhibitors as anti-cancer drugs.

**Clinical Studies of Plk1 Inhibitors**

**BI 2536**

A phase I first-in-humans study of BI 2536 was conducted in 40 patients with refractory or metastatic solid tumors previously treated with a median of 3 chemotherapy regimens (53). Most patients were treated with colorectal cancer (42.5%); the inclusion of patients with NSCLC was not reported. BI 2536 was administered i.v. once every 3 weeks following a dose-escalation design (25–250 mg). Reversible neutropenia with infection was the dose-limiting toxicity (DLT) that defined 200 mg as the maximum tolerated dose (MTD). Patients treated at the MTD (67.5% of all treated patients) developed grade 3/4 adverse events (Common Terminology Criteria for Adverse Events, version 3.0) including neutropenia (53.6%) and leukopenia (44.4%; ref. 53). Similar results were obtained in another phase I study of 70 patients with advanced solid cancers treated with BI 2536 on days 1 and 8 of a 3-week treatment course with MTD of 100 mg (54). Of patients treated with 200 mg or more in the once-per-3-weeks regimen, 23% showed stable disease for more than 3 months (53). This effect was more common with patients experiencing neutropenia (53).

Two clinical trials have investigated BI 2536 in NSCLC: a phase I study in combination with the antifolate agent pemetrexed (55) and a phase II study of BI 2536 monotherapy (56). In the combination trial, 33 patients with refractory or metastatic NSCLC who relapsed after first-line platinum-based therapy were treated with 500 mg/m² pemetrexed and escalating BI 2536 (100–325 mg) by i.v. on day 1 every 3 weeks. Grade 4 neutropenia and grade 3 rash were DLTs that established 300 mg as the MTD. Reversible grade 3/4 neutropenia occurred in 18%. The most common drug-related adverse events were mild to moderate nausea (36%), rash (33%), anorexia (27%), stomatitis (24%), and pruritus (24%). Two patients had a partial response. BI 2536 and pemetrexed exposures were not affected by coadministration (55).

In the monotherapy phase II trial, 95 patients with advanced or metastatic NSCLC who had progressed after, or failed, first- or second-line therapy received either a single i.v. dose of 200 mg on day 1 or a single i.v. dose of 50 to 60 mg on days 1, 2, and 3 of each treatment course (56). Neutropenia was the main grade 4 adverse event, and most of the reported adverse events transiently affected the hematopoietic system. Twelve patients had grade 3/4 complications associated with neutropenia, including sepsis or febrile neutropenia (1 patient each) and fever or infection...
associated with low neutrophil counts (10 patients). Four patients (4.2%) had an investigator-assessed partial response (36).

**Volasertib**

A phase I first-in-humans study of volasertib was conducted in 65 patients with advanced solid tumors, including 10 with NSCLC. Volasertib was administered i.v. once every 3 weeks following a dose-escalation design (12–450 mg; ref. 57). The study reported neutropenia, thrombocytopenia, and febrile neutropenia as DLTs and an MTD of 400 mg. Reversible and noncumulative hematotoxicity was the major adverse event. Three partial responses were observed in patients with urothelial and ovarian cancer and melanoma, and stable disease was reported in 48% (57). The study reported favorable pharmacokinetic parameters (linearity in the therapeutic dose range, large volume of drug distribution, and a half-life around 110 hours). A phase I trial of volasertib administered on days 1 and 8 every 21 days is currently ongoing (NCT00969553; ref. 58). These results prompted the initiation of multiple phase II studies, including a randomized trial of volasertib as monotherapy or in combination with pemetrexed in second-line NSCLC (NCT00824408).

The identification of neutropenia as the dominant adverse event in studies with BI 2536 and volasertib indicates a mechanism-based inhibition of bone marrow cellular proliferation. In addition, the absence of any reported cumulative or high-grade mucosal side effects suggests the feasibility of prolonged BI 2536 or volasertib administration.

Although the clinical development of BI 2536 has halted, volasertib is currently being investigated as a single agent in phase II trials in several different indications (NCT01023958 and NCT01121406) and as a combination with platinum (NCT00969761), pemetrexed (NCT00824408), or other targeted therapies (NCT01022853 and NCT01206816).

**GSK461364**

A phase I first-in-humans study of GSK461364 was conducted in 27 patients with advanced solid tumors (59). The agent was administered i.v. following 2 schedules with different dosing (50–225 mg on days 1, 8, and 15 or 25–100 mg on days 1, 2, 8, 9, 15, and 16) on a 28-day cycle. DLTs included grade 4 neutropenia, sepsis, or pulmonary embolism. Common adverse events included hematologic toxicity, phlebitis, nausea, and fatigue. Stable disease for more than 5 months was observed in 2 patients. GSK461364 increased phospho-histone H3 levels in circulating tumor cells, indicating antimitotic effects of the treatment. The recommended phase II dose for GSK461364 was 225 mg on days 1, 8, and 15 in a 28-day cycle. Because of the high incidence (20%) of venous thromboembolism, GSK461364 should involve coadministration of prophylactic anticoagulation for further clinical evaluation (59).

**HMN-214**

Two phase I studies of HMN-214 have been reported in patients with solid tumors (49, 60). DLTs of prolonged neutropenia, febrile neutropenia, neutropenic sepsis, electrolyte disturbance, neuropathy, and myalgia were observed at doses of 24 to 48 mg/m² for 5 consecutive days every 4 weeks. MTD was established at the range of 18 to 30 mg/m², based on previous patient treatment load (60).

In another study, 33 patients with advanced cancer received oral HMN-214 (3.0–9.9 mg/m²/d) in a continuous 21-day dosing schedule every 28 days (49). DLTs comprised severe myalgia and/or bone pain syndrome and hyperglycemia. MTD was 8.0 mg/m²/d. The study reported 24% of patients with stable disease and indicated that future development of HMN-214 would focus on patient populations with high tumor expression of Plk1.

**ON 01910.Na**

A phase I first-in-humans study of ON 01910.Na was conducted in 20 patients with advanced solid tumors (none with NSCLC; ref. 61). The agent was administered i.v. at 80 to 4,370 mg by accelerated titration design on days 1, 4, 8, 11, 15, and 18 in 28-day cycles. Grade 3 abdominal pain was reported as a DLT at an MTD of 3,120 mg. The most frequently reported adverse events (grade ≤3) during the first treatment cycle comprised skeletal, abdominal, and tumor pain; nausea and vomiting; urge to defecate; flatulence; and fatigue. Mild hematologic toxicity—anemia and lymphopenia—was reported in approximately 10% of patients, and this contrasts with other Plk1-targeting compounds. One partial response was observed in a patient with ovarian cancer (61).

Although early preclinical studies identified Plk1 as a main target of ON 01910.Na (52), its mode of action remains unknown (45). Current clinical studies of this agent concentrate on ovarian cancer and hematologic malignancies, with some ongoing phase I studies investigating ON 01910.Na as monotherapy or in combination with chemotherapies in patients with solid tumors. Its development in NSCLC in the near future is unlikely.

Of the Plk inhibitors discussed here, we believe that volasertib has several possible advantages. These include its high volume of distribution, long terminal half-life, and early signs of antitumor efficacy in single-agent studies (57, 58). However, because all of these agents are at an early development stage (Table 1), further clinical data are required to determine their potential.

The future investigation of Plk inhibitors should focus on combinations with chemotherapies. First, the tumor response to Plk inhibitors as a single agent appears limited. Second, biomarkers predictive of response to Plk inhibitors as a single agent have not been available so far. Third, the main side effect of Plk inhibitors is reversible, uncomplicated myelosuppression. Therefore, it is possible to combine a Plk inhibitor with other chemotherapeutic agent(s).
Conclusions

Monotherapy with Plk1 inhibitors, as demonstrated in phase I/II studies, has shown manageable, noncumulative, and predominantly hematologic toxicity that correlates with the predicted mode of action of agents. Preliminary signs of antitumor activity have been shown, although much work needs to be done to optimize treatment regimens, identify agents with potentially synergistic activity, and define markers of tumor response and patient characteristics that predict therapeutic success. Future clinical studies should include the detection of pharmacodynamic biomarkers, such as phospho-histone H3, to validate Plk1 as the tumor target. In the first-in-humans phase I study of GSK461364 in advanced solid tumors, Plk1 inhibition in tumor cells was confirmed on the basis of the analysis of phospho-histone H3 expression in circulating tumor cells (59). Other promising biomarker assays for Plk1 inhibition in vivo have been developed, such as an immunohistochemical approach that measures serine 46-phosphorylation of the translational controlled tumor protein by Plk1 (62) and an ELISA-based assay that measures phosphorylation of the PBD-binding protein 1 (PBIP1, also known as MLF1 or CENP-U) by Plk1 (63). Patient stratification based on Plk1 expression should be considered in future trials; NSCLC patients with poor prognosis whose tumors express high levels of Plk1 may be good candidates for Plk1-targeting therapy. Preclinical studies have shown that p53 and/or Ras-mutant cells may be more sensitive to Plk1 inhibitors. This hypothesis, relevant for several solid tumors (e.g., ovarian cancer and urothelial carcinoma), but particularly for NSCLC, also needs to be clinically validated.

Disclosure of Potential Conflicts of Interest

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Authors’ Contributions

The authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors, were fully responsible for all content and editorial decisions, and were involved in all stages of manuscript development.

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References


Table 1. Plk inhibitors in clinical development

<table>
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<th>DLT</th>
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<td>Neutropenia</td>
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<td>GlaxoSmithKline</td>
<td>Plk1 inhibitor</td>
<td>Neutropenia, thrombocytopenia, pulmonary emboli</td>
<td>Prolonged SD in phase I</td>
<td>Phase I</td>
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<td>Nippon Shinyaku Co. Ltd.</td>
<td>Plk1 interference, not direct inhibitor</td>
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<td>Abdominal pain</td>
<td>Prolonged SD in phase I</td>
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<td>61</td>
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</table>

Abbreviations: PR, partial response; SD, stable disease; ERK extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase.
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