

Roscovitine and Other Purines as Kinase Inhibitors. From Starfish Oocytes to Clinical Trials

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ABSTRACT

This article reviews the steps that have led us from very fundamental research on the cell division cycle, investigated with the starfish oocyte model, to the identification of drugs now being evaluated against cancer in the clinic. Among protein kinases activated during entry in M phase, the cyclin-dependent kinase CDK1/cyclin B was initially identified as a universal M-phase promoting factor. It was then used as a screening target to identify pharmacological inhibitors. The first inhibitors to be discovered were 6-dimethylaminopurine and isopentenyladenine, from which more potent and selective inhibitors were optimized (olomoucine, roscovitine, and purvalanols). All were cocrystallized with CDK2 and found to localize in the ATP-binding pocket of the kinase. Their selectivity and cellular effects have been thoroughly investigated. Following encouraging results obtained in preclinical tests and favorable pharmacological properties, one of these purines, roscovitine (CYC202), is now entering phase II clinical trials against cancers and phase I clinical tests against glomerulonephritis. CDK inhibitors are also being evaluated, at the preclinical level, for therapeutic use against neurodegenerative diseases, cardiovascular disorders, viral infections, and parasitic protozoa. This initially unexpected scope of potential applications and the large number and chemical diversity of pharmacological inhibitors of CDKs now available constitute a very encouraging stimulus to pursue the search for optimization and characterization of protein kinase inhibitors, from which we expect numerous therapeutic applications.

Introduction

Phosphorylation of serine, threonine, and tyrosine residues is now recognized as the main intracellular mechanism by which structural proteins and enzymes are regulated at the posttranslational level. The phosphorylation of a protein results from the balance between the activities of kinases and phosphatases (Figure 1). Anomalous phosphorylation is a very frequent feature associated

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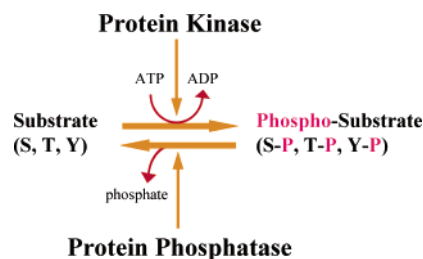


FIGURE 1. The phosphorylation/dephosphorylation reactions. Two opposing types of enzymes, kinases, and phosphatases regulate the phosphorylation level of proteins.

with human pathologies, and abnormal regulation of kinases and phosphatases appears to contribute directly to the onset of some diseases. These observations have encouraged the active search for pharmacological inhibitors of kinases and phosphatases, some of which have now been approved for clinical use.¹ In this article we will review, from a personal historical point of view, our contribution to the discovery, optimization, and use of pharmacological inhibitors of cyclin-dependent kinases (CDKs), a family of enzymes directly involved in cell cycle control, but also in many other physiological processes (see below). This work started in the late 1970's from very fundamental research carried out on marine invertebrate oocytes and embryos (L.M.) and is now leading to clinical tests against cancers (E.R.) and other diseases.

1. Starfish Oocytes

The cell division cycle is a universal process which allows cells to replicate their DNA hereditary message and to distribute it evenly between the daughter cells. Cell division is essential for embryonic development, for growth to the adult stage, and for continuous renewal of dying cells. Although cell division was discovered less than 150 years ago, we now have a good understanding of how this fundamental process is regulated. The very high conservation of cell division regulatory elements throughout evolution has allowed the use of a large variety of cellular models to investigate cell cycle control. Among these models, yeast, amphibian embryos, mammalian cell lines, and marine invertebrate eggs and embryos have

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FIGURE 2. Some of the figures involved in the 2,6,9-trisubstituted purine saga. Top row (left to right): Jaroslav Vesely, Steven Pelech, and Sung-Hou Kim. Middle row: Pierre Guerrier and Laurent Meijer (in submarine with George R. Pettit, seen upside-down!). Lower row: Nathanael Gray and Lionel Rebhun.

each shed light on the intracellular and molecular mechanisms which ensure the completion of cell division in every aspect.

Our contribution to this field started in Maurice Durchon's laboratory in Lille, when L.M. studied oocytes from the lugworm *Arenicola marina*.² In this marine worm, oocytes are naturally arrested in the first prophase stage of meiosis, and a brain hormone (still unidentified) triggers a very rapid and highly synchronous transition to the metaphase stage. This prophase/metaphase transition can be induced in seawater *in vitro*. This was our first contact with the cell cycle and marine invertebrate oocytes. During a postdoctoral stay in 1978–1979, in David

Epel's laboratory (Hopkins Marine Station, Stanford University, Pacific Grove, CA), L.M. investigated the global increase in protein phosphorylation (and associated activation of a "histone kinase"), which was found to occur during the prophase/metaphase transition of oocytes obtained from another marine worm, *Urechis caupo*.³ However, it was in the laboratory of Pierre Guerrier (Figure 2), at the "Station Biologique de Roscoff", that, in the early 1980's, L.M. discovered the starfish oocytes as an ideal model to investigate the functions of protein phosphorylation during the prophase/metaphase transition of the cell cycle.^{4–6} In short, starfish oocytes are very large (160 μm in diameter) and transparent cells which can be



FIGURE 3. Starfish oocytes, a unique source of CDK1/cyclin B and a cellular model of prophase/metaphase transition. Left: Starfish *Marthasterias glacialis*, collected off the Brittany coast. (center) Preparing starfish metaphase oocytes for the purification of CDK1/cyclin B. Isolated ovaries (bottom) are incubated with 1-methyladenine. This hormone triggers the spawning of metaphase oocytes, which are frozen in liquid nitrogen and stored until kinase extraction and purification. Right: Prophase starfish oocytes just prior to entry in metaphase.

isolated in huge amounts (>50 mL of packed cells/mature starfish) and cultured in natural seawater (Figure 3). They are naturally arrested in prophase, and 1-methyladenine, a hormone produced by the follicle cells,⁷ induces the transition to metaphase in vitro. This physiological shift is rapid (<20 min.) and highly synchronous, and these properties greatly contributed to the success of the starfish oocyte model in investigations of the biochemical regulation of the cell cycle prophase/metaphase transition.^{6,8}

2. Protein Kinase Activated during Entry in M Phase

The involvement of protein phosphorylation in cell cycle control came initially from circumstantial evidence, such as the effect of phosphatases and their inhibitors,^{9,10} and the transient activation of protein kinases during specific phases of the cell cycle. During a sabbatical leave in Edwin Krebs' laboratory (University of Washington, Seattle), Steven Pelech (Figure 2) and L.M. extensively characterized the M-phase specific histone kinase which is robustly activated as starfish oocytes transit from prophase to metaphase.^{11,12} Purification culminated in the late 1980's with the identification of the M-phase specific histone kinase as a heterodimer complex constituted of a catalytic subunit, cdc2 (later known as CDK1), and a regulatory subunit, cyclin B.^{13–15}

3. CDK1/Cyclin B Kinase as a Universal M-Phase Promoting Factor

How the prophase/metaphase transition is controlled had been studied by many researchers using various models, but mammalian cell fusions and oocyte cytoplasm transfers provided the first clues in the late 1960's—early 1970's. These experiments indeed revealed the existence of an intracellular "maturation-promoting factor" (later called M-phase promoting factor), or MPF, which appears in the cytoplasm as cells progress into metaphase. Purified MPF

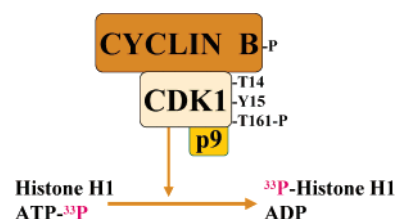


FIGURE 4. The CDK1/cyclin B screening assay. Active CDK1/cyclin B is dephosphorylated on Thr14 and Tyr15 but phosphorylated on Thr161 and cyclin. The complex is purified by affinity chromatography on recombinant p9^{CKShs1}. Its activity is assayed with radiolabeled ATP and histone H1, in the presence of various concentrations of the compounds to be tested. IC₅₀ values are determined from dose–response curves.

triggers the prophase/metaphase transition when microinjected in prophase-arrested oocytes.^{16,17} Its presence in all dividing cells, and its complete lack of species specificity pointed toward a universal factor responsible for entry into M-phase. However, it took over 20 years before MPF was found to be constituted, as the M-phase specific histone H1 kinase, of CDK1 and cyclin B.¹⁸

4. Screening for Pharmacological Inhibitors of CDK1/Cyclin B

The importance of MPF/M-phase specific kinase in cell cycle regulation led us to imagine that any inhibitor of this factor would be a potent antimitotic agent and that this property might be useful to treat cancers. We therefore decided to take advantage of starfish oocytes as an abundant source of native and highly active CDK1/cyclin B, free of monomeric CDK1, monomeric cyclin B, or inactive complex (Figure 3). Active CDK1/cyclin B was found to be easily purified to near homogeneity by affinity chromatography on p9^{CKShs1} or p13^{suc1} sepharose beads.^{15,18} A simple kinase assay was set up and optimized (Figure 4) which allowed the detection of potential inhibitors.¹⁹ Its selectivity was initially evaluated with a series of chemotherapeutic agents used in the clinic, all of which

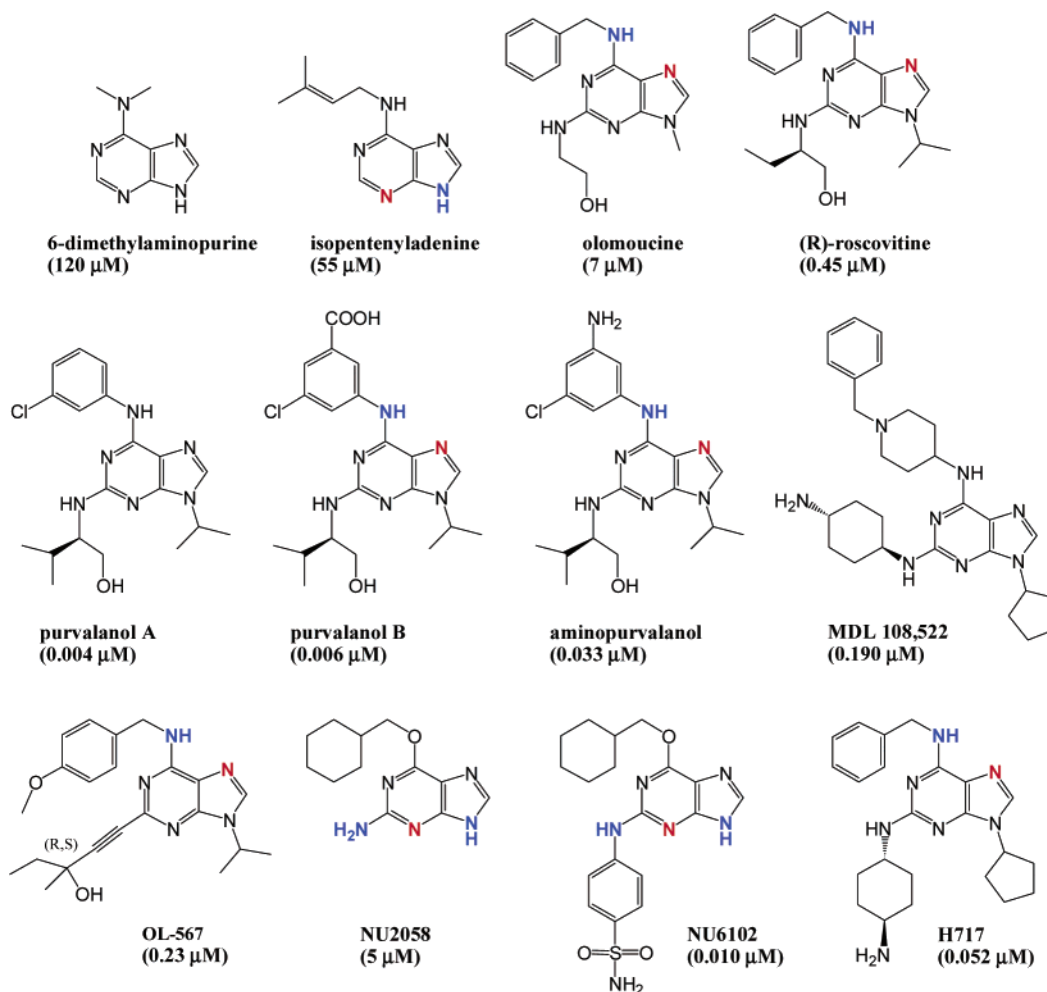


FIGURE 5. Structure of a selection of CDK inhibiting purines. For those compounds which have been cocrystallized with CDK2, the two or three atoms which interact with the backbone nitrogen and oxygen atoms of Leu83 and Glu81 are shown in red (H-bond acceptors) and blue (H-bond donors). IC_{50} values for CDK1/cyclin B are provided under each compound (CDK2/cyclin E for MDL108,522).

were inactive in the assay, and with staurosporine, a nonspecific inhibitor of protein kinases, which was quite potent on CDK1/cyclin B.¹⁹

5. The Discovery of the First Inhibitory Purines

Besides staurosporine, 6-dimethylaminopurine (6-DMAP) (Figure 5) was frequently used in the 1980's as a "non-specific kinase inhibitor". 6-DMAP was initially discovered by Lionel Rebhun (Figure 2) as a puromycin analogue which, in contrast to the parent structure, was unable to inhibit protein synthesis. However, 6-DMAP potently inhibited mitosis of the sea urchin embryo.²⁰ The mechanism of action of 6-DMAP on cell division remained a mystery until its inhibitory properties on M-phase specific phosphorylation and histone H1 kinase were discovered.^{21,22} This effect was confirmed in the early screening assays and the IC_{50} value was determined to be $120 \mu\text{M}$ (!).¹⁹ While screening through a few related purines, we found that isopentenyladenine (Figure 5) was slightly more active (IC_{50} : $55 \mu\text{M}$).¹⁹ Unfortunately both compounds were of limited interest because of their poor selectivity. Isopentenyladenine is a widely studied plant hormone (cytokinin), and this was the reason for the initial contact

with Jaroslav Vesely (Figure 2) and Miroslav Strnad. During a brief but labor-intensive stay in Roscoff, Jaroslav Vesely and L.M. tested all available isopentenyladenine analogues and other substituted purines on CDK1/cyclin B. The results remained frustrating until the discovery of a modestly active inhibitor, 2-hydroxyethylamino-6-benzylamino-9-methylpurine (Figure 5), which, for intralaboratory conversation convenience, we renamed olomoucine (from Olomouc, the home of Jaroslav's University in the Czech Republic).²³ This compound was commercially available as an antagonist of the plant cytokinin 7-glucosyltransferase²⁴ initially synthesized in Canberra, Australia, by David Letham, who, with Lionel Rebhun, can be considered as the (involuntary) grandfathers of purines as kinase inhibitors! Two features were particularly interesting with olomoucine, an improved efficiency (IC_{50} : $7 \mu\text{M}$) and, in contrast to 6-DMAP and isopentenyladenine, an unexpected selectivity for CDKs and, to a lesser extent, for MAP kinases. This was clearly against the (then) current dogma stating that no selectivity would ever be obtained with inhibitors targeting the ATP-binding pocket of kinases. Olomoucine was indeed found to inhibit CDK1 by competing with ATP binding to the enzyme. A struc-

ture–activity study with 81 purines showed that the kinase inhibitory properties were limited to the 2,6,9-trisubstituted purine sub-family.²³ Interestingly, we found, for reasons to be discovered later (see below), that a methyl substitution on position 7 (iso-olomoucine) led to inactivation of the inhibitor, while a substitution with isopropyl on position 9 was optimal.²³

6. Optimization by Classical Medicinal Chemistry and by Combinatorial Chemistry

These early optimization efforts were continued by classical medicinal chemistry in collaboration initially with Miroslav Strnad and later with Michel Legraverend (Institut Curie, Orsay). Among purines efficient in the sub-micromolar range, we selected 2-(*R*)-(1-ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine for further investigation^{25,26} (Figure 5). As a language convenience and by reciprocity, the chemical name was abandoned for an easier name, roscovitine. Roscovitine was potent at inhibiting CDK1/cyclin B (IC₅₀: 0.450 μ M) and still quite selective. (*R*)-roscovitine was slightly more efficient than (*S*)-roscovitine. Roscovitine generated a wide interest and was the start-point of an important combinatorial chemistry effort to generate even more efficient purine analogues. These efforts were also guided by the cocrystal structures of CDK2 with olomoucine and roscovitine (see below). They ultimately led Nathanael Gray (Figure 2), in Peter Schultz's laboratory in Berkeley, to the identification of purvalanols (Figure 5) which were very potent in vitro (IC₅₀ in the 0.004–0.04 μ M range) and highly selective.^{27–29} Many other related and potent purines were synthesized in numerous laboratories and confirmed the efficacy of 2,6,9-trisubstituted purines at inhibiting CDKs (refs 30 and 31 and reviewed in 32).

7. Cocrystallization with CDK2

Stimulated by discovery of the (relative) potency and the selectivity of olomoucine²³ and the crystal structure of CDK2 that had been solved³³ a few months earlier by Sung-Hou Kim (Figure 2) in Berkeley, we prompted him to attempt cocrystallizing olomoucine and isopentenyladenine with CDK2. He responded with great enthusiasm, and two months later, he had the cocrystal structures solved!³⁴ His results were accompanied by two surprises. First, although olomoucine occupied (as expected) the ATP-binding pocket of CDK2, its purine ring and that of ATP were not at all orientated in the same manner. Second, isopentenyladenine was positioned in yet a third orientation. Later, roscovitine,²⁵ purvalanols,^{27,29} and other purines^{31,32,35} were cocrystallized with CDK2. All these purines were orientated like olomoucine in the ATP-binding pocket of the kinase (Figure 6). In contrast, the apparently related O⁶-cyclohexylmethylguanine (NU2058) and its optimized derivative NU6102 (Figure 5) were found to bind to CDK2 in a different way than olomoucine/roscovitine/purvalanol.³⁶ The purine/CDK2 cocrystal structures generated a lot of interest and, complemented with extensive structure–activity relationship studies, greatly

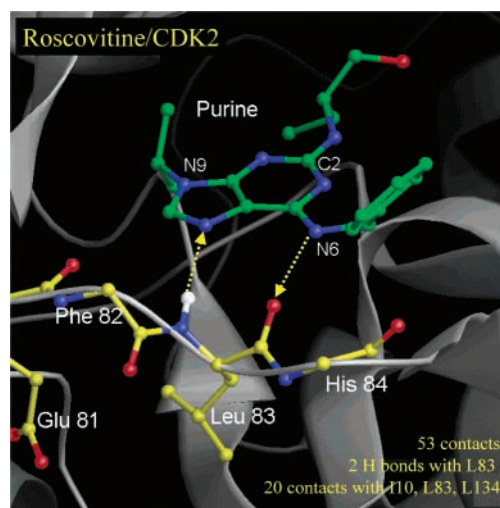


FIGURE 6. CDK2/roscovitine co-crystal structure.

stimulated the search for and rational optimization of new CDK inhibitors. Since olomoucine, more than 20 pharmacological inhibitors have been cocrystallized with CDKs (reviews in refs 37–39). Despite a surprising chemical diversity, they are all flat, hydrophobic heterocycles which bind in the ATP-binding pocket through 2–3 hydrogen bonds with the backbone atoms of Leu83 and Glu81 in the active site and hydrophobic and van der Waals interactions.

8. Selectivity: Investigation with Panels of Enzymes and by Affinity Chromatography

A frequently asked question about kinase inhibitors relates to their selectivity, especially in view of their molecular mechanism of action (competition with ATP) which, intuitively, does not seem to be very favorable for high selectivity. The selectivity issue is usually approached by testing the compounds on a panel of purified, usually recombinant kinases, a time-consuming and unsatisfying approach (considering that only a small fraction of the 850+ kinases present in the human genome can be evaluated, and that potential nonkinase targets are not tested!). Nevertheless, this approach showed that 2,6,9-trisubstituted purines were rather selective, essentially inhibiting CDK1, CDK2, CDK5, CDK7, and CDK9,^{23,26,27,29–41} but not CDK4 and CDK6. In addition to CDKs, the MAP kinases Erk1 and Erk2 were sensitive to 2,6,9-trisubstituted purines, although at much higher concentrations. In the absence of potent MAP kinase inhibitors, olomoucine was cocrystallized with Erk2.⁴²

As an alternative approach to purify and identify the targets of our purine CDK inhibitors, we developed an affinity chromatography method with Nathanael Gray.⁴³ Purines were first immobilized through a linker to sepharose beads. Extracts of various cell types and tissues were then incubated with this matrix, and after stringent washing of the beads, the bound proteins were resolved by SDS-polyacrylamide gel electrophoresis and identified by microsequencing of internal peptides. This global approach confirmed that CDK1, CDK2, CDK5, and CDK7 were targets of purvalanols.^{41,43} It revealed that Erk1 and

Erk2 are also important targets.^{43,44} Unexpectedly, this method showed that parasitic casein kinase 1 is a major, sometimes unique, target of purvalanol in unicellular parasites (*Plasmodium falciparum*, *Toxoplasma gondii*, *Leishmania mexicana*, and *Trypanosoma cruzi*).⁴³ The success of this method was a stimulus to extend it to other CDK inhibitors such as paullones⁴⁵ and indirubins (in preparation).^{39,46}

Undoubtedly new techniques will be developed in the future to determine the range of targets of any given inhibitor. Understanding what a pharmacological compound is targeting is of great help to improve its bioactivity but also to anticipate and reduce its side effects.

9. Cellular Effects of 2,6,9-Trisubstituted Purines

2,6,9-Trisubstituted purines have been used in a large variety of cellular models. Reviewing the results of these studies is beyond the scope of this Account. We can summarize the data by saying that the CDK inhibitory purines have four major actions.

First they clearly inhibit proliferation leading to cell cycle arrest in either G1 or G2, depending on the model and the conditions. For a few among many examples, see references.^{44,47–50} These antiproliferative effects are essentially due to CDK2 and CDK1 inhibition, but an effect on Erk1/2 has been demonstrated.⁴⁴

Second, they induce apoptosis in mitotic cells, usually when combined with another treatment. For example, roscovitine and olomoucine were found to synergize with a farnesyltransferase inhibitor to induce apoptosis of human cancer cell lines.⁵¹ This situation was also recently illustrated with purvalanol A and taxol.⁵² Microtubule stabilization with taxol followed by CDK1 inhibition with purvalanol A resulted in massive apoptosis in HeLa cells. Treatment with either taxol or purvalanol A alone and dual treatment but in the reverse order (purvalanol A followed by taxol) were inefficient, demonstrating an impressive ordered cooperativity between the two drugs. These effects were reproduced in animal models (see below).

Third, they induce differentiation in a few models.⁴⁷ For example, the *in vitro* differentiation of murine erythroleukemia cells was triggered by the combined sequential inhibition of CDK2 (with roscovitine) and CDK6 (not CDK4) (with p16^{INK4A}). The reverse sequence of inhibition was ineffective.⁵³

Fourth, they also protect cells from apoptosis by mechanisms yet to be identified (review in refs 39 and 54). Among many examples, purine CDK inhibitors prevent cAMP-induced apoptosis in rat leukemia cells,⁵⁵ etoposide-induced apoptosis in rat fibroblasts,⁵⁶ and HIV-induced cell death in HIV-induced syncytia.⁵⁷

10. Preclinical Tests

Following extensive testing, (*R*)-roscovitine (under the code CYC202) was selected for preclinical and clinical testing by Cyclacel Ltd., a company located in Dundee, Scotland, and directed by Professor Sir David Lane.

Interestingly, CYC202 offers a good oral bioavailability making oral treatment possible for extended periods of time at active plasma concentrations. On the basis of experiments performed in a variety of cultured cancer cells, IC₅₀ values ranged from 7.9 to 30.2 μ M with about 80% apoptosis at 20 μ M for 24h. Optimal cell death induction occurred between 8 and 24 h of CYC202 exposure, while the antiproliferative effects of (*R*)-roscovitine were not increased for exposures beyond 24 h.⁵⁸ Therefore, it was speculated that the targeted daily plasma concentration for significant *in vivo* activity would be 5–20 μ M. Interestingly, at these concentrations in mice, CDK2-dependent phosphorylation of Rb was abrogated for 96 h after the last dose in the tumor and peripheral blood lymphocytes, suggesting that Rb-phosphorylation could be used as a surrogate endpoint for the drug activity in future clinical trials.⁵⁸ On the basis of experiments performed in human cancer cell lines that showed time- and dose-dependent cytotoxicity of CYC202, the antitumor activity was investigated in nude mice and rats bearing a variety of human tumor xenografts using several protracted oral, intraperitoneal, and intravenous infusions (100–500 mg/kg \times 3 (tid)/day, duration 10 days).

The antitumor activity of CYC202 was investigated in athymic mice bearing human tumor xenografts including MESSA-DX uterine and LoVo colon carcinomas. Doses ranging 100–200 mg/kg tid were given orally for 21 days. In most cases, treatment with CYC202 was associated with limited tumor shrinkage but consistently slowed MESSA-DX tumor growth. As an example, a reduction in growth rate of 45% was observed using intraperitoneal administration in the Lovo colorectal xenograft.

Interestingly, in mice bearing MESSA-DX uterine xenografts, 200 mg/kg 3 times daily for 10 days resulted in 35% tumor growth, while 500 mg/kg 3 times daily for 4 days induced 62% reduction in tumor growth.⁵⁸ This suggested that maximum antitumor growth effects would occur when CYC202 is given at high dose over a short period of time. In addition to cytostatic effects due to cell cycle arrest when given at low dose for prolonged durations, CYC202 has the capability of driving cancer cells into apoptosis when given at high concentrations.

Laboratory results also showed that CYC202 does not cause Lovo cells to accumulate significantly at any point in the cell cycle suggesting that such a CDK inhibitor would not hold cycling cancer cells in stasis making possible the use of CYC202 in combination with chemotherapy and radiotherapy.

Recently, the sequential taxol-purvalanol A treatment was evaluated in a mouse xenograft model.⁵² MCF-7 cells were injected subcutaneously in immunocompromised SCID mice, giving rise to exponentially growing tumors. When mice were treated with taxol alone or purvalanol A (20 mg/kg) alone, no effect on tumor growth was observed and all mice died by 21–23 days. In contrast, sequential administration of taxol followed by purvalanol A resulted in tumor growth suppression and improved animal survival and even indefinite survival of all animals if the sequential treatment was continuous. The reverse com-

bination, purvalanol A followed by taxol, did not reduce tumor growth, and in fact enhanced tumor expansion. These encouraging results strongly support the evaluation of combination therapy to optimize the antitumor properties of CDK inhibitors.

A third purine, MDL108,522 (Figure 5), was also found to display a potent antiproliferative effect in a xenograft PC-3 prostate tumor model in mice, when dosed orally at 3 mg/kg.⁵⁹

11. The First Clinical Trials against Cancer

Dose escalation studies in mice and rats suggested that a daily oral dose of 200 mg CYC202 would represent about 1/10 of the maximal tolerated dose in human. Furthermore, pharmacokinetic analysis in mice showed that a single dose of 500 mg/kg (corresponding to about 2750 mg/m² in human) allowed the plasma concentration to reach levels above 10 μ M for 24 h.

From animal experiments, it appeared that the dose was essential for maximal antitumor activity. Although durations of exposure of more than 24 h were associated with higher antitumor effects, schedules using duration of exposure for more than 5 days were not associated with significantly better activity. A preliminary phase I clinical study showed that fixed doses of up to 200 mg were well tolerated and led to a drug exposure in serum that was sufficient for enzymatic effects. Therefore, on the basis of those preclinical and preliminary clinical data, two phase Ib trials were initiated with the aim of defining the toxicity profile, the maximal tolerated dose, and pharmacokinetics of oral CYC202 in patients with advanced solid tumors. CYC202 was administered at fixed doses twice daily for either 5 or 7 consecutive days every 3 weeks. The starting dose was 100 mg 2 \times daily. Further dose escalation was based on toxicity at cycle 1 with 1–3 patients per dose level and 100% dose-escalation until mild toxicity and 25% dose escalation in case of moderate toxicity. So far, total daily doses of up to 2500 mg have been explored. CYC202 was well tolerated up to 2000 mg/day. Preliminary pharmacokinetic data showed good oral bioavailability with inter/intra-patient variability. Sustained 6-month tumor stabilization was observed in two patients. Further dose escalation is ongoing to define the maximum tolerated dose and the recommended dose of CYC202 in human using the 5 \times and 7 \times daily schedules and longer durations of exposure ranging 10–60 days. In addition, future studies will be considered combining CYC202 with other cytotoxic and cytostatic agents to optimize its clinical antitumor effects.

12. A Large Variety of Potential Applications

CDK1 was initially discovered as *cdc2* in yeast for its role in cell cycle regulation. Other CDKs, such as CDK2, CDK3, CDK4, CDK6, and CDK7, also play essential functions in cell cycle control. However, CDKs are involved in other processes such as apoptosis (CDK1, CDK5), neuronal functions (CDK5, CDK11), and transcription (CDK7, CDK8, CDK9). The human genome appears to encode about 13

CDKs and 25 cyclins, many of which have no identified function.³⁹ We can thus expect quite a large variety of effects, and therefore of applications, from selective inhibitors of CDKs. Their potential applications have been recently reviewed.³⁹ Beside cancers, they include neurodegenerative disorders (Alzheimer's and Parkinson's disease, stroke, ALS), cardiovascular diseases (restenosis), viral infections (HIV, Herpes, cytomegalovirus, papillomavirus), various unicellular parasites (*Plasmodium*, Trypanosomes, *Leishmania*), and use in in vitro reproduction and cloning techniques. These potential applications are being currently investigated at the cellular and animal levels. Following encouraging results obtained in a rat model of nephritis,⁶⁰ CYC202 is now entering phase I clinical trials for glomerulonephritis, a group of inflammatory kidney diseases, that can cause end-stage renal disease.⁶¹

Conclusion

Fundamental research on the mechanisms regulating the cell cycle has led to the discovery of cyclin-dependent kinases as key regulators. These enzymes have been turned into mechanism-based screening targets which led to the discovery of a large variety of chemically diverse, small molecular weight, pharmacological inhibitors. These compounds are currently being evaluated as potential drugs in a growing number of therapeutic areas. This scientific adventure nicely illustrates the famous phrase of Louis Pasteur: "There is no applied research, there are only applications of research".

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