Cancer Chemother Pharmacol (2008) 62:1091–1101
DOI 10.1007/s00280-008-0771-1

ORIGINAL ARTICLE

Toxicity and toxicokinetics of the cyclin-dependent kinase inhibitor AG-024322 in cynomolgus monkeys following intravenous infusion

Alan P. Brown · Cynthia L. Courtney · Kay A. Criswell · Christopher L. Holliman · Winston Evering · Bart A. Jessen

Received: 30 January 2008 / Accepted: 8 May 2008 / Published online: 29 May 2008

Abstract
Purpose Cyclin-dependent kinases (CDKs) play a significant role in the control of cell-cycle progression and exhibit aberrant regulation in various neoplastic diseases. AG-024322 is a potent inhibitor of CDK1, CDK2, and CDK4 that produces cell-cycle arrest and antitumor activity in preclinical models. This study evaluated the toxicity of AG-024322 when given by intravenous (IV) infusion to cynomolgus monkeys, including reversibility of effects.
Methods Male and female monkeys received AG-024322 by 30-min IV infusion once daily for 5 days at doses of 2, 6, and 10 mg/kg (24, 72, and 120 mg/m^2, respectively). Controls received vehicle alone which was aqueous 5% dextrose, pH 3.8. Three animals/sex/group were necropsied on day 6, and two animals/sex/group at 6 and 10 mg/kg were necropsied on day 22 (reversal cohort). Doses were based upon the results of a dose range-finding study in monkeys; decreased white blood cells occurred at ≥3 mg/kg and 12 mg/kg produced central nervous system effects and was above the maximum-tolerated dose.
Results No deaths occurred and clinical signs of toxicity, including swelling at the IV administration site, were seen at ≥6 mg/kg. AG-024322 at ≥6 mg/kg produced pancytic bone marrow hypocellularity, lymphoid depletion, and vascular injury at the injection site. Renal tubular degeneration occurred at 10 mg/kg. These changes were either reversible or in a process of repair following the 17-day recovery period. Hematology changes included decreases in reticulocytes and/or granulocytes at ≥6 mg/kg, which were reversible and consistent with changes in the bone marrow. Lymphoid and bone marrow depletion are consistent with pharmacologic inhibition of CDKs by AG-024322 and were expected findings. On day 22, vacuolar degeneration of pancreatic acinar cells with increased serum amylase and lipase levels occurred in one female at 10 mg/kg. Neither sex-related differences in toxicokinetics nor plasma accumulation over 5 days of dosing were seen. Terminal phase overall mean half-life on day 5 ranged from 6.69 to 8.87 h (across dose levels) and was not dose dependent.
Conclusion The no-adverse-effect dose of AG-024322 was 2 mg/kg and associated with overall mean plasma AUC(0–24.5) of 2.11 μg h/mL.

Keywords Oncology · Anticancer · CDK · Cyclins · Myelotoxicity · Vascular irritation

A. P. Brown (✉) · C. L. Courtney · K. A. Criswell
Drug Safety Research and Development,
Pfizer Global Research and Development,
Ann Arbor, MI 48105, USA
e-mail: abrown@namsa.com; apbrown07@comcast.net

C. L. Holliman
Pharmacokinetics, Dynamics, and Metabolism,
Pfizer Global Research and Development,
Ann Arbor, MI 48105, USA

W. Evering · B. A. Jessen
Drug Safety Research and Development,
Pfizer Global Research and Development,
San Diego, CA 92121, USA

Introduction

Mammalian cell division typically involves an orderly progression through four distinct phases (known as the cell cycle) that are identified as G1 (growth phase 1), S (DNA synthesis), G2 (growth phase 2), and M (mitosis). Cyclin-dependent kinases (CDKs), of which there are various forms, are cellular proteins that play a key role in regulating

cell-cycle progression. During the G1 phase, cells respond to extracellular signals by moving towards cell division or entering into a quiescent state (G0). Movement of a cell from G1 to S phase is dependent upon the cell passing a restriction point late in G1, which is regulated by CDKs [17]. D-type cyclins are cellular proteins which are induced by mitogens during G1 and assemble with the major CDKs (such as CDK4 and CDK6) to produce holoenzymes that phosphorylate key proteins, such as the retinoblastoma (Rb) protein, thereby facilitating the cells commitment to S phase [5, 16]. Cyclin D-CDK4, cyclin D-CDK6, and cyclin E-CDK2 protein complexes facilitate the G1 to S phase transition, whereas cyclin A-CDK2 and cyclin B-CDK1 protein complexes are involved in S-phase progression and G2–M transition, respectively [14].

Aberrant control of the cell cycle is a hallmark of neoplastic cells and extensive evidence suggests that unregulated control of CDKs is associated with various human cancers [17]. Pharmacologic inhibition of CDKs by small molecule inhibitors has recently become an attractive strategy for cancer chemotherapy with the intent of blocking tumor cell-cycle progression [4, 8]. The first inhibitor of CDKs to enter clinical trials was flavopiridol, which is administered by intravenous (IV) infusion. This compound directly competes with the ATP substrate and inhibits multiple CDKs including CDK1, CDK2, CDK4, CDK6, and CDK7 with IC_{50} values of 100–400 nM [15]. Another CDK inhibitor to enter clinical trials is PD-0332991, which is a highly specific inhibitor of CDK4 and CDK6, and has demonstrated antitumor activity against a range of tumor types and induces G1 arrest in cancer cells [6].

Data have suggested that cells can recover from targeted inhibition of a single CDK form by compensatory activity from other CDKs [20]. Therefore, a potent multitargeted CDK inhibitor should minimize this compensatory activity and display significant inhibition of cell-cycle activity. AG-024322 (Fig. 1) is a potent ATP-competitive inhibitor of CDK1, CDK2, and CDK4 with Ki values in the 2–3 nM range and selectivity over other non-CDKs. This compound has been shown to inhibit Rb phosphorylation in cells, elicit cell-cycle arrest, and have antiproliferative activity in multiple human tumor cell lines (IC_{50} values from 30 to 200 nM) [9, 21]. In human tumor xenograft studies conducted in mice, AG-024322 produced dose-dependent antitumor activity, including induction of apoptosis in tumor cells [22]. AG-024322 was identified as a second-generation CDK inhibitor to the prototype molecule, AG-012986, which exhibited a broad range of preclinical toxicities including bone marrow independent acute leukocyte toxicity. AG-024322 demonstrated decreased peripheral leukocyte toxicity in comparison with AG-012986 and was subsequently selected for clinical development [9].

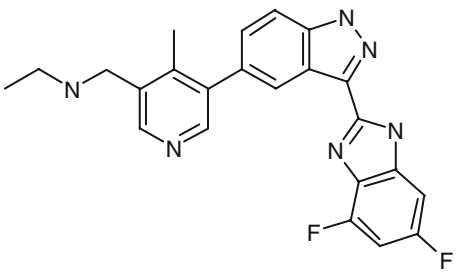

Fig. 1 AG-024322 chemical structure

The cynomolgus monkey was selected as the non-rodent species for evaluating the intravenous toxicity of AG-024322. Toxicity has been previously observed in monkeys administered with another CDK-inhibitor compound (AG-012986) [9], and nonhuman primates are acceptable to regulatory agencies as a nonrodent model for drug safety testing. Intravenous infusion is the intended clinical route of administration for AG-024322 and was incorporated in the toxicology studies. Initially, a dose range-finding study in monkeys was performed to provide dose-selection information prior to the pivotal IND-enabling toxicology study, which was conducted in compliance with the US FDA Good Laboratory Practice Regulations. The intravenous dosing period (5 days) and non-treatment phase (17 days) in the toxicology study were designed to mimic the initial Phase 1 clinical trial protocol. The Phase 1 clinical trial of AG-024322 was designed as a single-agent, dose-escalation study in patients with various advanced solid tumors or non-Hodgkin's lymphoma. AG-024322 was given once daily for 5 consecutive days as a 30-min IV infusion, with repeated cycles at 21-day intervals.

Materials and methods

Experimental drug

AG-024322 (molecular weight of 418 Da; Fig. 1) was synthesized by Pfizer Global Research and Development and has the chemical name of *N*-({5-[3-(4,6-difluoro-1*H*-benzimidazol-2-yl)-1*H*-indazol-5-yl]-4-methylpyridin-3-yl}methyl)ethanamine. Dosing solutions for the dose range-finder study were prepared as follows. AG-024322 was dissolved in aqueous 5% dextrose (D5W) with the aid of glacial acetic acid, and the pH was adjusted to 4.5 ± 0.5. The final solutions (0.6–3 mg/mL) were filter-sterilized through an approximately 0.2 μm filter and given intravenously in dose volumes of 4–6 mL/kg, depending upon final dose. Dosing solutions for the toxicology study were prepared by dissolving AG-024322 in D5W with the aid of glacial acetic acid and sterile water to a stock concentration of 5 mg/mL, followed by dilution with D5W to final solutions of 0.33, 1, and 1.67 mg/mL, pH of 3.8 ± 0.3. Dosing

solutions were filter-sterilized through an approximately 0.2 μm filter and given intravenously in a dose volume of 6 mL/kg.

Animals

Male and female cynomolgus monkeys were obtained from Charles River Laboratories. Animals were approximately 3- to 4-years old and weighed 2.7–6.4 kg at the start of dosing. Animals were housed individually in stainless steel cages, and standard procedures/conditions applied for animal care, feeding, and maintenance of room, caging, and environment. Lab Diet 5K91 Certified HI-Fiber Primate Diet (toxicology study) or Lab Diet 5048 Certified Primate Diet (dose-range finder) were offered daily, and supplemented with fruit and/or vegetables. Water was supplied ad libitum via an automatic system. In the toxicology study, animals were fasted overnight prior to collection of blood samples for clinical laboratory tests and prior to scheduled euthanasia. Both studies were conducted in accordance with the current guidelines for animal welfare (National Research Council *Guide for the Care and Use of Laboratory Animals*, 1996; Animal Welfare Act, 1966, as amended in 1970, 1976, and 1985, 9 CFR Parts 1, 2, 3). Animals were individually housed for the purpose of individual clinical observations, study procedures, and sample collection; however, social interactions between animals were still maintained. The procedures used in this study were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee.

Dose-range-finding study design

Two monkeys/sex received AG-024322 once daily (for up to 5 days per dose level) by intravenous injection in a dose-escalation schedule and one animal/sex received the vehicle alone, which was aqueous 5% dextrose, pH 4.5 ± 0.5. Day 1 constituted initiation of dosing. Animals received AG-024322 from days 1 to 5 at 3 mg/kg (36 mg/m^2), from days 22 to 26 at 6 mg/kg (72 mg/m^2), on day 36 at 18 mg/kg (216 mg/m^2), and days 44 and 45 at 12 mg/kg (144 mg/m^2). Animals were not dosed on interim days. Either drug or vehicle were administered intravenously over an approximate 6-min period in dose volumes of either 5 mL/kg (3- and 6-mg/kg dose levels), 4 mL/kg (12-mg/kg dose), or 6 mL/kg (18-mg/kg dose). Control animals received an equivalent dose volume of vehicle to match the dose volume administered to the drug-treated animals. The vehicle and drug solutions were administered with a catheter placed in the saphenous vein and delivered with a syringe and infusion pump. The required volume of drug or vehicle alone for each animal was based on the most recent individual body weight.

Animals were observed predose, immediately after dosing, and approximately 1 and 7 h postdose daily for clinical signs. Animals were observed once daily on nondosing days. Physical examinations were conducted pretest and at termination. Body weights were recorded prior to dosing on day 1 and weekly thereafter. Daily food consumption was estimated at the end of the workday by visual inspection. Animals were given primate biscuits of approximately 100 g/day. Reduced food consumption was considered when the animal consumed less than approximately 50% of the daily ration and anorexia was considered when there was the absence of food consumption for that day. Hematology, coagulation, and serum chemistry parameters (including troponin I and troponin T levels) were evaluated in samples collected from nonfasted animals pretest and approximately 24 h after the last day of dosing for each dose level (i.e., days 6, 27, 37, and 46). Hematology parameters were also measured on days 10, 23, and 45. In addition, serum troponin levels were measured prior to dosing on day 1, immediately prior to each new dose level, and approximately 7 h postdose on each dosing day. Frequent measurement of serum troponins was conducted to monitor for potential myocardial injury which was seen with another CDK inhibitor (AG-012986). Drug-treated animals were sacrificed and necropsied on day 46. A gross examination was conducted, and various tissues collected and examined microscopically. Control animals were returned to the stock animal colony on day 46.

Toxicology study design

Male and female cynomolgus monkeys received AG-024322 by IV infusion once daily for 5 days. Doses administered were 2, 6, and 10 mg/kg (24, 72, and 120 mg/m^2, respectively) in a dose volume of 6 mL/kg. Doses were selected based upon the results obtained in the dose-range-finding study. Control animals received vehicle alone which was aqueous 5% dextrose, pH 3.8 ± 0.3. This pH was consistent with the intended clinical formulation. The required volume of drug or vehicle alone for each animal was based on the most recent individual body weight and administered by infusion over an approximate 30-min period. Dosing solutions were administered with a catheter placed in the saphenous (primary site) and/or cephalic vein, and delivered via a syringe and infusion pump. The cephalic vein was used if dosing would have been difficult to occur in a saphenous vein (due to bruising for example). Treatment groups were comprised of three animals/sex/group for the control and 2 mg/kg groups, and six animals/sex/group for the 6 and 10 mg/kg groups. Three animals/sex/group were necropsied on day 6 (following the last day of dosing). In addition, three animals/sex/group at 6 and 10 mg/kg were necropsied on day 22 following a 17-day

period in the absence of dosing. A control group was not included in the reversal phase. Day 1 constituted initiation of dosing.

Animals were observed predose, immediately after dosing, and approximately 1 and 7 h postdose daily for clinical signs. Animals were observed once daily on nondosing days for clinical signs. Observations included the injection (dosing) sites. Physical and ophthalmic examinations were conducted pretest, and on days 5 and 21. Body weights were determined on days 1, 6, 15, and 22. Food consumption was assessed daily by visual inspection. Hematology, coagulation, and serum chemistry parameters were evaluated pretest, and on days 6, 10 (hematology only), 15, and 22. Hematology parameters included complete blood count, differential, and blood cell morphology (days 6–22). Coagulation parameters included prothrombin time, activated partial thromboplastin time, and fibrinogen. Serum was analyzed for alanine aminotransferase, albumin, albumin/globulin ratio (calculated), alkaline phosphatase, aspartate aminotransferase, bilirubin (total), amylase, calcium, chloride, cholesterol, creatinine, gamma glutamyltransferase, globulin (calculated), lipase, glucose, potassium, phosphorus, protein (total), sodium, urea nitrogen, lactate dehydrogenase (total and isoenzymes), and troponin I. Urine was analyzed pretest, and days 6 and 22. Bone marrow analysis (differential count) by flow cytometry was performed at necropsy.

Toxicokinetic parameters were assessed on day 5. Blood samples (approximately 1 mL) were collected within the last 2 min of dosing, and at approximately 1, 3, 8, and 24 h after completion of dosing. In addition, an approximate 1-mL blood sample was collected from all animals within the last 2 min of dosing on day 1. Samples collected within the last 2 min of dosing were considered as the 0.5-hour time-point sample (based upon a 30-min infusion time). Blood samples from control animals were discarded without being analyzed. Blood samples were collected into a tube containing EDTA as an anticoagulant, immediately placed on ice, and centrifuged under refrigerated conditions. The plasma was separated and stored frozen at $\leq -20°C$ pending analysis.

Plasma samples were analyzed for AG-024322 concentration(s) using a validated LC/MS/MS method with a lower limit of measurement of 1.00 ng/mL. Toxicokinetic parameters calculated included maximum observed plasma concentration (Cmax), half-life, and area under the concentration–time curve from initiation of dosing until 24 h after the 30-min infusion period [AUC(0-24.5)]. Because a 0-h time point was not taken on day 5, toxicokinetic parameters were calculated by setting the concentration at time zero equal to the concentration measured immediately prior to the end of dosing.

Blood samples were inadvertently not collected within the last 2 min of dosing on day 5 from males at 10 mg/kg which resulted in undetermined Cmax and AUC values for this group. However, because sex-related differences in exposure data at all other times were not observed, toxicokinetic parameters in females at 10 mg/kg are considered representative of exposures in males at the same dose.

All animals underwent a complete gross necropsy, and tissues were collected, weighed, and evaluated microscopically. The toxicology study was conducted in compliance with US FDA Good Laboratory Practice regulations.

Statistical analysis

Statistical analyzes were conducted for body weight and weight change, organ weight, and quantitative clinical laboratory data in the toxicology study. Treatment comparisons were performed on rank-transformed data using a dose-trend test sequentially applied at the two-tailed 1 and 5% significance levels within one-factor analysis of variance (ANOVA), and were conducted on pretest and day 6 data. Dunnett's test replaced the sequential-trend test if the overall linear-trend test was not significant at the 5% level and a quadratic trend was significant at the 1% level. All parameters were analyzed separately for each sex and time period. Supplemental analyzes including analysis of the original (non-rank-transformed) data, and comparisons to pretest measure for clinical pathology data were used to aid with interpretation.

Results

Dose-range-finding study

Clinical signs were not observed at doses of 3 or 6 mg/kg (Table 1), and drug-related body weight changes (data not shown) were not observed in the study. Following the 18-mg/kg dose on day 36, emesis occurred in all animals within approximately 1 h after dosing, and signs of ataxia, hypoactivity, hunched posture, and/or pallor were observed on that day. Animals that were hypoactive or had ataxia appeared to be sedated. One male had repeated emesis on day 36. Because of the severity of these signs, additional dosing at 18 mg/kg was suspended and recovery from the clinical toxicity occurred by day 37. Following the 12-mg/kg dose on day 44, emesis occurred within approximately 1 h after dosing in one male and both females, and hypoactivity was observed in both females. Following a second 12-mg/kg dose on day 45, clinical signs increased in severity and included emesis, hypoactivity, decreased skin turgor, anorexia, and/or stereotypic behavior (females only). Stereotypic behavior included self-directed aggression. Because of the severity of the changes, all drug-treated animals were euthanized on day 46.

Table 1 Dose-range-finding study: summary of drug-related clinical signs

Clinical sign	Dose (mg/kg)				
	0	3	6	12[e]	18
Emesis (within 1 h postdose)	–	–	–	2M, 2F	2M, 2F
Emesis[d]	–	–	–	2M, 2F	1M
Hunched posture	–	–	–	–	1M
Hypoactive[a]	–	–	–	2M, 2F	1M, 2F
Pallor	–	–	–	–	1M
Decreased skin turgor	–	–	–	1M	–
Ataxia	–	–	–	–	1M[b]
Anorexia	–	–	–	1F	–
Stereotypic behavior[c]	–	–	–	2F	–

[a] Animals appeared sedated

[b] Animal appeared sedated

[c] Animals exhibited self-directed aggression

[d] Emesis recorded at the 7-h clinical observation period

[e] Animals were dosed at 12 mg/kg after having received a single 18-mg/kg dose

Percent change for hematology parameters described below are with respect to individual animal pretest values (data not shown). Decreases in white blood cells occurred in a dose-dependent fashion at ≥3 mg/kg. On day 6, decreased neutrophil counts of 71 and 85% occurred in one male and female, respectively. Whereas recovery occurred in the female by day 10, neutrophil counts remained decreased 76% in the male on that day. Neutrophil counts continued to be decreased in this animal by 85 and 65% on days 23 and 27, respectively, following 6 mg/kg doses. On day 37, following a single 18-mg/kg dose, all animals had decreased total white blood cell counts of 69–84%. Decreases in neutrophils (43–88%), lymphocytes (65–82%), and monocytes (17–82%) were observed. Two animals had decreases in total white blood cells (48–65%) and neutrophils (59–92%) on days 45 and 46 following doses at 12 mg/kg. No other changes in clinical laboratory tests were considered noteworthy and drug-related gross or microscopic changes were not observed.

The conclusions of the dose-range-finding study were the following. Daily intravenous dosing of AG-024322 to monkeys at 3 and 6 mg/kg for 5 days did not produce clinical signs of toxicity, with decreased white blood cells as the only toxicity observed. Doses ≥12 mg/kg produced central nervous system effects and were above the maximum-tolerated dose. Based upon these results, doses for the toxicology study of AG-024322 in monkeys were selected to be 2, 6, and 10 mg/kg (24, 72, and 120 mg/m^2, respectively). The high dose was anticipated to produce significant toxicity, and the low dose was expected to be a no-adverse-effect level. The mid dose was expected to produce changes in hematology parameters and aid in the development of dose–response relationships.

Toxicology study

Clinical responses

No deaths occurred in the study. On day 5, one 10 mg/kg male was unable to be dosed due to swelling and/or bruising of the limbs, and clinical signs of anorexia, cool-to-touch, decreased skin turgor, hypoactivity, pallor, and systolic murmur (Table 2). This animal was necropsied on day 6. Swelling of the limbs, associated with the injection sites, was also seen on days 4–6 in two other males at 10 mg/kg and one male at 6 mg/kg. Emesis occurred on the day of dosing, in a dose-dependent fashion, in males and females at ≥6 mg/kg. On days 2–4, generalized tremors were seen immediately after dosing in one female each at 2, 6, and 10 mg/kg, but were not observed by approximately 1 h postdose indicating reversibility. Tremors were not seen in the males. No other observations were considered drug-related, and reversal of clinical signs of toxicity occurred within 1 or 2 days following cessation of dosing. Reduced food consumption was observed during the 5-day treatment period at all doses, including control, and likely reflected a stress-related response to the animal handling procedures (i.e., restraint, dosing, blood collection). Other observations were either present prior to study initiation, incidental to monkeys, or related to the IV infusion procedure (e.g., bruising of the limbs). Drug-related ophthalmic changes did not occur. Drug-related changes in body weight or weight gain did not occur (data not shown). Body weight loss

Table 2 Toxicology study: summary of drug-related clinical signs

Clinical sign	Dose (mg/kg)			
	0	2	6	10
Animals/sex	3	3	6	6
Anorexia	–	–	–	1M[a]
Cool-to-touch	–	–	–	1M[a]
Decreased skin turgor	–	–	–	1M[a]
Emesis (within 1 h postdose)	–	–	1M, 2F	3M, 3F
Emesis[b]	–	–	1M, 2F	5M, 2F
Hypoactivity	–	–	–	1M[a]
Systolic murmur	–	–	–	1M[a]
Pallor	–	–	–	1M[a]
Swelling (hindlimbs, forelimb)	–	–	1M	3M
Tremor (generalized)[c]	–	1F	1F	1F

[a] The same male monkey

[b] Emesis recorded at the 7-h clinical observation period

[c] Immediately postdose only

occurred in the majority of animals, including vehicle controls, by day 6 (approximately 2–5% from day 1). These changes were reversible and may have reflected animal response (i.e., decreased food consumption) to the frequent dosing and handling procedures.

Clinical laboratory tests

Percent changes for hematology parameters described below are with respect to mean pretest values. Absolute reticulocyte counts were decreased in both sexes by 46–71% at ≥6 mg/kg on day 6 and by 75% in males at 10 mg/kg on day 10 (Table 3). The effects on reticulocyte count were reversed by day 15 and demonstrated a mild overcompensation over pretest. Statistically significant decreases in red blood cells, hemoglobin, or hematocrit were not seen. Red blood cell morphology was similar between control and drug-treated groups. Toxicologically relevant changes in platelet counts were not seen. Although statistically significant increases in red blood cell counts were seen on day 6 in males at ≥6 mg/kg, this was likely due to the low mean count in the control group and therefore not considered toxicologically relevant.

Mean total white blood cell count showed variability amongst groups, changes were not dose-dependent, significant decreases were not seen, and values remained within laboratory historical reference range. In 10 mg/kg males, mean neutrophil count was decreased 63% on day 10 (mean count of 1,530/μL compared to pretest mean of 4,170/μL). This decrease was largely due to a marked decrease in neutrophils in one male at this dose (count of 800/μL on day 10). Decreases in eosinophils of 68% were observed in males at 10 mg/kg on days 10 and 15 (mean count of 70/μL on both days compared with pretest mean of 220/μL), consistent with depression of granulocytic cell production. Effects on neutrophils and eosinophils were completely reversed by day 22.

With regards to hematology changes, 2 mg/kg was considered a no-effect dose and 6 mg/kg was considered a minimal effect dose with depression of reticulocyte production

Table 3 Peripheral blood absolute reticulocyte and red blood cell (RBC) counts

Parameter and sex	Study day	Dose (mg/kg)			
		0	2	6	10
Reticulocytes (1,000/μL)					
Males	Pretest	21.3 ± 3.21	28.7 ± 16.80	30.2 ± 11.86	32.0 ± 14.21
	6	35.7 ± 21.08	56.7 ± 10.07	15.0 ± 6.03*	10.3 ± 6.71**
	10	–[a]	–	59.7 ± 7.51	8.0 ± 3.61
	15	–	–	58.3 ± 15.50	92.0 ± 2.65
	22	–	–	58.7 ± 18.72	74.0 ± 11.53
Females	Pretest	35.0 ± 5.57	49.3 ± 21.39	32.5 ± 10.31	46.2 ± 25.76
	6	68.7 ± 53.12	43.7 ± 10.97	17.5 ± 12.50*	13.5 ± 7.50**
	10	–	–	45.0 ± 10.58	56.7 ± 7.09
	15	–	–	41.0 ± 16.52	123.7 ± 26.10
	22	–	–	88.0 ± 22.11	78.7 ± 23.76
RBC (10^6/μL)					
Males	Pretest	6.370 ± 0.1453	6.707 ± 0.4248	7.072 ± 0.4373	6.618 ± 0.2215
	6	6.010 ± 0.0985	6.240 ± 0.4543	6.570 ± 0.2631*	6.740 ± 0.4914**
	10	–	–	6.613 ± 0.1436	6.063 ± 0.6070
	15	–	–	6.823 ± 0.3889	5.873 ± 0.3523
	22	–	–	6.873 ± 0.6278	6.170 ± 0.2893
Females	Pretest	6.547 ± 0.4561	6.660 ± 0.1819	6.708 ± 0.2081	6.600 ± 0.5846
	6	5.777 ± 0.4759	6.037 ± 0.3870	6.142 ± 0.1991	6.257 ± 0.5973
	10	–	–	5.913 ± 0.2001	5.827 ± 0.4735
	15	–	–	5.913 ± 0.2397	6.083 ± 0.5254
	22	–	–	5.510 ± 0.1874	6.160 ± 0.4331

Values are mean ± standard deviation

* Value significantly different from concurrent control at 5% level

** Value significantly different from concurrent control at 1% level

[a] Data not available, dose group not included in reversal phase

only. Hematology changes occurred to a greater extent in males than females. Changes in coagulation parameters were not seen.

Bone marrow analysis was conducted by flow cytometry (Table 4). On day 6, the mean bone marrow myeloid-to-erythroid (M:E) ratio was increased 387 and 255% in males and females, respectively, at 10 mg/kg. Mean M:E ratio was 1.50 and 1.10 in male and female controls, respectively, and 7.30 and 3.90 in 10 mg/kg males and females, respectively. This is considered a moderate-to-marked change in cellular distribution. Consistent with the depression in peripheral blood reticulocytes, total erythroid cells were decreased 71% in males and 53% in females at 10 mg/kg. Total myeloid cells were increased 43 and 34% in males and females, respectively, at 10 mg/kg. Relative numbers of lymphocytes and megakaryocytes were unchanged. Because the bone marrow differential reflects only relative changes in cell percentages, the results indicate that erythroid cells were more sensitive than myeloid cells to the cytotoxic effect of AG-024322. All bone marrow effects were reversible by day 22.

Group mean changes in serum biochemistry parameters were minimal and occurred on day 6 only (data not shown for brevity). Percent changes indicated below are with respect to mean pretest values. Serum phosphorus was decreased 23–35% in both sexes at ≥6 mg/kg. There were minor increases in serum bilirubin in both sexes of 48–168% at ≥6 mg/kg. Serum creatinine was increased 36% in males at 10 mg/kg (1.43 mg/dL vs. pretest mean of 1.05 mg/dL). Reversal from these changes occurred thereafter. One female at 10 mg/kg had markedly increased serum amylase and lipase levels on day 22 only. Amylase and lipase values for this animal on day 22 were 2,229 and 5,781 U/L, respectively, in comparison with pretest values of 290 and 42 U/L, respectively.

Toxicokinetics

A summary of plasma toxicokinetic parameters of AG-024322 is presented in Table 5. AG-024322 was systemically available to all monkeys at all doses. There were no observed sex-related differences in Cmax or AUC(0-24.5). In general, plasma exposures increased in a dose-proportional fashion. Although Cmax and AUC(0-24.5) could not be calculated for males at 10 mg/kg (due to inadvertently not collecting samples prior to end of dosing), drug levels at the various collection times were similar to females at 10 mg/kg, indicating no sex-related differences in exposure. Terminal phase overall mean half-life on day 5 ranged from 6.69 to 8.87 h across dose levels and did not appear to be dose or sex-dependent. In addition to the low plasma drug concentrations at 24 h after dosing on day 5 (combined-sex means of 7.48, 26.9, and 104 ng/mL at 2, 6, and 10 mg/kg, respectively), plasma concentrations immediately prior to the end of infusion were similar on days 1 and 5, suggesting a lack of AG-024322 accumulation after 5 days of dosing.

Pathology

There were no biologically significant drug-related effects on organ weights (data not shown). Drug-related pathologic changes occurred in the kidney, gastrointestinal tract (tongue, esophagus, and large intestine), bone marrow, lymphoid organs, and injection sites at ≥6 mg/kg. These

Table 4 Summary of bone marrow parameters

Parameter and sex	Study day	Dose (mg/kg)			
		0	2	6	10
M:E ratio (:1)[a]					
Males	6	1.50 ± 0.200	1.37 ± 0.252	1.63 ± 0.569	7.30 ± 0.917*
	22	–[b]	–	1.50 ± 0.954	1.83 ± 1.137
Females	6	1.10 ± 0.361	1.67 ± 0.115	1.50 ± 0.200	3.90 ± 2.100*
	22	–	–	1.67 ± 0.252	1.43 ± 0.115
Total myeloid cells (%)					
Males	6	53.93 ± 3.156	51.80 ± 4.703	56.27 ± 9.819	77.33 ± 3.591*
	22	–	–	53.23 ± 12.687	58.00 ± 13.115
Females	6	47.23 ± 8.598	53.67 ± 3.623	53.23 ± 3.668	63.33 ± 9.074
	22	–	–	57.80 ± 2.707	53.23 ± 2.155
Total erythroid cells (%)					
Males	6	37.03 ± 3.677	38.53 ± 4.319	35.90 ± 7.255	10.67 ± 1.155*
	22	–	–	39.80 ± 11.616	36.73 ± 12.947
Females	6	44.33 ± 6.268	32.97 ± 1.701	36.00 ± 1.873	21.00 ± 13.000**
	22	–	–	34.67 ± 3.837	37.43 ± 2.053

Values are mean ± standard deviation

* Value significantly different from concurrent control at 5% level

** Value significantly different from concurrent control at 1% level

[a] *M:E* myeloid:erythroid ratio

[b] Data not available, dose group not included in reversal phase

Table 5 Mean plasma toxicokinetic parameters of AG-024322

Day	Dose (mg/kg)	Sex	Cmax (μg/mL)	Half-life (h)	AUC (0–24.5) (μg h/mL)
1[a]	2	Male	0.663	–	–
		Female	0.669	–	–
		Overall	0.666	–	–
	6	Male	2.12	–	–
		Female	3.09	–	–
		Overall	2.61	–	–
	10	Male	3.65	–	–
		Female	4.05	–	–
		Overall	3.85	–	–
5	2	Male	0.583	7.96	1.26
		Female	2.34	7.13	2.96
		Overall	1.46	7.55	2.11
	6	Male	2.27	7.02	5.18
		Female	3.32	6.35	6.22
		Overall	2.80	6.69	5.70
	10	Male	ND	10.4	ND
		Female	3.69	7.63	11.5
		Overall	ND	8.87	ND

ND not determined

[a] Plasma samples collected immediately prior to the end of dosing only

Table 6 Incidence of drug-related microscopic changes, end of the dosing period (day 6)

Microscopic changes	Dose (mg/kg)							
	0		2		6		10	
	M	F	M	F	M	F	M	F
Kidney—renal tubular degeneration	–	–	–	–	–	–	2	1
Bone marrow—pancytic hypocellularity	–	–	–	–	1	1	3	3
Lymphoid depletion								
Spleen	–	–	–	–	–	2	2	3
Mesenteric lymph node	–	1	–	–	–	–	2	2
Gut-associated lymphoid tissue	–	–	–	–	–	–	2	2
Thymus	–	–	–	–	–	–	1	–
Cephalic vein—transmural necrosis	–	–	–	–	–	1	2	3
Saphenous vein								
Thrombus	–	–	–	–	–	3	–	–
Transmural necrosis	1	2	2	3	3	3	3	3
Esophagus—epithelial hyperplasia	–	–	–	–	3	1	3	3
Tongue—epithelial hyperplasia	–	–	–	–	2	1	3	3
Large intestine								
Karyomegaly, prominent nucleoli	–	–	–	–	3	–	3	3
Adrenals—cortical cell hypertrophy	–	–	–	–	1	–	3	1

$N = 3$ animals/sex/group

changes were considered reversed or reversing within the 17-day period following dosing. Pancreatic changes were seen in one female at 10 mg/kg at the end of the reversal period (day 22).

End of dosing necropsy—day 6: Renal tubular degeneration (mild) occurred in two males and one female at 10 mg/kg only (Table 6). Degeneration occurred principally within medullary rays and was characterized by the presence of detached epithelial cells in tubular lumens, tubules lined by epithelial cells with karyomegaly (enlarged nucleus), and increased prominence of nucleoli. Increased serum creatinine levels (1.3–1.6 mg/dL) were seen in these animals on day 6.

Dose-related bone marrow pancytic hypocellularity occurred in one male and female at 6 mg/kg, and all monkeys at 10 mg/kg. Severity was minimal at 6 mg/kg and minimal to moderate at 10 mg/kg. These changes correlated with bone marrow data obtained by flow cytometry as described previously.

Drug-related lymphoid depletion (minimal to mild) was seen in spleen, mesenteric lymph node, and gut-associated lymphoid tissue in the majority of monkeys at 10 mg/kg. Minimal lymphoid depletion was also present in the spleen of two females at 6 mg/kg. Depletion was most conspicuous in the B-cell follicular areas. Mild thymic lymphoid depletion occurred in the 10-mg/kg male with the most severe renal and bone marrow changes. Minimal lymphoid depletion was seen in the mesenteric lymph node of one control female and considered a background finding.

Catheter/injection site alterations (cephalic and/or saphenous veins) consisting of necrosis, inflammation, hemorrhage, and thrombosis were present in varying degrees in all drug-treated and vehicle control animals. However, the severity and distribution of transmural vascular necrosis (mild to marked) and thrombosis were more severe in animals at ≥6 mg/kg and correlated with clinical signs of limb swelling. Vascular injury was considered to be drug-related at the 6 and 10 mg/kg doses.

Epithelial hyperplasia occurred in the basal layer of the squamous epithelium of the tongue and esophagus, and was characterized by increased epithelial mitoses and hypertrophic epithelial cells with enlarged vesicular nuclei and prominent nucleoli. Large intestine crypts had epithelium with nuclear alterations of karyomegaly and prominent nucleoli. These changes were present in both sexes at ≥6 mg/kg.

Minimal to mild hypertrophy of adrenal cortical cells of the zona fasciculata occurred in one male at 6 mg/kg, all males and one female at 10 mg/kg, and was likely due to stress rather than direct drug-induced toxicity.

End of reversal phase necropsy—day 22: Following a 17-day reversal period, reparative drug-related effects were evident in the kidney of one male at 10 mg/kg only (Table 7). This male had multifocal renal tubular basophilia

Table 7 Incidence of drug-related microscopic changes, end of the reversal period (day 22)

Microscopic changes	Dose (mg/kg)			
	6		10	
	M	F	M	F
Kidney—tubular basophilia	–	–	1	–
Pancreas				
vacuolar degeneration, acinar cells	–	–	–	1
Cephalic vein				
canalized thrombus	1	–	–	3
perivascular fibrosis	–	–	–	3
Saphenous vein				
canalized thrombus	3	2	2	3
perivascular fibrosis	3	3	3	3
intima necrosis	1	–	–	–
transmural necrosis	–	1	–	1

$N = 3$ animals/sex/group

within the medullary rays, consistent with tubular regeneration. No other drug-related effects in kidney, bone marrow, lymphoid tissues, or gastrointestinal tract were present in the reversal monkeys.

Vascular changes present at the catheter site were present in all monkeys and consisted of various degrees of repair that included canalization of vascular thrombi and perivascular fibrosis. Transmural or intima necrosis was present in three animals at ≥6 mg/kg.

Diffuse moderate vacuolar degeneration of pancreatic acinar cells, along with interlobular edema, mixed inflammation, and increased apoptosis, were noted in one female at 10 mg/kg. The pancreatic lesions correlated with increased serum amylase and lipase levels on day 22 in this animal.

Discussion

AG-024322 is a multitargeted cyclin-dependent kinase (CDK) inhibitor that demonstrated antitumor activity in preclinical models. This compound was subsequently evaluated in toxicology studies to support initiation of clinical testing in oncology patients with various solid tumors or non-Hodgkins lymphoma. Cynomolgus monkeys were selected as the nonrodent species for conducting safety studies of this compound because this species demonstrated toxicity to a related CDK-inhibitor compound [9]. The dosing and recovery (reversal) phases used in the toxicology study were selected to mimic the intended clinical protocol of daily dosing for 5 days followed by a 17-day recovery period, which would constitute a dosing cycle.

Initially AG-024322 was evaluated in a dose-range-finding study in monkeys that involved a dose-escalation scheme, the purpose of which was to identify a maximum-tolerated dose (MTD) and provide dose-selection information for the pivotal toxicology study. In this study, AG-024322 was administered by intravenous injection (over a 6-min period) at doses from 3 to 18 mg/kg (36–216 mg/m^2), with at least a 7-day drug-free period between changes in dose level. Daily dosing for 5 days up to 6 mg/kg (72 mg/m^2) did not result in clinical signs of toxicity. Single doses at ≥12 mg/kg (≥144 mg/m^2) resulted in significant clinical effects, indicating that 12 mg/kg was above the MTD. Following single or repeated doses at ≥12 mg/kg, the animals exhibited clinical signs indicating effects on the central nervous system. In vitro binding studies have indicated interactions of AG-024322 with various receptors of the central nervous system (CNS) that may be related to the observed in vivo effects. Receptor ligand studies have indicated that AG-024322 is an antagonist of muscarinic, μ-opioid, serotonin-2B, and dopamine-2 receptors. Although plasma drug levels were not determined in this study, it is possible that the CNS effects were due in part to high Cmax associated with the 6 min drug infusion time.

In the dose-range-finding study, decreases in white blood cells occurred at ≥3 mg/kg, and included changes in neutrophils, monocytes, and lymphocytes. Although the changes generally occurred in a dose-dependent fashion, the dose-escalation design of the study (within the same animals) confounded exact association between hematologic changes and a given dose level, and may have been influenced in part due to total drug administration. Microscopic evaluation of bone marrow and lymphoid tissues was unremarkable and therefore evidence of direct bone marrow suppression was not apparent. In summary, 6 mg/kg was identified as the MTD in the dose-range-finding study.

In the pivotal toxicology study, AG-024322 was administered by IV infusion over an approximate 30-min period to mimic the intended clinical administration and to minimize potential Cmax-related toxicities. Doses of up to 10 mg/kg (120 mg/m^2) were given for 5 consecutive days, and CNS-related clinical signs were limited to transient tremors (seen in one female each at 2, 6, and 10 mg/kg) and emesis at ≥6 mg/kg. Daily intravenous administration of AG-024322 for 5 days resulted in dose-dependent pancytic bone marrow hypocellularity (by microscopic evaluation) and lymphoid depletion in lymph nodes, spleen, and/or thymus at ≥6 mg/kg. Bone marrow analysis by flow cytometry indicated marrow suppression, primarily within the erythroid series, at 10 mg/kg. These changes correlated with hematology data in which reticulocytes were decreased at ≥6 mg/kg, and neutrophils and eosinophils decreased at 10 mg/kg. Recovery from these changes occurred by day

22, following a 17-day reversal period in the absence of dosing. The hematology changes in the toxicology study were less pronounced than in the dose-range-finding study, which may have been due in part to lower Cmax obtained with the longer infusion period.

Cyclin-dependent kinases (CDKs) play a prominent role in controlling progression through the cell-cycle and pharmacologic inhibition of CDKs is postulated to result in cell-cycle arrest [5]. At a cellular level, cytostasis may be defined as the inhibition of cell growth and/or proliferation, and this initial event can be followed by cell death if cell-cycle arrest or cytostasis is prolonged [14]. Alternatively, cells that undergo cytostasis may maintain viability and eventually recover. Cellular responses are dependent upon dose, schedule of drug administration, cell type, and cell-cycle phase during exposure [14]. CDKs and associated cell-cycle control proteins have been demonstrated to exist in bone marrow progenitor cells and likely participate in the control of hematopoiesis [3, 7]. Intravenous administration of the CDK inhibitor flavopiridol in mice resulted in lymphoid depletion in spleen, thymus, and lymph nodes, and decreased white blood cells [1]. In a Phase 1 clinical trial of flavopiridol in cancer patients, dose-limiting neutropenia developed following daily IV infusion [19]. Specific inhibition of CDK4 and CDK6 in primary bone marrow myeloma cells by the small molecule inhibitor PD-0332991 results in cell-cycle arrest in G1 [2]. In a Phase 1 clinical trial of PD-0332991 in patients with various solid tumors, myelosuppression was the principal dose limiting toxicity [12]. Bone marrow hypocellularity and lymphoid depletion produced by AG-024322 in monkeys is consistent with inhibition of CDKs in these tissues, and the pharmacology of an anticancer agent that targets populations of cells that are actively moving through the cell cycle.

Reversible epithelial changes were seen in the intestinal tract after the dosing period only that included nuclear alterations (enlarged vesicular nuclei, karyomegaly, prominent nucleoli). Similar changes were seen in intestinal glands/crypts of rats and dogs administered PD-0332991 (unpublished) and may represent cell-cycle arrest due to the pharmacology of AG-024322 as cyclin proteins and CDKs are present in the intestinal tract [10, 11, 23].

Diffuse vacuolar degeneration of pancreatic acinar cells, along with increased apoptosis, were observed at the end of the toxicology study in one female at 10 mg/kg, which correlated with markedly elevated levels of serum amylase and lipase. Degenerative changes were not evident in the pancreas of other drug-treated animals and changes in serum amylase or lipase were not observed prior to day 22. Because the study ended on day 22, reversibility of these changes was not assessed. Intravenous administration of a pan-CDK inhibitor (AG-012986) that is structurally dissimilar to AG-024322 to male Sprague–Dawley rats produced single-cell death in exocrine and endocrine (lesser extent) pancreatic cells [13]. Lesions in the exocrine pancreas were associated with increased serum amylase and lipase levels in the rats and immunohistochemistry indicated positive staining for active-caspase 3 in the affected regions of pancreas, indicating that apoptosis had occurred [13]. It is not known whether pancreatic toxicity observed with these compounds is due to direct inhibition of CDKs or off-target effects.

Intravenous administration of AG-024322 at ≥6 mg/kg (dose solution concentrations of 1–1.67 mg/mL) resulted in vascular lesions at the catheter sites consisting of transmural necrosis and thrombosis. The injection site changes were in a process of repair at the end of the study (day 22). Thrombus formation can have potentially serious consequences. In a Phase 2 clinical trial in patients with metastatic renal cancer, intravenous administration of flavopiridol resulted in grade 3 or 4 vascular thrombotic events [18]. These included myocardial infarction, transient neurologic ischemic attacks, deep venous thrombosis, and pulmonary emboli. Although the relationship between flavopiridol administration and thromboses was uncertain, the frequency observed in the trial was higher than expected [18]. In a Phase 1 trial in cancer patients, administration of AG-024322 required central IV access for delivery due to injection site reactions observed initially. These data indicate that intravenous administration of a CDK inhibitor can result in vascular changes/irritation at the injection site, which should be monitored during clinical use.

Plasma drug levels were measured and toxicokinetic parameters calculated in the toxicology study. The minimal toxic dose was 6 mg/kg (72 mg/m^2) which was associated with AG-024322 overall mean plasma AUC(0–24.5) of 5.70 μg h/mL. The no-adverse-effect dose was 2 mg/kg (24 mg/m^2) which was associated with an overall mean plasma AUC(0–24.5) of 2.11 μg h/mL and Cmax of 0.666 μg/mL (day 1). Based upon tumor growth inhibition in murine models, the targeted efficacious AG-024322 plasma AUC(0–24) is 2.66 μg h/mL (at murine dose of 10 mg/kg or 30 mg/m^2), which is similar to the no-adverse effect level (NOAEL) in the toxicology study. The human dose estimated to achieve this exposure is 140 mg/day or 2 mg/kg (74 mg/m^2) when administered as a single 30-min IV infusion. The human Cmax at this dose is estimated to be 0.75 μg/mL, which is similar to Cmax observed at the NOAEL. The estimation of human dose was based upon prediction of pharmacokinetics in man using interspecies allometric scaling of single-dose animal (rat, dog, and monkey) pharmacokinetic data of AG-024322.

In conclusion, intravenous administration of AG-024322 to monkeys at ≥6 mg/kg resulted in pancytic bone marrow hypocellularity, lymphoid depletion, and vascular injury at

the injection site. Renal tubular degeneration occurred at 10 mg/kg. These changes were reversible or in a process of repair following a 17-day recovery period. Pancreatic changes were seen at the end of the reversal phase at 10 mg/kg. Intravenous doses $\geq$12 mg/kg produced central nervous system effects and were not tolerated. The no-adverse-effect dose was 2 mg/kg.

References

1. Arguello F, Alexander M, Sterry JA, Gabriela T, Smith EM, Kalavar NT, Greene JF, Koss W, Morgan CD, Stinson SF, Siford TJ, Alvord WG, Klabansky RL, Sausville EA (1998) Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity in vivo against human leukemia and lymphoma xenografts. Blood 91(7):2482–2490
2. Baughn LB, Di Liberto M, Wu K, Toogood PL, Louie T, Gottschalk R, Niesvizky R, Cho H, Ely S, Moore MAS, Chen-Kiang S (2006) A novel orally active small molecule potently induces G_1 arrest in primary myeloma cells and prevents tumor growth by specific inhibition of cyclin-dependent kinase 4/6. Cancer Res 66(15):7661–7667
3. Fink JR, LeBien TW (2001) Novel expression of cyclin-dependent kinase inhibitors in human B-cell precursors. Exp Hematol 29:490–498
4. Fischer PM, Gianella-Borradori A (2003) CDK inhibitors in clinical development for the treatment of cancer. Expert Opin Investig Drugs 12(6):955–970
5. Fry DW, Bedford DC, Harvey PH, Fritsch A, Keller PR, Wu Z, Dobrusin E, Leopold WR, Fattaey A, Garrett MD (2001) Cell cycle and biochemical effects of PD 0183812, a potent inhibitor of the cyclin D-dependent kinases CDK4 and CDK6. J Biol Chem 276(20):16617–16623
6. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, Albassam M, Zheng X, Leopold WR, Pryer NK, Toogood PL (2004) Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther 3(11):1427–1437
7. Furukawa Y, Kikuchi J, Nakamura M, Iwase S, Yamada H, Matsuda M (2000) Lineage-specific regulation of cell cycle control gene expression during haematopoetic cell differentiation. Br J Haematol 110:663–673
8. Garrett MD, Fattaey A (1999) CDK inhibition and cancer therapy. Curr Opin Genet Dev 9:104–111
9. Jessen BA, Lee L, Koudriakova T, Haines M, Lundgren K, Price S, Nonomiya J, Lewis C, Stevens GJ (2007) Peripheral white blood cell toxicity induced by broad spectrum cyclin-dependent kinase inhibitors. J Appl Toxicol 27:133–142
10. Kim JH, Kang MJ, Park CU, Kwak HJ, Hwang Y, Koh GY (1999) Amplified CDK2 and cdc2 activities in primary colorectal carcinoma. Cancer 85(3):546–553
11. Ko TC, Bresnahan WA, Thompson EA (1997) Intestinal cell cycle regulation. Prog Cell Cycle Res 3:43–52
12. O'Dwyer PJ, LoRussso P, DeMichele A, Gupta V, Barbi A, Dials H, Chen I, Courtney R, Wilner K, Schwartz GK (2007). Phase I dose escalation trial of a daily oral CDK 4/6 inhibitor PD-0332991. J Clin Oncol. 2007 ASCO annual meeting proceed part 1. vol 25. no 18S (abstract 3550)
13. Ramiro-Ibanez F, Trajkovic D, Jessen B (2005) Gastric and pancreatic lesions in rats treated with a pan-CDK inhibitor. Toxicol Pathol 33:784–791
14. Rixe O, Fojo T (2007) Is cell death a critical end point for anticancer therapies or is cytostasis sufficient? Clin Cancer Res 13(24):7280–7287
15. Shapiro GI (2004) Preclinical and clinical development of the cyclin-dependent kinase inhibitor flavopiridol. Clin Cancer Res (suppl) 10:4270s–4275s
16. Sherr CJ (1995) D-type cyclins. Trends Biol Sci 20:187–190
17. Sherr CJ (1996) Cancer cell cycles. Science 274:1672–1677
18. Stadler WM, Vogelzang NJ, Amato R, Sosman J, Taber D, Liebowitz D, Vokes EE (2000) Flavopiridol, a novel cyclin-dependent kinase inhibitor, in metastatic renal cancer: a University of Chicago Phase II consortium study. J Clin Oncol 18(2):371–375
19. Tan AR, Headlee D, Messman R, Sausville EA, Arbuck SG, Murgo AJ, Melillo G, Zhai S, Figg WD, Swain SM, Senderowicz AM (2002) Phase I clinical and pharmacokinetic study of flavopiridol administered as a daily 1-hour infusion in patients with advanced neoplasms. J Clin Oncol 20(19):4074–4082
20. Tetsu O, McCormick F (2003) Proliferation of cancer cells despite CDK2 inhibition. Cancer Cell 3:233–245
21. Zhang CC, Kephart S, McAlpine I, Nonomiya J, Higgins J, Arango ME, Yan Z, Knighton D, Ferre RA, Tikhe J, Verkhivker G, Xu M, Romines W, Palmer C, Park J, Reich S, Chong W, Li L, Los G, Lewis C (2005) AG-024322 is a potent and selective multi-targeted CDK inhibitor with broad spectrum anti-proliferative activity. Proc Am Assoc Cancer Res 46 (abstract 4415)
22. Zhang CC, Troche G, Yan Z, Arango ME, Higgins J, Romero D, Kephart S, McAlpine I, Koudriakova T, Skaptason J, Nonomiya J, Knighton D, Ferre RA, Tikhe J, Verkhivker G, Xu M, Romines W, Palmer C, Park J, Reich S, Los G, Lewis C (2005) AG-024322 is a multi-targeted CDK inhibitor with potent antitumor activity in vivo. Proc Am Assoc Cancer Res 46 (abstract 4413)
23. Zhang T, Nanney LB, Luongo C, Lamps L, Heppner KJ, DuBois RN, Beauchamp RD (1997) Concurrent overexpression of cyclin D1 and cyclin-dependent kinase 4 (Cdk4) in intestinal adenomas from multiple intestinal neoplasia (Min) mice and human familial adenomatous polyposis patients. Cancer Res 57:169–175