Preclinical Development

BAY 1000394, a Novel Cyclin-Dependent Kinase Inhibitor, with Potent Antitumor Activity in Mono- and in Combination Treatment upon Oral Application

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Abstract

Deregulated activity of cyclin-dependent kinases (CDK) results in loss of cell-cycle checkpoint function and increased expression of antiapoptotic proteins, which has been directly linked to the molecular pathology of cancer. BAY 1000394 inhibits the activity of cell-cycle CDKs CDK1, CDK2, CDK3, CDK4, and of transcriptional CDKs CDK7 and CDK9 with IC₅₀ values in the range between 5 and 25 nmol/L. Cell proliferation was inhibited at low nanomolar concentration in a broad spectrum of human cancer cell lines. In cell-based assays, the inhibition of phosphorylation of the CDK substrates retinoblastoma protein, nucleophosmin, and RNA polymerase II was shown. Cell-cycle profiles were consistent with inhibition of CDK 1, 2, and 4 as shown in cell-cycle block and release experiments. The physicochemical and pharmacokinetic properties of BAY 1000394 facilitate rapid absorption and moderate oral bioavailability. The compound potently inhibits growth of various human tumor xenografts on athymic mice including models of chemotherapy resistance upon oral dosing. Furthermore, BAY 1000394 shows more than additive efficacy when combined with cisplatin and etoposide. These results suggest that BAY 1000394 is a potent pan-CDK inhibitor and a novel oral cytotoxic agent currently in phase I clinical trials. Mol Cancer Ther; 11(10); 2265–73. ©2012 AACR.

Introduction

Loss of cell-cycle control and increased resistance to apoptosis represent major hallmarks of cancer (1). Cyclin-dependent kinases (CDK) belong to a family of serine/threonine protein kinases that associate with an activating cyclin regulatory subunit. CDKs are involved in the regulation of fundamental cellular processes such as cell division cycle and gene transcription. Cell-cycle CDKs 1, 2, 4, and 6 are required for the correct timing and order of the events of the cell-cycle division cycle. CDK7 is a component of the CDK-activating complex that contributes to the assembly of CDK1/cyclin B. In addition, CDK7 functions as a transcriptional CDK, as well as CDKs 8 and 9, which have been shown to be involved in gene transcription via regulation of RNA polymerase II activity (2). Deregulated CDK activity results in loss of cell-cycle checkpoint function and increased expression of antiapoptotic proteins, which has been directly linked to the molecular pathology of cancer (3). Inhibition of CDK activity by low-molecular weight chemical compounds appears as an attractive option for cancer treatment. Results from CDK gene targeted mice provoked a discussion that cell-cycle kinase should be preferentially targeted for cancer therapy. Whereas interphase CDKs CDK2, CDK4, and CDK6 are only required for proliferation of specific cell types, CDK1 is essential for cell division in the embryo and can substitute for the genetic loss of interphase CDKs (4, 5). However, recent results with genetically engineered human cells harboring CDK2 variants that are selectively sensitive to inhibition by bulky adenine analogs revealed a nonredundant role of CDK2 in restriction point passage and S phase entry in tumor cells as well as in nontumorigenic cells (6). Specific inhibition of CDK4 and CDK6 may be of advantage in certain tumor settings, such as Her-2 positive breast cancers (7), but is critically reliant on the presence of an intact retinoblastoma tumor suppressor gene, excluding indications with a high frequency of retinoblastoma loss of function mutations such as small cell lung cancer (SCLC; ref. 8). The heterogeneity of the cell population within the tumor tissue with respect to their position in the cell cycle may argue for a broad spectrum CDK inhibition profile.

Several low molecular weight inhibitors of CDKs with various inhibitor profiles on the panel of CDKs (9–11), some with additional non-CDK targets (12, 13), have entered clinical trials (for recent review see ref. 3). However, the therapeutic efficacy was modest and none has
reached the market yet. This may be at least in part because of insufficient potency, pharmacokinetic and physicochemical properties, off-target toxicities, and sub-optimal dosing schedules (14).

BAY 1000394 is a new CDK inhibitor with low nanomolar activity against cell-cycle CDKs and transcriptional CDKs. The physicochemical and pharmacokinetic properties of the compound facilitate rapid absorption and potent efficacy in human tumor xenograft models upon oral application. Here, we present the in vitro and in vivo pharmacological profile of BAY 1000304.

Materials and Methods

Chemicals

BAY 1000394, (R)-S-Cyclopropyl-S-4-[[4-[[1(R,2R)-2-hydroxy-1-methylpropyl][oxy]-5-(trifluoromethyl)pyrimidin-2-yl]amino[phenyl]sulfoximide, was synthesized at Bayer Pharma AG. Paclitaxel was purchased from Bristol-Myers Squibb, cisplatin from Sigma, and etoposide from Hexal.

Kinase assays and counterscreening

Recombinant CDK1 and CycB-GST fusion proteins, and CDK2 and CycE-GST fusion proteins, purified from baculovirus-infected Sf9 insect cells, were purchased from ProQinase. Histon H3S (Sigma) was used as kinase substrate. Inhibition of kinase activity was determined by substrate phosphorylation assays using [33P]-gamma adenosine triphosphate (for details, see Supplementary Methods). BAY 1000394 was counter screened against a panel of 220 kinases using the Millipore Kinase profiler screen at 500 nmol/L. Kinases, which were inhibited to less than 10% residual activity, were retested at 10 and 100 nmol/L, and IC50 values were estimated.

Cell lines and cell-based assays

Authentication of all human cell lines used was conducted at the German Collection of Microorganisms and Cell Cultures via PCR-based DNA profiling of polymorphic short tandem repeats (for cell line details, see Supplementary Table S1). Cells were propagated under the suggested growth conditions in a humidified 37°C incubator.

Proliferation assays were conducted in 96 well plates at densities between 1,000 and 5,000 cells per well in the appropriate medium containing 10% fetal calf serum (FCS). Cells were treated in quadruplicates with serial dilutions of BAY 1000394 for 96 hours following by quantification of relative cell numbers upon crystal violet staining (15). IC50 values were calculated by means of a 4 parameter fit using the company’s own software. Activated caspase-3 and -7, as well as DNA fragmentation were determined using the ApoONE Caspase-3/7 Assay (Promega) and the Cell Death Detection ELISA Plus (Roche Diagnostics). BAY 1000394 was profiled on panels of 40 human lung and 24 human breast cell lines using the Invitrogen Oncology Profiling Service.

Assessment of pRb in tumor samples

Frozen xenograft tumor pieces (approximately 5 mm3) were homogenized in lysing buffer including phosphatase inhibitor I + II and protease inhibitor (Meso Scale Discovery) using a tissue lyser machine (Qiagen). 20 μg/well protein lysate, determined using a bicinchoninic acid protein assay (Pierce), was analyzed using a Phospho (Ser780)/Total Rb Whole Cell Lysate Kit (Meso Scale Discovery) and a Meso Scale Discovery MSD-SEC-TOR reader.

Pharmacokinetic investigations

Pharmacokinetic studies in rats and mice were conducted in 3 animals/time point and blood was taken by exsanguination via the carotid artery. In dogs, 3 individual animals were used and blood was drawn from a limb vein. BAY 1000394 was solubilized in 50% polyethylene glycol 400, 5% ethanol, 45% water for studies with rats and dogs. For studies in mice, the compound was solubilized in 30% polyethylene glycol, 5% ethanol, 65% water for i.v. application, and in 40% polyethylene glycol, 60% water for intragastric (i.g.) dosing. In pharmacokinetic studies, plasma samples were collected at various time points between 2 min and 24 hours after i.v. administration and between 15 min and 24 hours after i.g. administration and precipitated with ice-cold acetonitrile (1:5). Supernatants were analyzed for BAY 1000394 via liquid chromatography-tandem mass spectroscopy. Pharmacokinetic parameters were estimated from the plasma concentration data, for example, using the lin-log trapezoidal rule for AUC estimation. Maximal plasma concentrations (Cmax) and time thereof (Tmax) were taken directly form the concentration time profiles.

Plasma protein binding was determined by ultrafiltration using the 14C-labelled compound at 0.1 to 2.5 μmol/L.

Animal efficacy studies

Housing and handling of animals was in strict compliance with European and German Guidelines for Laboratory Animal Welfare. For tumor xenograft studies female athymic nu/nu mice (Taconic), 50 days old, average body weight 20 to 22 g, were used after an acclimatization period of 14 days. Feeding and drinking was ad libitum 24 hours/d. Human tumor cells derived from exponentially growing cell cultures were resuspended in 50% Matrigel (BD Biosciences)/50% growth medium (without FCS) to a final concentration of 1.5 x 106 cells/mL. Subcutaneous implants of 1.5 x 106 cells were inoculated into the inguinal region of mice. Tumor area (product of the longest diameter and its perpendicular) measured with a caliper, and body weight were determined twice a week. When tumors reached a size of approximately 21 mm2, the animals were randomized to treatment and control groups (8 mice/group) and treated per os with BAY 1000394, vehicle (40% polyethylene glycol, 60% water), or chemotherapeutic agents as indicated in tables and figure legends. Animals were euthanized according to the German Animal Welfare Guidelines. Data were expressed...
as means ± SD. Statistical analysis included one way analysis of variance, and differences to the control were compared versus control group by pair-wise comparison procedure, using the Sigmastat software.

Results and Discussion

Kinase inhibition profile and cellular mechanism of action studies

In biochemical assays, BAY 1000394 (Fig. 1A) inhibits the kinase activity of the cell-cycle CDKs CDK1/cyclin B, CDK2/cyclin E, and CDK4/cyclin D with IC50 values of 7, 9, and 11 nmol/L, respectively (Supplementary Table S2). The transcriptional CDKs CDK9/cyclin T1 and CDK7/cyclin H/MAT1 were inhibited in a similar range (5 and 25 nmol/L), as well as the other CDK family members tested, classifying BAY 1000394 as a pan-CDK inhibitor. In particular, the compound inhibits cell-cycle and transcriptional CDKs with similar IC50s, indicating simultaneous inhibition of cell-cycle progression and of RNA polymerase II-mediated gene transcription. On a panel of 214 non-CDK Ser/Thr and Tyr kinases, 16 additional kinases were found to be inhibited by BAY 1000394 with IC50 values below 100 nmol/L (Supplementary Table S2).

The antiproliferative activity of BAY 1000394 was tested on a panel of 25 human cancer cell lines that represent various tumor types. It showed broad and uniform inhibitory activity on cell proliferation with IC50 values between 8 and 33 nmol/L (mean 16 nmol/L, Supplementary Table S3) and IC50 values between 13 and 110 nmol/L. These results were further corroborated by data from a panel of 40 human lung tumor cell lines (IC50s between 9 and 79 nmol/L, mean 39 nmol/L) and a panel of 24 human breast tumor and immortalized cell lines (IC50s between 6 and 84 nmol/L, mean 37 nmol/L; Supplementary Table S4). Within these panels, which represent a broad range of genetic backgrounds (p53, pRB, K-Ras, PGP, etc.), no cell line could be identified that was poorly sensitive towards treatment with BAY 1000394 (i.e., with an IC50 value >100 nmol/L).

Similar in vitro cell line panels have been previously used to identify genomic markers that may help to select the patient population that could benefit most from the treatment, an approach that is highly recommended for molecularly targeted agents (16). For the CDK4/6 inhibitor PD 0332991 IC50 values in the range of 4 nmol/L up to more than 1,000 nmol/L were reported for a panel of 44 breast cancer cell lines. Cell lines with IC50 less than 150 nmol/L were classified as sensitive and those with IC50 more than 1,000 nmol/L as insensitive. A statistical analysis resulted in the identification of differentially expressed genes in sensitive and insensitive cell lines (17). In contrast to the CDK4/6 inhibitor PD 0332991, BAY 1000394 is characterized by a remarkably
uniform inhibition pattern on the cell line panels that does not provide any basis for a clear-cut classification into sensitive cells and insensitive cells, a feature of BAY 1000394 that can most likely be attributed to the potent inhibition of multiple CDKs. A statistical analysis against genomic data failed to reach the level of statistical significance. Of note, a couple of PD 0332991 insensitive cell lines respond quite well to treatment with BAY 1000394. In summary, BAY 1000394 has shown a broad and potent antiproliferative activity on a wide range of human tumor cells representing various oncogenic backgrounds.

In wash out experiments in HeLa-MaTu cells IC50 values declined from 1,000 to 12 nmol/L with increasing time of exposure to BAY 1000394. A time of exposure of approximately 16 hours achieved almost the maximal effect (IC50 of 14 nmol/L; Supplementary Fig. S1), indicating that CDKs have to be inhibited for a sufficiently long period of time to achieve antitumor activity. Similar results had been obtained for the CDK1 inhibitor RO-3306 that showed fully reversible effects on cell viability up to an exposure time of 20 hours (18).

Next, we investigated whether the antiproliferative activity of BAY 1000394 was associated with the induction of apoptotic cell death. A concentration-dependent increase in activity of caspase-7 and -9 as well as in DNA fragmentation was observed upon treatment of HeLa cells for 17 hours, suggesting the induction of apoptotic cell death (Fig. 1B). These results were further corroborated by the observation that 32 of 66 lung and breast cancer cell lines within the antiproliferation panels showed a reduced cell number relative to the starting cell number upon exposure to BAY 1000394 at 111 nmol/L for 72 hours, indicating cell death (Supplementary Fig. S2).

Ample of evidences indicate that activity of individual CDK isoforms is required for transition through the phases of the cell cycle (for recent review, see ref. 19). Exposure of asynchronously growing HeLa cells to BAY 1000394 for 24 hours reduced the fraction of cells with 2N content, indicative for G0–G1 cell-cycle phase, from 69% to 52% compared with vehicle treated cells and strongly increased the fraction of cells with less than 2N DNA content from 1% to 16%, indicative of apoptotic cell death (Fig. 1C). Only minor shifts were observed for cells in S, G2, or M phase (2 < N < 4, or 4N DNA content). For a more detailed analysis of the effects of BAY 1000394 on cell-cycle distribution, HeLa cells released from a double-thymidine block were used. At release about 60% of the cells had a 2N DNA content. Twenty-four hours after release into vehicle, 33% of the cells were found with 2N whereas 44% were found with 4N DNA content, indicating cell-cycle progression. In contrast, 24 hours after release into BAY 1000394 containing medium, 50% of the cells were 2N and only 11% were 4N, indicating that the compound arrests the majority of cells at the G1 to S boundary. Similarly, cells treated with vehicle 3, 5, or 8 hours after release progressed or completed the cell cycle. BAY 1000394 treated cells were found with intermediate (S phase) or 4N DNA contents and with cell-cycle distribution patterns that resembled more the pattern at the time of treatment than the pattern of vehicle treated cells. These data suggest that BAY 1000394 arrests the cells at various CDK-dependent steps within the cell cycle, which differentiates BAY 1000394 from compounds with a more pronounced selectivity toward specific CDK isoforms such as the CDK1-selective compound RO-3306 that did not affect transition through G1 and S phases but arrested the cells at the G2 to M boundary (18).

The mechanism of action of BAY 1000394 was further investigated by analysis of downstream targets of the cell-cycle kinases CDK1, CDK2, and CDK4, and of the transcriptional kinase CDK9. BAY 1000394 at 30 nmol/L completely suppressed the phosphorylation of retinoblastoma protein (P-pRb) in MCF7 human breast cancer cells stimulated to enter the cell cycle from quiescence indicative for cellular inhibition of CDK4 and CDK2 (Supplementary Fig. S3A). T199-phosphorylated nucleophosmin, a mitotic target of CDK1/cyclin B (20), was reduced at a concentration of 30 nmol/L of BAY 1000394 and completely abolished at 100 nmol/L in nocodazole-arrested HeLa cells (Supplementary Fig. S3B). BAY 1000394 inhibited Ser-2 phosphorylation of RNA polymerase II, a key substrate of CDK9/cyclin T1 (21), in a concentration-dependent manner in A549 cells and reduced the level of myeloid cell leukemia sequence 1 protein, which is under transcriptional control of RNA polymerase II (22), at 100 nmol/L (Supplementary Fig. S3C).

The concentrations of BAY 1000394 that were sufficient to inhibit phosphorylation of the substrate proteins of CDK1, CDK2, and CDK9 in the cellular mechanistic assays were in the range of the IC50/IC90 values determined in antiproliferation assays, suggesting that the antiproliferative activity of BAY 1000394 is mediated by intracellular inhibition of kinase activity of the CDKs. Cell-cycle analyses, which indicated cell-cycle arrest at CDK-dependent transition events, further suggest that the predominant mode of action of BAY 1000394 is through CDK inhibition despite biochemical inhibition of some non-CDK kinases.

**In vivo pharmacokinetic parameters and pharmacodynamic mechanism of action of BAY 1000394**

Pharmacokinetic parameters were determined in mouse, rat, and dog. After intravenous administration, BAY 1000394 showed a low clearance and an intermediate half-life across species (Supplementary Table S5). A high volume of distribution suggests extensive tissue distribution of the compound. After oral application, BAY 1000394 was readily absorbed and showed an intermediate oral bioavailability of approximately 50%. The compound is characterized by a high solubility in water of 170 mg/L at pH 7.0 and by moderate plasma protein binding with a fraction unbound between 5% and 7% in mouse, rat, dog, and human.
Pharmacokinetic/pharmacodynamic relationship and in vitro mechanism of action were investigated in athymic mice bearing established HeLa-MaTu human cervical tumors. In vehicle treated tumors concentration of P-pRb remained constant over the time course of the study (Fig. 2). One hour after treatment with BAY 1000394 P-pRb was barely detectable and remained at a low level for 7 hours. Forty eight hours after dosing, phosphorylation of pRb recovered to the level of the vehicle controls. Unbound concentrations of BAY 1000394 reached approximately 70 nmol/L 1 hour postdosing and declined over time. Seven hours after dosing, when Rb protein still remains dephosphorylated, the unbound concentration declined to approximately 20 nmol/L, and 24 hours after dosing, when phosphorylation of pRb partially recovered, the unbound concentration was approximately 12 nmol/L. As tumor and serum concentrations ran in parallel over time, the unbound serum levels were put in relation to the observed P-pRb inhibition in tumor tissue. This result shows that BAY 1000394 inhibits CDK2 and CDK4 activity within the tumor tissue. The time course of pRb phosphorylation is consistent with the time course of the unbound concentration of BAY 1000394. Furthermore, the unbound concentration of BAY 1000394 after a single oral application of a dose of 2 mg/kg exceeded the IC50 of inhibition of HeLa-MaTu cell proliferation in vitro of 11 nmol/L for 24 hours.

The pharmacokinetic and pharmacodynamic data indicate that by once daily dosing of BAY 1000394 a coverage of the cellular antiproliferative IC50 can be achieved for 1 day. By twice daily dosing, an almost complete target inhibition should be feasible over a time frame that is sufficient to cause profound antiproliferative and cell death inducing effects.

BAY 1000394 in vivo efficacy

The dose–response relationship was investigated using the HeLa-MaTu cervical cancer xenograft model. Athymic mice bearing established HeLa-MaTu human cervical tumors of approx. 25 mm² in size were treated orally with BAY 1000394 at doses of 0.5, 1.0, 1.5, and 2.0 mg/kg once daily for 21 days (Fig. 3A). A clear dose–response relationship was observed with T/C values of 0.61 at the lowest dose and of 0.03 and signs of tumor regression at the 2 mg/kg dose (Fig. 3C). Treatment was well tolerated as no body weight loss below the initial body weight was observed (Fig. 3D). Additional groups of mice were treated on a cyclic intermittent dosing schedule at doses of 1.5, 2.0, and 2.5 mg/kg twice daily for 2 days followed by 5 days without treatment (2 on/5 off). In total, 3 treatment cycles were completed (Fig. 3B). Tumor growth was strongly inhibited in a dose-dependent manner with T/C values of 0.19 at the lower dose and of 0.02 (tumor regression) at the higher dose. Furthermore, BAY 1000394 strongly inhibited growth of HeLa-MaTu tumors that had been grown to a size of approximately 50 mm² before start of treatment (day 8 after inoculation). T/C values of 0.06 and 0.13 were achieved upon once daily 2 mg/kg and twice daily intermittent 2.5 mg/kg (2 on/5 off) treatment schedule (data not shown).

These data indicate that BAY 1000394 can produce similar in vivo responses upon various dosing schedules ranging from continuous once daily dosing to cyclic intermittent dosing. The full coverage of the antiproliferative IC50 for about 24 hours observed upon a single dose of 2 mg/kg and the suppression of P-pRb are in line with the observed efficacy upon once daily dosing in the xenograft study. The higher exposure upon twice daily treatment leads to a more pronounced target inhibition over the treatment period and allows introduction of drug holidays.

Next, we extended our in vivo xenograft studies to models that are refractory to standard of care drugs. Treatment with BAY 1000394 of the triple negative MX-1 human breast cancer model, of the A2780-cis cisplatin-resistant human ovarian cancer model, and of the OVCAR-8-ADR (also known as NCI-ADR-Res) multidrug-resistant human ovarian cancer model was much more efficacious than treatment with the comparator drugs doxorubicin, cisplatin, and paclitaxel (Supplementary Table S6), indicating that BAY 1000394 was not recognized by the molecular mechanisms conveying resistance to the comparator drugs.

SCLC represents a disease that is characterized on the molecular level by inactivation of the Rb tumor suppressor gene in more than 90% of the cases, indicating a crucial role of the Rb-cell-cycle pathway in tumorigenesis (8). BAY 1000394 was evaluated in cell line-derived and patient-tumor-derived SCLC xenograft models (Supplementary Table S6). The compound strongly reduced tumor growth with T/C values between 0.12 and 0.19 showing that BAY 1000394 was similar or even more efficacious as compared with cisplatin (T/C values between 0.06 and 0.35).
In summary, BAY 1000394 showed potent tumor growth inhibition in monotreatment upon oral application in various dosing schedules in a dose-dependent manner, activity in models of treatment-refractory tumors, and efficacy in cell-line–derived as well as in patient tumor–derived models.

**BAY 1000394 shows more than additive efficacy in combination with cisplatin and etoposide**

On the basis of the good antitumor activity of BAY 1000394 in SCLC models, we asked whether cotreatment with BAY 1000394 could improve the treatment outcome of the standard of care first line medication for extensive-stage SCLC patients that consists of a combination treatment with platin derivatives (either cisplatin or carboplatin) and etoposide (23). Monotreatment of NCI-H82 SCLC xenograft tumors with either cisplatin (6 mg/kg) or etoposide (12 mg/kg) on a 14 days treatment cycle was only weakly effective with \( T/C \) values of 0.58 and 0.88, respectively (Fig. 4A and B). The combination of cisplatin and etoposide produced a \( T/C \) value of 0.51. Note that cisplatin and etoposide were administered at the maximal tolerated doses. Treatment with BAY 1000394 at doses of 1.5 and 1 mg/kg slowed tumor growth to \( T/C \) values of 0.15 and 0.62, respectively. Addition of BAY 1000394 to cisplatin resulted in a strong tumor growth inhibition with \( T/C \) values of 0.01 (1.0 mg/kg BAY 1000394) and –0.02 (1.5 mg/kg BAY 1000394; Fig. 4A). The combination of etoposide (12 mg/kg) with a dose of 1.5 mg/kg of BAY 1000394 resulted in a tumor growth inhibition with a \( T/C \) of –0.05 (Fig. 4B). Calculating expected additive \( T/C \) values on the basis of the monotreatment results according to the Bliss algorithm (0.31 and 0.08 for the cisplatin/BAY 1000394 combination; ref. 24) revealed that the observed \( T/C \) values were below the calculated values, indicating more than additive efficacy of the combination. Similar results were found for the BAY 1000394/etoposide combination. Long-term tumor growth control (stasis) was observed in the cisplatin/BAY 1000394 combination groups over an observation period of 48 days. Treatments were well tolerated with acceptable body weight loss.

Next, we asked whether a triple cisplatin/etoposide/BAY 1000394 combo would be feasible. The selected dose of 0.75 mg/kg of BAY 1000394 administered in mono produced a \( T/C \) value of 0.69. Cisplatin/etoposide combination treatment did also not achieve significant tumor growth inhibition (\( T/C \) 0.58). The triple combination of cisplatin (6 mg/kg), etoposide (12 mg/kg), and BAY 1000394 (0.75 mg/kg) treatment resulted in a strong tumor
growth inhibition with a $T/C$ value of 0.09 (Fig. 4C), which was clearly below expected additive $T/C$ of 0.40 calculated according to the Bliss algorithm, indicating that BAY 1000394 adds efficacy to the cisplatin/etoposide combination in a more than additive manner. The strong tumor growth inhibition continued over the observation period of 32 days. Remarkably, BAY 1000394 did not increase the body weight loss in the triple combination over the body weight loss observed in the cisplatin/etoposide combination group (20%), indicating that the main drivers of toxicity are the standard of care drugs cisplatin and etoposide.

The pan-CDK inhibitor BAY 1000394 strongly improved the efficacy of the standard of care combination treatment with the DNA-damaging agents cisplatin and etoposide in the Rb negative NCI-H82 SCLC xenograft model without further worsening the tolerability of the therapy. CDKs play multiple roles in the DNA damage response pathway including activation of the DNA damage checkpoint, activation of DNA repair pathways, and G2 cell-cycle arrest (recently reviewed in ref. 25). In line with the role of CDKs in DNA-damage response signaling combination treatment NCI-H460 NSCLC xenograft tumors with BAY 1000394 and carboplatin was more efficacious when carboplatin was administered a day before BAY 1000394 treatment compared with the schedules BAY 1000394 followed by carboplatin or concomitant administration of both drugs (data not shown).

Overall, the novel pan-CDK inhibitor BAY 1000394 is characterized by a broad range of on target activity and potent efficacy in various in vitro and in vivo models of human cancer; however, a contribution of inhibition of non-CDK kinases cannot be excluded. Although various low molecular weight inhibitors of CDKs have entered clinical trials in the past years the therapeutic efficacy was modest and none has reached the market yet. This may at least in part be because of insufficient potency, pharmacokinetic and physicochemical properties, off-target toxicities, and suboptimal dosing schedules (14). R-roscovitine, for example, has low cellular activity, whereas i.v. drugs administered on a 3-week-treatment cycle, such as R547 or SCH 727965, have to overcome the hurdle to cover the whole length of the cell-division cycle at sufficient exposures (3, 26). Another example is ZK 304709, an oral multitargeted CDK inhibitor, which showed a promising activity profile in various human tumor xenografts at high daily doses of up to 100 mg/kg (13, 27). In phase I clinical trials, blood concentrations of ZK 304709 increased in a subdose proportional manner with high interpatient
variability. Concentrations expected to deliver meaningful pharmacological activity were not reached and the trials were closed before the maximum tolerated dose was determined (28, 29). The most likely reasons for the suboptimal human pharmacokinetics of ZK 304709 were its low solubility and the high doses to be administered. BAY 1000394 clearly overcomes these limitations and is characterized by a high solubility in water even at neutral pH and low efficacious oral doses in the range of 1.5 to 2.5 mg/kg in mice. BAY 1000394 is efficacious in human tumor xenograft models including patient-derived models that are considered to provide a high probability of correct prediction of human tumor response (30). The compound is active upon once daily dosing as well as upon intermittent dosing providing the room to optimize human dosing schedules with respect to efficacy and tolerability. Furthermore, BAY 1000394 has proven anti-tumor activity in xenograft models resistant to standard of care drugs such as doxorubicin, cisplatin, or paclitaxel, and has shown its potential for combination treatment with drugs on the market. BAY 1000394 is currently in phase I clinical trials (ClinicalTrials.gov identifier: NCT01188252).

Disclosure of Potential Conflicts of Interest
All the authors are employees of Bayer Pharma AG.

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