REVIEW

Polo-like kinase 1 inhibition in NSCLC: mechanism of action and emerging predictive biomarkers

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Abstract: Non-small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide. Due to often unspecific disease symptoms, locally advanced or metastatic disease is diagnosed in the majority of all cases. Palliative treatment options comprise of conventional cytotoxic agents, immunotherapy with checkpoint inhibitors and the use of specific small-molecule tyrosine kinase inhibitors (TKI). However, these TKIs are mainly restricted to a small proportion of patients with lung cancer that harbor activating driver mutations. Still, the effectiveness and favorable safety profile of these compounds have prompted a systematic search for specific driver mechanisms of tumorigenesis and moreover the development of corresponding kinase inhibitors. In recent years, the Polo-like kinase (PLK) family has emerged as a key regulator in mitotic regulation. Its role in cell proliferation and the frequently observed overexpression in various tumor entities have raised much interest in basic and clinical oncology aiming to attenuate tumor growth by targeting the PLK. In this review, we give a comprehensive summary on the (pre-) clinical development of the different types of PLK inhibitors in lung cancer and summarize their mechanisms of action, safety and efficacy data and give an overview on translational research aiming to identify predictive biomarkers for a rational use of PLK inhibitors.

Keywords: non-small cell lung cancer, Polo-like kinase, targeted therapy, therapy resistance

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide.[1] Due to often unspecific disease symptoms, locally advanced or metastatic disease is diagnosed in the majority of all cases.[2] Among others, adenocarcinoma is the most common histopathological subtype and palliative treatment options comprise of conventional cytotoxic agents, immunotherapy with checkpoint-inhibitors and the use of specific small-molecule tyrosine kinase inhibitors (TKI). However, these TKIs are mainly restricted to a small proportion of patients with lung cancer that harbor activating driver mutations, such as activating *EGFR* mutations or alterations in *ALK, ROS1* and *BRAF* genes. Still, the effectiveness and favorable safety profile of these compounds have prompted a systematic search for specific driver mechanisms of tumorigenesis and moreover the co-evolutionary development of corresponding kinase inhibitors. In the last two decades, the Polo-like kinase (PLK) family has emerged as a key regulator in mitotic regulation, being involved in the complex process from mitotic onset to its termination. The key role in cell proliferation and the frequently observed overexpression in various

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tumor entities have raised much interest in basic and clinical oncology aiming to attenuate tumor growth by targeting the PLK. In this review, we give a comprehensive summary on the (pre-) clinical development of the different types of PLK inhibitors in lung cancer and summarize their mechanisms of action, safety and efficacy data and give an overview on translational research aiming to identify predictive biomarkers for a rational use of PLK inhibitors.

Biological function and structure of Polo-like kinases and historical overview

The human homolog of the Polo gene was independently cloned by three research groups in 1993/1994.[3–5] All groups reported a 603 amino-acid polypeptide with several nucleotide differences that were all classified as polymorphisms. The product of the human PLK1 gene is a 66KD serine/threonine kinase protein.[5] Today, altogether 5 isoforms of PLK (PLK1-5) are known; however, PLK1 is by far the best characterized isoform (see Figure 1A).[6–9] PLKs (with the exception of PLK5) contain a catalytic N-terminal serine/threonine kinase domain and a C-terminal tandem-Polo-box region with regulatory functions.[10] The catalytic site incorporates most of the highly conserved hallmarks of serine/threonine protein kinases.[11] The tandem Polo-boxes of the N-terminal domain are involved in substrate-binding and in determining the correct subcellular localization of PLK1.[12]

First insights on the cell-cycle-dependent expression of PLK1 were provided by Lake and Jelinek who showed that PLK1 mRNA is nearly absent in the G1 phase of the cell cycle, but reaccumulates in the S phase and reaches highest levels during the G2/M phase,[3] linking its function to mitotic activity. Its role in mitosis was further elucidated in 1995, when Goldsteyn et al confirmed increased transcription at all stages of mitosis.[13] They localized PLK1 juxtaposed to the spindle apparatus in confocal microscopy analyses and concluded that PLK1 plays a role in chromosome condensation, spindle dynamics and chromosome segregation. Aside of regulatory functions regarding mitosis onset, PLK1 was found to be involved in the assembly of key components of the contractile ring (eg, ECT2, RhoA GTPAse, CYK4) at the equatorial cortex during anaphase onset[14,15] and finally in the exit process participating in controlling chromosome segregation and G1 phase entry.[16,17] Other physiological roles of PLK1 have been recognized, involving telomere stabilization,
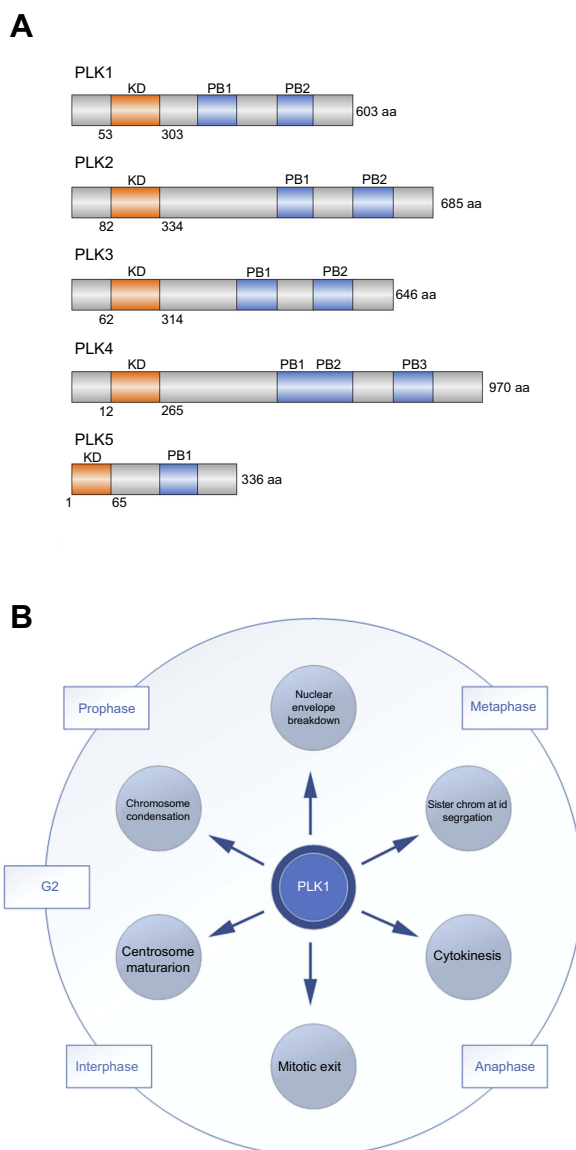

Figure 1 Structure and function of the human Polo-like kinases: (**A**) Polo-like kinases in human cells. Schematic representation of the five identified PLKs in human cells. The open reading frame amino-acid lengths are shown on the right, the kinase domain is shown in red color with the corresponding amino-acid position. Polo-box domains are shown in blue color. (**B**) Schematic diagram of the cell cycle functions of PLK1.
Abbreviations: PLK, Polo-like kinases; KD, kinase domain; PB, Polo-box domain; aa, amino acids.

extracellular matrix invasion and regulation of topoisomerase IIa in cell cycle progression (see Figure 1B).[18–21] For example, Cyclin B1, a key component of the prophase initiation, was identified as an important target structure of PLK1, promoting its (Cyclin B1) nuclear translocation after phosphorylation.[22] Activation of PLK1 in turn is a complex process, requiring phosphorylation of a conserved threonine residue (Thr 210) within the PLK1 kinase domain. The Aurora A kinase, a member of the Aurora serine/threonine kinase family, was found to phosphorylate

PLK1 during G2/M phase in synergistic action with Bora, a known cofactor of Aurora A.[23,24]

Given the nature of PLK1 and its involvement in mitosis, unsurprisingly, Holtrich et al provided evidence, that expression in normal human tissue is limited to highly proliferative organs, such as the placenta, colon and the testis. However, the transcripts are detectable in tumors of various origins, including but not limited to lung cancer, colon and stomach cancer as well as non-Hodgkin lymphomas,[4] firstly pointing to a possible relevance in malignant processes. Smith et al later showed that cells transformed with PLK1 were capable to produce tumors in nude mice.[25] Liu and Erikson and others used vector-based siRNA and antisense oligonucleotides to deplete PLK1 in various cancer cell lines and found that transfected cells arrested in G2/M phase and became highly apoptotic.[26–29] The formation of dumbbell-like chromatin and the presence of 4 N DNA content further suggested the inability of successfully transfected cells to completely separate their chromatin. PLK1-depleted cells enter mitosis, but accumulate in a pre-anaphase state with absence of focused spindle poles and chromosomes that do not become stably attached to the spindle apparatus.[30] Interestingly, normal human epithelial cells transfected with siRNA showed no impaired survival.[28] Conclusively, the preclinical data unequivocally pointed to a central role in regulating mitotic actions, which prompted a series of attempts to pharmacologically target PLK1.

Frequency of PLK1 expression in NSCLC and its prognostic relevance

In an early study on 111 resected lung cancer specimens from patients suffering from limited disease stages, a considerable transcription of PLK1 mRNA (PCR-based method) was documented in all but 4 patients. With a proportion of >80%, the majority of the study population consisted of male smokers with clinical stage I-II NSCLC. Additionally, high PLK1 expression was an independent risk factor for inferior survival in this cohort.[31] Allera-Moreau et al performed gene expression profiling of 77 genes involved in DNA replication in 93 tumor specimens collected from chemo-naïve early-stage patients and analyzed its prognostic relevance. They could show that most genes analyzed were significantly deregulated compared to juxtaposed normal lung tissue. PLK1 was found to be overexpressed in 92% of all probes analyzed, and a transcriptive "replication signature" consisting of 5 specific genes involving PLK1 was associated with inferior

relapse-free and overall survival.[32] The prognostic relevance of PLK1 expression was also confirmed by Wang et al, who additionally found a positive correlation with the presence of lymph node metastases and advanced clinical stage (approximately 50% of all cases tested).[33] Another study focused on squamous cell NSCLC and evaluated PLK1 expression by immunohistochemistry and PCR screening.[34] PLK1 was upregulated in 72 of 132 tumor probes (55%) and overexpression markedly correlated with disease stage and tumor size. In line with previous data, high expression of PLK1 was a negative prognostic factor correlating with inferior survival in univariate and multivariate analysis.

Preclinical and clinical-pharmacological PLK-1 targeting in NSCLC

The biological function of PLK1 as a key regulator of mitosis and its expression in various tumor entities overall supported the potential role of PLK1 disruption in cancer therapy. Zhou et al performed PLK1 antisense oligonucleotide (ASO) (pcDNA3-PLK1) studies in A549 lung cancer cells and determined cell proliferation, cell cycle distribution and apoptosis.[35] The number of viable cells was markedly reduced 48 hrs after successful transfection as compared with control cell lines. Flow cytometry revealed a significant increase of cells in G2/M phase and apoptosis in the transfected cell lines. Mixing studies furthermore revealed a sensitization to vinorelbine quantified by a reduction of the IC_{50} by more than 40% in the transfected cell lines, implying a synergistic effect of chemotherapy and PLK1 disruption. Another study was carried out with low-proliferative NCI H596 lung carcinoma cell lines. PLK1 function was blocked with an adenoviral delivery of a dominant-negative (dnPLK1) gene.[36] The expression of the dnPLK1 caused significant apoptosis at relatively low wild-type:dominant-negative expression ratios despite low proliferation rates of the H596 cell line. In another study, in vitro cell growth of A549 lung cancer cell lines transfected with PLK1 ASOs was reduced by more than 50% with a moderate induction of G2/M phase arrest.[29] Moreover, systemical treatment of A549 xenograft nude mice with bolus injections of PLK1 ASOs inhibited tumor growth by 70–86%. Kawata et al used a lung cancer xenograft model to determine PLK1 influence on metastatic tumor spread: A549 cells were inoculated into the murine spleen and subsequent liver

metastasis was compared between control animals and mice treated with PLK1 small interfering RNA.[37] Macroscopic analysis of mice exsanguinated 70 days after cancer cell inoculation showed a significantly lower volume of liver metastases. Finally, in another xenograft model (nude mice) with subcutaneously inoculated A594 cells, treatment with PLK1 silencing small hairpin DNA resulted in a significant delay in tumor growth compared to sham treatment, and the combination with gemcitabine caused a strong inhibition of tumor cell proliferation by enhanced apoptosis in an additive manner.[38]

In summary, disruption of PLK1 was feasible in preclinical models and showed encouraging anticancer activity, thus leading the way to the clinical evaluation of PLK1 inhibitors.

The development of small molecules to target PLK1

Inhibitory molecules of the kinase domain may be distinguished in ATP-competitive and non-ATP-competitive inhibitors, depending on their mechanism of action. The deep cavity of the PLK1 ATP binding site is an obvious target; however, the highly conserved structure of binding sites makes the identification of specific inhibitors a challenging endeavor due to potential off-target side effects.

The first specific inhibitor described in the literature is scytonemin, an extracellular matrix pigment synthesized by cyanobacteria. It is supposed to be an effective absorber of damaging ultraviolet rays, while allowing the transmittance of wavelength necessary for photosynthesis.[39] This natural marine product was found to inhibit PLK1 in a dose-dependent manner while sparing toxic effects in nonproliferating cells, providing a possible template for the upcoming development of PLK1 inhibitors.[40] However, scytonemin showed similar inhibitory effects on other kinases (CDK1, CHK1, MYT, PKC), and the inhibitory indices indicated additional non-competitive modes of action. An overview on PLK1 inhibitors that had been tested clinically in patients with lung cancer gives Table 1. A comprehensive summary enumerating all conducted trials with PLK1 inhibitors in lung cancer patients is depicted in Table 2.

BI 2536

BI 2536 was a first-in-class highly selective ATP-competitive small molecule based upon a dihydropteridone derivative that accomplished inhibiting the enzymatic activity of PLK1 by blocking the ATP-binding site. This compound inhibits PLK1 with an at least 1.000-fold selectivity against a panel of other kinases and an IC_{50} of 0.8 nM. Irrespective of the tumor origin, the agent inhibits the proliferation of various cell lines in culture assays,[41] including the *KRAS* mutated A549 in addition to NCI-A460 and the *TP53* mutated NCI-520 lung cancer cell lines. Comparable to previous RNA-based silencing studies, BI 2536 induced mitotic arrest and cells accumulate 4 N DNA content, indicative of G2/M arrest. Subsequently, massive tumor cell death and apoptosis occurred after 48 hrs of treatment. The growth inhibitory potential was also tested in an A549 xenograft model by using a twice-weekly i.v. schedule, and excellent tolerability and meaningful tumor regression were observed.

A first in human Phase I open-label dose-escalating PLK1 inhibitor study was conducted in patients with refractory and/or metastatic solid tumors.[42] A total of 40 patients were treated with at least one cycle of parenteral BI 2536 following a 3+3 escalation scheme starting with 25 mg on day 1 every 3 weeks (D1Q3W). Patients included suffered from colorectal and renal cancer as well as sarcoma among several other cancer entities. The inclusion of patients with lung cancer, however, was not reported. Dose-limiting toxicities were observed at the 250 mg dose level, comprising of neutropenic infection and neutropenia; therefore, the maximum tolerated dose was set to 200 mg for further enrollment. Other namable toxicities were gastrointestinal side effects, fatigue, arthralgia and γ-glutamyltransferase increase without additional signs of hepatic toxicity. Antitumor activity was encouraging with a reported disease control rate of 42% with one patient achieving a partial response.

A total of three clinical trials with BI 2536 were conducted in which patients with NSCLC were enrolled. A Phase I trial aimed to define the maximum tolerated dose of single-agent BI 2536 and overall safety in various cancer entities, including each one patient diagnosed with SCLC and NSCLC, respectively. Toxicity was comparable to previous data with mainly hematotoxic side effects and no new safety issues raised. The patient suffering from SCLC experienced disease stabilization for more than 2 cycles.[43]

Two clinical trials enrolled patients with relapsed or refractory NSCLC only, a Phase II trial evaluating the efficacy of single-agent BI 2536 and a Phase I trial aiming to determine the recommended Phase II dose of pemetrexed in combination with BI 2536. Both clinical trials showed only modest efficacy in NSCLC patients with tumor response limited to a few patients each. Sebastian

Table 1 PLK1 inhibitors involved in clinical trials with lung cancer

Compound	Company	Mode of action	Target structure	PLK1 IC50	Clinical trial DLT	Status
BI 2536	Boehringer Ingelheim	ATP-competitive	PLK1, PLK2, PLK3	0.8 nM	Neutropenia, neutropenic infection, GIT	On hold
BI 6727 (Volasertib)	Boehringer Ingelheim	ATP-competitive	PLK1	0.87 nM	Neutropenia, thrombocytopenia	Ongoing in solid and hemat. malignancies
GSK461364A	Glaxo-Smith-Kline	ATP-competitive	PLK1, PLK2, PLK3	2.2 nM	Neutropenia, thrombocytopenia	On hold
ON01910.Na (Rigosertib)	Onconova Therapeutics	non ATP-competitive	PLK1, PLK2, PI3K, Abl, Flt-1, PDGFR	9 nM	Neutropenia, hyponatriemia, delirium, urinary symptoms, pelvic pain	Ongoing in hemat. malignancies
MK-1496	Merck	ATP-competitive	PLK1	n/a	Neutropenia, thrombocytopenia, anemia	On hold
NMS-1,286,937	Nerviano Medical Sciences	ATP-competitive	PLK1	2 nM	Neutropenia, thrombocytopenia, hypokaliemia	On hold
TAK-960	Takeda	ATP-competitive	PLK1, PLK2	0.8 nM	Not reported	Clinical trial was terminated, on hold
HMN-214	Nippon Shinyaku Co. Ltd.	Not reported	PLK1	0.12 µM	GIT, arthralgia, myalgia	On hold

Abbreviations: PLK, Polo-like kinases; GIT, gastrointestinal toxicity; ATP, adenosine triphosphate.

and co-authors concluded that despite the objective response rates (ORR, 2%) following single-agent BI 2536 administration in R/R NSCLC patients being low, the capability of BI 2536 to achieve response rates with low treatment-induced mortality suggests clinical activity of Plk-1 inhibitors in relapsed NSCLC.[44] Importantly, most of the adverse events affected the hematopoietic system without causing relevant clinical problems to the majority of patients, thus potentially warranting combination strategies. However, although not powered for response rate, the combination of BI 2536 with pemetrexed in the Phase I trial has similarly shown only modest anti-tumor activity with an ORR of 5% (n=2).[45] Still, these two patients had a progression-free survival (PFS) of 20.5 months and a censored PFS of 20.1 months, raising the questions of valid biomarkers to more precisely allocate PLK1 inhibitory strategies.

BI 6727 (volasertib)

The second-generation PLK1 inhibitor volasertib (BI 6727), an additional dihydropteridinone derivative, is a potent and selective agent with a favorable pharmacokinetic profile.[46] In comparison to BI 2536, volasertib shows improved tissue penetration leading to a high volume of distribution and a prolonged terminal half-life, which offers a theoretic therapeutic advantage, thus leading to prioritization of volasertib in clinical development. Preclinical efficacy was determined in several cell culture assays and its derivative xenograft models, including the lung cancer cell line NCI-H460. Furthermore, it was shown that volasertib (and BI 2536) was effective in *EGFR* mutant cell lines, and drug-induced cell death was increased in those gaining acquired resistance, especially due to epithelial-to-mesenchymal transition, thus providing a rationale for volasertib and EGFR TKI combination strategies.[47–49] Therapeutical potential of volasertib in preclinical assays has also been demonstrated for SCLC cell lines, in which PLK1 inhibition significantly attenuated cell proliferation and induced apoptosis in a dose-dependent manner.[50]

The clinical development of volasertib in patients suffering from NSCLC consists of 6 clinical trials, 5 of which were Phase I trials enrolling various solid tumor entities in the relapsed or refractory state.[51–56] Two of these trials evaluated increasing doses of volasertib in combination with other target therapy compounds, afatinib[54] and nintedanib,[56] respectively. In total, 25 patients with NSCLC were enrolled in these 5 trials, of which 2 patients

Table 2 Clinical trials involving PLK1 inhibitors and patients with lung cancer

Compound	Author	Year	Phase	Lung cancer patients enrolled	Primary and secondary study aims	Efficacy	Intervention/ treatment	Safety issues/ DLT
BI 2536	Sebastian et al[44]	2010	II	R/R NSCLC, n=59	ORR, PFS, OS	ORR, 2%; PFS, 49 days; OS, 201 days;	150–200 mg total dose/cycle	Neutropenia, fatigue, GIT
BI 2536	Hofheinz et al[43]	2010	I	R/R solid tumors, n=1 NSCLC, n=1 SCLC	MTD, safety, ORR, PK	No response in SCLC	50–200 mg total dose/ cycle	Neutropenie, thrombopenia, exanthema
BI 2536	Ellis et al[45]	2013	I	R/R NSCLC, n=41	MTD, safety, ORR, PK	ORR (5%)	100–325 mg total dose/cycle + Pemetrexed	Neutropenia, fatigue, exanthema, GIT
BI 6727	Gil et al[51]	2010	I	R/R solid tumors, n=10 NSCLC	MTD, safety, ORR, PK	No response in NSCLC	12–450 mg total dose/ cycle	Neutropenia, thrombocytopenia, fatigue
BI 6727	Lin et al[53]	2014	I	R/R solid tumors, n=4, NSCLC	MTD, safety, ORR, PK	No response in NSCLC	100–400 mg total dose/cycle	Neutropenia, Thrombopenia, anemia
BI 6727	Machiels et al[54]	2015	I	R/R solid tumors, n=1 NSCLC	MTD, safety, PFS, PK	PR in NSCLC	150–300 mg total dose/cycle + Afatinib	Neutropenia, thrombocytopenia, GIT
BI 6727	Ellis et al[45]	2015	II	R/R NSCLC, n=131	PFS, ORR, safety, PK	PFS, ORR 3.3 m, 21% (Volasertib + Pemetexed) vs 5.3 m, 10.6% (Pemetrexed) vs 1.4 m, 8.1% (Volasertib)	300 mg total dose/ cycle + Pemetrexet vs Volasertib mono vs Pemetrexet mono	Fatuige, neutropenia
BI 6727	Braud et al[56]	2015	I	R/R solid tumors, n=5, NSCLC	MTD, safety, PFS, PK	1 PR in NSCLC	100–400 mg total dose/cycle + Nintedanib	Neutropenia, thrombopenia
BI 6727	Nokihara et al[55]	2015	I	R/R solid tumors, n=5, NSCLC	MTD, safety, ORR, PK	No response in NSCLC	200–350 mg total dose/cycle	Neutropenie, thrombopenia
HMN-214	Garland et al[62]	2006	I	R/R solid tumors, n=7, NSCLC	MTD, safety, ORR, PK	No response in NSCLC	3–9.9 mg/m² total dose/cycle	Fatigue, arthralgia, GIT
NMS-1286937	Weiss et al[63]	2018	I	R/R solid tumors, n=2, NSCLC	MTD, safety, ORR, PK, biomarker	No response in NSCLC	6–48 mg/m² total dose/cycle	Neutropenia, thrombocytopenia
ON01910.Na	Ohnuma et al[60]	2013	I	R/R solid tumors, n=2, NSCLC	MTD, safety, ORR, PK	No response in NSCLC	50–1,700 mg total dose/cycle	Neutropenia, delirium, confusion, GIT
ON01910.Na	Bowles et al[61]	2014	I	R/R solid tumors, n=1, NSCLC	MTD, safety, ORR, PK, biomarker	No response in NSCLC	70–700 mg total dose/ cycle	Urinary symptoms, pelvic pain, fatigue

Abbreviations: R/R, relapsed or refractory; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; PK, pharmacokinetics; MTD, maximum tolerated dose; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

achieved a partial remission as a best response. Transient hematologic side effects, fatigue and gastrointestinal toxicity were the most common dose-limiting adverse events; however, treatment was overall reported as well-tolerated.

A three-armed Phase II trial evaluating volasertib single agent or in combination with pemetrexed against pemetrexed monotherapy was conducted by Ellis et al. The trial enrolled 131 patients with relapsed or refractory NSCLC. Although the overall response rate of 21% was highest in patients treated in the combination trial arm compared to volasertib (8.1%) or pemetrexed (10.6%) monotherapy, PFS with the combination strategy (3.3 months) was inferior to pemetrexed single agent treatment. In an exploratory comparison between volasertib monotherapy and pemetrexed monotherapy, the HR was 2.045 (95% CI, 1.27–3.292; p=0.0027) favoring pemetrexed, although surprisingly the response rates in both single-agent trial arms were similar. The discordance between response rate and PFS was attributed to chance imbalances within this small clinical trial.

Although all these trials could convincingly demonstrate that the toxicity profile of volasertib was very well manageable, even in combination strategies, the overall efficacy in NSCLC patients was lower than expected.

ON01910 (rigosertib)

ON01910 was the first-of-its class non-ATP-competitive PLK1 inhibitor binding near or at the PLK1 substrate binding site, since substrates compete for its inhibitory activity.[57] In vitro studies with normal human cell lines showed surprisingly low growth attenuation and were very resistant to the apoptotic effect of ON01910. In several tumor cell culture assays (including two lung cancer cell lines: A549, H157), however, ON01910 induced mitotic arrest characterized by spindle abnormalities leading to apoptotic death. Of interest, it was tested if co-culturing of tumor cells with sublethal amounts of ON01910 may induce resistance to the compound, but such cell lines could not be isolated, indicating that acquired resistance to ON01910 itself may cause a survival disadvantage. Furthermore, ON01910 has been identified as an effective inhibitor of the PI3K/Akt/mTOR pathway in preclinical series.[58,59]

The clinical development of ON01910 in NSCLC is based on two Phase I clinical trials, that enrolled in total of 3 NSCLC cases,[60,61] all of which did not respond to this non-ATP-competitive agent. Aside of transient hematologic side effects, dose-limiting toxicities consisted of disturbances in electrolyte balances, pelvic pain and urinary tract symptoms in addition to the occurrence of neurocognitive dysfunction. These side effects differed to former experiences with PLK1 inhibitors, presumably owing to the different mechanism of action of ON01910. Priority of the clinical development of rigosertib is currently restricted to malignant hematologic diseases.

NMS-1286937/HMN-214

Two early-phase clinical trials had been conducted aiming to define the MTD and overall safety with additional PLK1 inhibiting compounds: HMN-214 and NMS-1286937.[62,63] Altogether 9 lung cancer patients were recruited with no documented response in these patients. Safety and toxicity were overall comparable to other ATP-competitive PLK1 inhibitors.

Other ATP and non-ATP competitive PLK1 inhibitors

There is an ongoing list of ATP-competitive and non-competitive compounds in preclinical or early phase clinical development (eg, MLN0905,[64] Ro3280,[65] CFI-400945,[66] CYC140[67]); however, patients with lung cancer have not been enrolled in trials conducted with these compounds; thus, detailed information on preclinical and/or trial results are not presented here. A list of ongoing clinical trials involving PLK1 inhibitors in solid malignancies depicts Table 3.

The new class of polo-box-domain inhibitors

The highly conserved nature of the ATP-binding pocket and the anticipated or experienced side effects of its inhibition gave rise to a new class of PLK1 targeting compounds that target the PLK1 tandem polo-box domain (PBD). Of interest, unlike PLK1-3, PLK5 only contains a single PBD. Polo-box binding inhibitors fall into 2 categories, peptide-based and small-molecule based. Peptides primarily provide functional information on protein–protein interactions and give rise to the identification of surface structures and potential binding pockets for targeting the PBD, that might be allosterically masked in the absence of a peptide ligand. However, peptides are in general not cell-permeable and are susceptible to proteolysis. These inhibitors serve as proof-of-concept models to accelerate the development of pharmacological agents. Small molecule inhibitors on the other hand provide the advantages of cell permeability, are characterized by a high degree of selectivity and represent inhibitors for potential cancer-therapy.

Table 3 Active clinical trials with PLK1 inhibitors in solid malignancies/NSCLC

Compound	Study start date	Estimated study completion date	Phase	Intervention/treatment	Key eligibility criteria (planned enrollment)	Primary and secondary study aims	NCT number
BI 6727 (Volasertib)	04-11-2005	26-08-2022	I	BI6727 dose escalation	R/R solid tumors (n=65)	MTD, ORR, PFS, PK	NCT02273388
BI 6727 (Volasertib)	13-05-2014	15-04-2019	I	BI6727+ Itraconazol	R/R solid tumors (n=28)	PK	NCT01772563

Abbreviations: R/R, relapsed or refractory; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; PK, pharmacokinetics; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer.

The first phosphopeptide designed to target the PDB was PoloBoxtide optimal that was not specifically designed to target the PBD, but was co-identified with the regulatory domain of the PLKs themselves.[12,68] The definition of the optimal binding motif of the PBD in these pivotal studies provided the basis for the development of PBD targeting agents. Other peptide-based inhibitors, PLHSpT, FDPPLHSpTA, and their modified sister compounds were able to induce mitotic arrest in a high proportion of cancer cells after microinjection in culture assays, supporting their mechanism of action in the context of malignancy.[69] The first small-molecule inhibitor of the PBD was Poloxin that was found through systematic screening of chemical libraries.[70] The compound induced mitotic arrest and apoptosis in HeLa cells with an IC_{50} of 4.8 μM and suppressed tumor growth in xenograft mouse models.[71] A handful of comparable compounds have been developed/identified so far: Poloxin-2,[72] Purpurogallin,[69] Poloxipan,[73] bg-34,[74] Poloppin[75] and Poloppin-2.[75] To our knowledge, there is currently no clinical trial recruiting with this new type of PLK inhibitors. However, one of the main green tea polyphenols (epigallocatechin) was found to be an inhibitor of the PLK1 PBD,[76] thus falling into this category of PLK inhibitors. The only clinical trial that was conducted in 17 patients with advanced lung cancer treated with green tea extract found low efficacy with no objective responses.[77]

The search for predictive biomarkers

The clinical activity of PLK1 inhibitors in NSCLC has only shown modest activity. Still, a small proportion of patients derives the meaningful clinical benefit that might be masked among a larger proportion of patients who fail to benefit from PLK1 inhibition. Therefore, the identification of predictive biomarkers might be the key to enrich patients who benefit from this class of inhibitors. Unfortunately, translational research and exploratory biomarker analysis were not part of the study objectives in most early phase clinical trials that had involved patients with NSCLC. Still, there is some preclinical and clinical evidence on molecular mechanisms that support the usefulness of PLK1 inhibition in specific (NSCL) cancer subentities.

Preclinical evidence for emerging biomarkers

It has been consistently shown that *TP53* defective cancer cell lines were more prone to reduced survival than their

wild-type counterparts.[78–84] Although *TP53* usually acts in an anticancer capacity, it was recently shown that functional *TP53* reduces the sensitivity of cell lines to PLK1 inhibitors and maintains centrosome separation and completion of mitosis in the presence of PLK1 inhibition.[85] Cells lacking *TP53* show an increased proportion of abnormal spindle formation and arrest in G1 phase upon treatment with PLK1 inhibitors. Therefore, the presence of inactivated *TP53* might serve as a predictive biomarker in NSCLC patients, that is present in approximately 50% of all cases.[86]

KRAS mutations are one of the most common genetic alterations in patients with NSCLC; however, its prognostic role as a single or co-mutation has extensively been investigated with inconsistent results (eg, reviewed in[87]). There have been attempts to therapeutically address *KRAS* mutated tumors (such as NSCLC) which have been proven to be largely refractory to those target treatment approaches. An RNAi screen performed by Luo et al identified PLK1 and components of the APC/C ubiquitin-conjugating holoenzyme as a survival critical cofactor in *KRAS p.G12C* cell lines and xenografts.[88] Increased apoptosis in *KRAS* mutated cell lines compared to their wild-type counterparts was also seen after treatment with the PDB inhibitor Pollopin,[75] BI 6727 and BI2536.[47] Furthermore, it has been shown that ON01910 (rigosertib) has the ability to bind to the RAS-binding domain of the RAF family proteins and thus effectively blocking RAS downstream signaling.[89] The PI3K-mTor pathway as a second key regulator of cell growth cross-talks with the RAS-RAF-ERK pathway and positively and negatively co-regulate each other.[90] As mentioned above, rigosertib has been identified as an effective inhibitor of PI3K. In xenograft models of colorectal and lung cancer (A549 cell line xenograft), reduction in tumor growth after rigosertib treatment was accompanied by downregulation of RAS-RAF-ERK and PI3K-mTor pathways. This has also been shown for other preclinical models of hepatocellular carcinoma, head and neck and prostate cancers.[79,91,92] Whether this effect is highly specific for rigosertib or is also traceable in other PLK1 inhibitors is not conclusively clarified; however, a direct targeting of PI3K has not been shown for the other compounds. In summary, tumors harboring activating *KRAS* mutations or show increased activity of RAS downstream signaling pathways are supposed to be more sensitive to PLK1 targeting. In case of rigosertib, (co-) activation of the PI3K pathway, as indicated by *PIK3CA* expression or *PTEN* loss (a tumor suppressor

gene counteracting to the PI3K pathway), might additionally function as a predictive marker for tumor response in PLK1 inhibition. However, although *KRAS* and *TP53* alterations preclinically indicate a sensitization to PLK1 inhibition, the high proportion of patients with NSCLC harboring these mutations and the low tumor response rates contradict a sufficient sensitivity of these markers.

Cyclin B1, a physiologic target structure of PLK1, is a key mitotic cyclin in the G2/M phase transition of the cell cycle, is overexpressed in various tumor entities and has consistently been described as a negative prognostic factor in NSCLC with a frequency of approximately 20%, particularly in squamous cell NSCLC.[93–97] In one study, the dynamic reduction of mRNA transcription of cyclin B1 upon treatment with ON01910 positively correlated with response to PLK1 inhibition in patient-derived pancreatic cancer xenografts.[98] Thus, although absolute expression of cyclin B1 did not conclusively predict tumor response in xenografts or in those patients who were subsequently treated with ON01910, early dynamic changes were highly predictive for treatment response. A similar relationship has been described in patients with hematologic malignancies treated with ON01910 and the dynamic changes of cyclin D1 expression measured by flow cytometry.[99] However, this relationship has not been proven in lung cancer (models) yet. Other biomarkers with predictive potential have been evaluated in solid malignancies other than NSCLC. The centrosomal protein CEP55 is a downstream target of the MAPK pathway and a key regulator of cytokinesis.[100] Its overexpression is associated with inferior survival in NSCLC and breast cancer.[101] In xenograft models of triple negative breast cancer, the combined inhibition of MEK1/2 (AZD6244) and PLK1 (volasertib) substantially impacted tumor growth.[100] However, the role of CEP55 as a potential biomarker for PLK1 inhibition in NSCLC has not been elucidated yet. Additionally, head and neck squamous cell cncer (HNSCC) cell lines and xenograft models with mutated *AJUBA*, presumably an essential activator of the Aurora Kinase A, were highly sensitive to knockdown or inhibition of PLK1.[102] Presence of activating AJUBA mutations might therefore play a role as a predictive biomarker in head and neck cancers and other solid malignancies for successful PLK1 targeting.[102]

Finally, there is emerging evidence that PLK1 activation plays a key role as an acquired mechanism of resistance, particularly in the process of epithelial-to-mesenchymal transition (EMT). EMT is a complex molecular and cellular process involved in tissue remodeling

that has been linked to tumor progression and metastasis and precludes a high plasticity of cancer cells.[103] Importantly, EMT has been found to be associated with inherited and acquired drug resistance,[104–106] in case of NSCLC particularly in refractoriness to platinum therapy and acquired resistance to EGFR TKIs.[107–109] Presence of an EMT gene signature determined by analyzing 264 different genes in lung cancer cell lines predicted sensitivity to PLK1 inhibition.[47] This could also be shown in two erlotinib-resistant cell lines with activating EGFR mutations that harbored an EMT gene profile.[49] Of note, it was recently shown that NSCLC cell lines (HEK293) with epithelial gene signature and constitutive cMET activation were highly resistant to volasertib treatment, whereas PLK1 inhibition prevented cMET phosphorylation in mesenchymal isogeneic cell pairs leading to high apoptosis rates.[110] The combination of cMET and PLK1 inhibition in this setting led to sustainable xenograft tumor regression possibly paving the way for combination strategies involving PLK1 and cMET targeting therapy. Conclusively, acquired resistance to target therapies or conventional cytotoxic agents involving EMT might be a domain of PLK1 inhibition in the future.

Clinical and patient-derived evidence for predictive biomarkers

The largest set of tumor samples for exploratory biomarker screening were obtained from the phase I trial conducted by Sebastian et al with BI 2536.[44] Breitenbuecher and colleagues performed broad spectrum posthoc analyses with archival tumor samples from 47 patients using immunohistochemistry and DNA sequencing.[111] Sequence analysis of all mutational hotspot exons of *KRAS, EGFR, BRAF* and *PIK3CA* was successfully conducted in 26 patients, immunohistochemistry analysis for the expression of PTEN, HER2, PLK, p-AKT and p-ERK was informative in 20 patients. Exploratory correlation analyses showed notably long PFS in some patients harboring *KRAS* mutations in the cohort of nonsquamous NSCLC, but this relationship failed to meet statistical significance. There was no survival difference in patients with *KRAS*-mutated lung cancer compared to the *KRAS* wildtype cohort. Partially owing to the small sample size, other outcome associations with genetic alterations could not be identified. When testing for PI3K/AKT/mTOR pathway activation indicated by positive ERK, AKT and (loss of) PTEN IHC, there was no significant relationship with PFS or OS outcomes.

In the Phase I trial in relapsed or refractory solid malignancies (NSCLC, n=1) conducted by Bowles et al, archival tumor tissue blocks from 32 participants were assessed with DNA sequencing for the presence of common mutations in 15 hotspot genes.[61] Additionally, in a subset of patients, FISH analyses were performed to determine the *PIK3CA* and *PTEN* gene copy number. In a subset of patients with squamous cell cancers (n=6; NSCLC, n=1), a PI3K pathway activation via *PIK3CA* amplification and *PTEN* loss in combination with an inactivated *TP53* was seen in those 2 patients with therapy response. However, others with these mutations failed to respond to therapy indicating that both mutations may be necessary but are not sufficient to induce a response to PLK1 inhibition.

Conclusions

The captivating position of PLK1 as a central key regulator of mitosis leads to numerous attempts to pharmacologically disrupt PLK1 signaling in various cancer entities, including lung cancer. Currently, three different types of small-molecule inhibitors – ATP-competitive, non-ATP competitive and PBD inhibitors – have found their way into clinical trials; however, most experiences were gained with the ATP-competitive compounds BI 2536 and BI 6727 (Volasertib). The overall toxicity of PLK inhibitors was judged as well manageable with dose-dependent hematotoxicity as the limiting side effect in most clinically tested compounds. The overall efficacy in patients with NSCLC however was limited and in the context of the rapidly broadening landscape of immunotherapy (combinations) and small-molecule strategies, further development of PLK1 inhibitors is currently primarily restricted to more promising areas, such as hematologic malignancies. Still, a small proportion of patients shows an enduring benefit of PLK1 inhibition, probably masked by a higher proportion of patients showing refractoriness to this small-molecule class. There have been few clinical attempts to define predictive biomarkers to enrich lung cancer patients that may derive increased benefit from PLK1 inhibition, but in summary, these suggested biomarkers performed weakly in predicting tumor responses in NSCLC patients and more research is needed to define the molecular frame of effective PLK1 targeting. In recent years, the role of PLK1 in acquired therapy resistance has been investigated and has been linked to resistance to cytotoxic agents as well as target therapies. In light of these observations, the future domain of PLK1 inhibitors in the treatment of lung cancer may be found in combination strategies to prevent early treatment failure and delay obligatory acquired resistance in advanced or metastatic disease. More studies are

inevitably needed to define the optimal role of PLK1 inhibition in lung malignancies.

Disclosure

Dr Jan A Stratmann reports personal fees from Bristol-Myers & Squibb, personal fees from Novartis, outside the submitted work. Dr Martin Sebastian reports personal fees from Lilly, personal fees from Astra-Zeneca, personal fees from Bristol-Myers & Squibb, personal fees from Merck Sharp & Dohme, personal fees from Pfizer, personal fees from Takeda, personal fees from Roche, personal fees from AbbVie, personal fees from Boehringer-Ingelheim, personal fees from Celgene, personal fees from Novartis, outside the submitted work. The authors report no other conflicts of interests in this work.

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