

Advances on Cyclin-dependent Kinases (CDKs) as Novel Targets for Antiviral Drugs

L. M. Schang*

Department of Biochemistry and Department of Medical Microbiology and Immunology, Signal Transduction Research Group, Molecular Mechanisms of Growth Control Research group, University of Alberta, 315C Heritage Medical Research Center, Edmonton, Alberta T6G 2S2, Canada

Abstract: Although targeting viral proteins has led to many successful antiviral drugs, these antivirals have certain limitations. They rapidly select for resistance, tend to be active against only a few related viruses and the proteins of a pathogen must be characterized before such drugs can be developed. Consequently, a long period is required from the identification of a new pathogen to the development of relevant antivirals, a major concern for emerging diseases. Cellular proteins are now considered as potential targets for antivirals. Drugs that target cellular proteins required for several viral functions might not easily select for drug-resistance. They may also be active against a variety of unrelated viruses, which commonly require the same cellular proteins, and against viral strains resistant to conventional antiviral drugs. These antivirals could be promptly tested against emerging viruses because even distantly related viruses commonly require the same cellular proteins.

Cellular cyclin-dependent kinases (CDKs) are required for replication of many viruses and specific pharmacological CDK inhibitors (PCIs) are proving to have surprisingly few negative side effects in clinical trials (against cancer). PCIs inhibit replication of wild-type and multi-drug resistant strains of HIV, HSV-1, HSV-2, HCMV, EBV and VZV. Two PCIs, roscovitine and flavopiridol, were recently proven active in a mouse model of HIV-induced nephropathy. Because the antiviral mechanisms of PCIs require no viral proteins, mutations in viral genes may not easily overcome inhibition by these drugs. In fact, no PCI-resistant viral mutant has been reported. PCIs are scheduled to enter clinical trials as antivirals in 2005.

Key Words: CDK, PCI, protein kinase inhibitors, HIV, herpesvirus, antiviral drugs, gene transcription, gene expression.

CYCLIN-DEPENDENT KINASES (CDKs) AS POTENTIAL TARGETS OF NOVEL ANTIVIRAL DRUGS

Because viruses are strongly selected to replicate with small genomes, they have evolved to replicate employing many cellular proteins. For example, replication of most viruses that use DNA as a template for genome synthesis require cellular cyclin-dependent kinases (CDKs). CDKs are a family of well conserved serine/threonine protein kinases that usually phosphorylate a serine or threonine followed by a proline and which are only active in complexes with regulatory subunits called cyclins [1]. The human genome encodes 13 putative CDKs and 25 putative cyclins [2]. To date, 10 CDKs have been shown to interact with cyclins or similar proteins. CDK1, 2, 3, 4, 6 and 7 are involved in regulation of the cell cycle. CDK7 is also involved in regulation of transcription, as are CDK8 and 9. CDKs 5 and 11 are involved in neuronal functions. CDK2, 5, 6 and 9 are also involved in cell differentiation and CDK1, 2, 4, 5, 6 and 11 participate in apoptosis [recently reviewed in 3]. CDKs likely participate in other cellular functions.

Each CDK is regulated preferentially by proteins, usually cyclins. CDK1 interacts with cyclins B or A, whereas CDK2 interacts with cyclins A or E. CDK3 interacts with yet

unknown proteins, whereas CDK4 and CDK6 interact with the D-type cyclins. CDK5 interacts with a non-cyclin protein, known as p25 or p35. CDK7 interacts with cyclin H and Mat3 and CDK8 interacts with cyclin C. Lastly, CDK9 interacts with cyclins T and K.

PHARMACOLOGICAL CDK INHIBITORS (PCIs)

The realization that upregulation of subsets of CDKs would lead to unrestricted cell-cycle progression, and therefore could play major roles in carcinogenesis, stimulated the search for specific PCIs. This search resulted in the discovery of 6-aminopurines as semi-specific, but not very potent, CDK inhibitors, which then triggered a search for other purine-related PCIs. The first specific and relatively potent PCI discovered was the 6-benzylamino-2-(2-hydroxyethylamino)-9-methylpurine (olomoucine- Olo) [4], which had originally been synthesized as an inhibitor of cytokinin 7-glucosyl-transferase (an enzyme that inactivates growth promoting hormones in plants) [5]. Elegant structure activity relationship (SAR) studies soon led to the discovery of the more potent, yet equally specific, 2-(1-D,L-hydroxymethylpropylamino)-6-benzylamino-9-isopropylpurine (roscovitine - Rosco) [6]. Further SAR analyses and combinatorial chemistry led to the discovery of other second and then third generation PCIs, including the 6-[(3-Chloro)anilino]-2(1R)-(isopropyl-2-hydroxyethylamino)-9-isopropylpurines (purvalanols) [7]. The flavonoid 4H-1-Benzopyran-4-one, 2-(2-chlorophenyl)-5,7-dihydroxy- 8-(3-

*Address correspondence to this author at the 315C Heritage Medical Research Center, Edmonton, Alberta T6G 2S2; CANADA. Tel:(780) 492-6265; Fax:(780) 492-3383; E-mail: luis.schang@ualberta.ca

hydroxy-1-methyl-4-piperidinyl)-hydrochloride, (-)-cis-(flavopiridol-Flavo), which was previously known to inhibit cell replication, was found simultaneously with the discovery of Olo to be a specific inhibitor of CDKs [8]. Many other non-purine related PCIs were soon developed, including other flavonoids, paullones, indirubines and aloisines [9-12]. The development of novel PCIs still continues and new compounds are continuously added to this group. The structures of the PCIs most relevant to this review are presented in Fig. (1).

PCIs are chemically diverse, low molecular weight (<600 Da) flat, hydrophobic heterocycles, see Fig. (1). No PCI has been shown to compete with the peptide co-substrate of the target CDKs, whereas most PCIs compete with the ATP co-substrate. As an exception, Flavo inhibits CDK9 either in a non-competitive manner or as a result of high affinity binding that is too tight to be outcompeted by physiologically relevant concentrations of ATP [13].

PCIs can be classified according to their specificities as non-specific, pan-specific, oligo-specific, or mono-specific. Non-specific PCIs inhibit CDKs and other protein kinases with similar potencies. Pan-specific PCIs preferentially inhibit CDKs over other non-CDK kinases, but fail to discriminate among different CDKs. Oligo-specific PCIs preferentially inhibit a subset of CDKs and can be further subdivided into “transcription specific” (preferentially inhibit CDKs involved in transcription), or “cell cycle specific” (inhibit CDKs involved in cell cycle regulation preferentially) [14-16]. No mono-specific PCIs have yet been discovered.

PURINE-TYPE PCIs

Compounds containing a purine-like ring are arguably the best characterized oligo-specific PCIs. This group includes Rosco, Olo, the purvalanols and related compounds. Although the specificities of each of these drugs varies slightly, purine-type PCIs as a group preferentially inhibit CDK1, 2, 5 and 7, but not CDK4, 6, or 8 [4,17-19]. They also inhibit ERK1, ERK2 and DYRK1a, although only at

concentrations approximately 50- to 1,000-fold higher than those that inhibit CDKs. Purine-type PCIs fail to inhibit 53 other protein kinases, phosphatases, DNA polymerases, ATPases, or topoisomerases [4,18-20]. Olo and Rosco appear to inhibit CDK2 activity also *in vivo*, as first suggested by immunokinase assays [21,22] and recently confirmed by an elegant vital luminescence system [23]. These drugs may also inhibit CDK1 activity *in vivo*, as suggested by immunokinase assays [21,22]. *In vivo* inhibition of CDK2 and 1 is further supported by the abilities of purine-type PCIs to inhibit cell cycle progression at G1/S and G2/M, when CDK2 and CDK1 are required [18,21,22,24-26]. Purine-type PCIs also inhibit cellular DNA synthesis and phosphorylation of known CDK substrates [18,19,27-29]. High, cell-type dependent, concentrations of PCIs are required to achieve biological effects, as expected from their competitive mechanisms of action and the high intracellular concentrations of ATP. For example, 10 to 180 μM Rosco has been required in different cell types to yield the biological responses expected from inhibition of the target CDKs [30-37]. Lower concentrations appear to be usually required in non-dividing or primary cells than in transformed or rapidly dividing cells.

NON-PURINE-TYPE PCIs

One of the best characterized PCIs is the pan-specific Flavo. Flavo is a semi-synthetic flavonoid derived from rohitukine, a natural alkaloid originally isolated from leaves and stems of *Amoora rohituka* [38] and later from stem bark of *Dysoxylum binectiferum* [39] [recently reviewed in 40]. Both *A. rohituka* and *D. binectiferum* are tropical trees of the Meliaceae family, autochthonous from India, China and other parts of Asia. Flavo was originally submitted to the National Cancer Institute anti-cancer screen as a potential inhibitor of epidermal growth factor receptor (EGFR) or protein kinase A (PKA) [recently reviewed in 41]. However, Flavo was found to inhibit cell replication at far lower concentrations than those required to inhibit EGFR or PKA (IC_{50} 66 nM versus 21 μM and 122 μM , respectively). Further analyses of

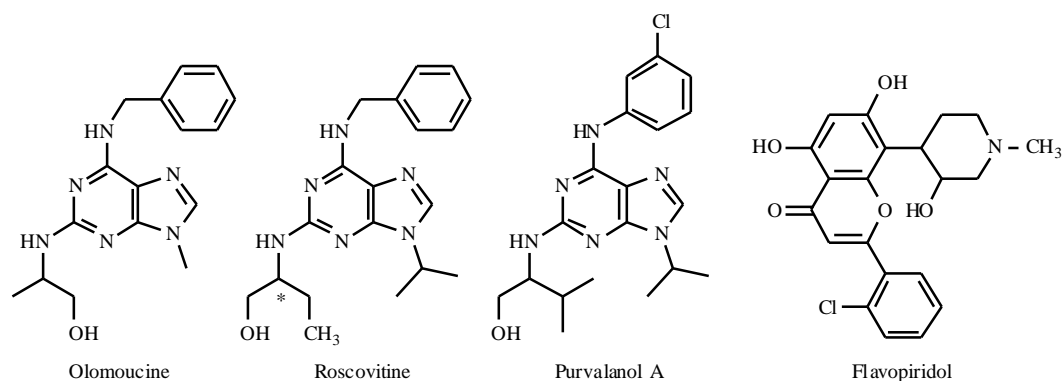


Fig. (1). Chemical structures of three purine-type PCIs, 6-benzylamino-2-(2-hydroxyethylamino)-9-methylpurine (*olomoucine*), 2-(1-D,L-hydroxymethylpropylamino)-6-benzylamino-9-isopropylpurine (*roscovitine*), 6-[(3-Chloro)anilino]-2(1R)-(isopropyl-2-hydroxyethylamino)-9-isopropylpurine (*purvalanol A*) and a non-purine-type PCI, the flavonoid 4H-1-Benzopyran-4-one, 2-(2-chlorophenyl)-5,7-dihydroxy- 8-(3-hydroxy-1-methyl-4-piperidinyl)-, hydrochloride, (-)-cis- (*flavopiridol*). The asterisk in the structure of roscovitine indicates the chiral center, which determines the (*R*)- and (*S*)- isomers. Purified (*R*)-roscovitine (Cyc202, seleclitib) is being developed as anticancer and potential antiviral drug by Cyclacel (www.cyclacel.com).

the specificity of Flavo indicated that at nanomolar concentrations it inhibits CDK1, 2, 4, 9 and likely 6, but not CDK7 [42-46]. As other PCIs, Flavo competes with ATP for binding to CDK1 and 4 and likely also to CDK2, since it binds to the ATP binding pocket [47]. Although Flavo may also bind to the ATP binding pocket of CDK9, it inhibits this kinase non-competitively [44]. Flavo inhibits several non CDK kinases, such as GSK-3, GSK3B is the example of a kinase and glycogen phosphorylase (a and b forms) [20,48,49], binds to cytosolic aldehyde dehydrogenase and duplex DNA and stimulates the ATPase activity of MRP1 [50-53].

Flavo inhibits cell cycle progression *in vivo*, perhaps by inhibiting CDK1 and 2 [23,42,54,55], and induces or represses apoptosis, probably by inhibiting CDK1, 2 or 9 [56]. Similar concentrations of Flavo suffice to inhibit cell-cycle progression in the presence of millimolar concentrations of ATP (IC_{50} 66 nM) and to inhibit any purified CDK in the presence of micromolar concentrations of ATP (IC_{50} 8-40 nM, depending on the specific CDK). Flavo may be efficiently concentrated in intracellular compartments, or it may inhibit cell-cycle progression as a result of inhibition of yet unidentified kinases. It was recently confirmed that Flavo inhibits CDK2 in cultured cells and in mice [23], but whether such inhibition mediates Flavo effects on the cell-cycle was not tested. Flavo also inhibits *in vitro* transcription by RNA PII [44] and at high concentrations (1 μ M) it inhibited expression of 5.6% of genes in cultured cells (284 of 5,032 genes) [57]. These inhibitory effects appear to be direct results of inhibition of CDK9 and resemble the effects of global transcriptional inhibitors, such as actinomycin D and DRB [58].

TOXICITY STUDIES OF PCIs IN PRE-CLINICAL AND CLINICAL TRIALS

CDKs have become especially attractive as targets for antiviral drugs since September 2003, when it was demonstrated that CDK2 is not essential for mammalian cells. Knock outs of CDK2 in mice indicated that, contrary to expectations, mammals show a single phenotypic defect in the complete absence of CDK2, inhibition of meiosis (and therefore, of gametogenesis) [59,60]. These results might have been anticipated in light of previous reports which indicated that mice knocked-out in the genes encoding CDK4 or E- or D type cyclins were also viable, although they too had some phenotypic defects [61,62]. It is perhaps surprising that PCIs had already been shown to be apparently well tolerated by animals and humans before certain CDKs were shown to be non-essential for mammals. In retrospect, the apparent safety of PCIs observed in pre-clinical and clinical trials [63-85], which was so puzzling when reported, may have been a consequence of the non-essential nature of certain CDKs.

The first PCI evaluated *in vivo* was Flavo, which has been tested in animal models, has completed phase I and is currently in phase II clinical trials (all against cancer). In three different studies, Flavo had minimal negative side effects for animals at doses that inhibited growth of transplanted tumors (up to 100% tumor stasis) and even could induce remissions [63-65]. Flavo was also apparently well tolerated in phase I human clinical trials, in which it

was administered *i.v.* as a continuous infusion for 72 hours every 2 weeks. Plasma concentrations of 300 to 500 nM were achieved in these studies, which is higher than the concentrations required *in vitro* to repress cell cycle progression and viral replication, and lower than those required to inhibit cellular transcription [44,63,70,71,82,83,86,87]. The most common side effect of Flavo was fatigue, whereas the major side effect was secretory diarrhea that responded to standard treatments [68,69,71]. Although continuous *i.v.* infusion of Flavo resulted in a high rate of thrombotic events in two phase II clinical trials, daily *i.v.* bolus doses of Flavo did not induce similar negative effects in another more recently completed phase II trial [73,88,89]. No major immuno-suppressive effects of Flavo have been reported in animals or humans and no negative side effects reported for Flavo have been linked to inhibition of CDKs [69,71]. Flavo has shown very limited anti-tumor activity in patients with different cancers [69,71-73,77,82], just sufficient to indicate that anti-proliferative concentrations of Flavo can be used in humans in the absence of major negative side effects.

Like Flavo, Rosco also decreased cell proliferation at concentrations that were well tolerated in several animal models. For example, Rosco administered to rats at doses of 2.8 mg/Kg per day (*i.p.*) for five days decreased mesangial cell proliferation without obvious negative side effects. Diarrhea developed at doses of 3.0 mg/Kg per day or higher, a far lower concentration than in all other studies reported. In another report, Rosco was non-toxic for mice in doses of up to 20.0 mg/Kg *i.v.*, 2,000 mg/Kg *p.o.*, or 200 (3 times a day for 10 days), to 500 (3 times a day for 4 days) mg/Kg *p.o.* [85]. These last two treatments were effective at inhibiting tumor growth, indicating that Rosco is well tolerated at concentrations that have anti-proliferative effects (i.e., concentrations that presumably inhibit the target CDKs). Such concentrations are sufficient to inhibit viral replication *in vitro* [90].

In phase I clinical trials, Rosco was well tolerated orally up to 1,600 mg/day [78,81], or 2,500 mg/day for 5 days every 3 weeks. The maximum tolerated dose was 3,200 mg/day with vomiting as a limiting toxicity [78]. In these trials, plasma concentrations higher than those that inhibit HIV replication *in vitro* were reached in humans without obvious negative side effects [78]. The purified (*R*)-isomer of Rosco (Cyc202, seliciclib) is currently in phase II trials against glomerulonephritis, breast cancer and small cell lung cancer (www.cyclacel.com).

Since PCIs are apparently well tolerated (in animal experiments and human clinical trials against cancer), one could speculate that non-toxic concentrations of these drugs may soon be tested as clinical antivirals and, in fact, PCIs are scheduled to enter clinical trials as antivirals in 2005 (www.cyclacel.com).

ANTIVIRAL ACTIVITIES OF PCIS *IN VITRO*

Replication of HIV, HTLV, KSHV, HCMV, VZV, HSV-1, HSV-2, EBV, adeno- and other viruses require CDK activities. Expectedly, viral replication functions of these viruses are efficiently inhibited by PCIs [13,17,26,90-96].

Because PCIs display these antiviral activities *in vitro* at concentrations that appear to have only limited negative side effects *in vivo*, PCIs are now scheduled to enter clinical trials as antivirals. In this context, a complete characterization of the targets and antiviral mechanisms of PCIs would foster their development as antivirals. Several groups are performing such characterizations, including ours.

The first antiviral activity demonstrated for any PCI was inhibition of HIV replication by CDK9-specific drugs [97]. Unfortunately, these drugs have been further evaluated in only two other publications, the last 4 years ago, and apparently are no longer considered as potential clinical drugs [98,99]. The first antiviral activity demonstrated for any PCI that continues to be considered as a potential clinical drug was inhibition of HCMV replication by Rosco [100]. Since HCMV was known to replicate in dividing cells, such antiviral effects were expected. Soon after, Rosco was shown to inhibit HSV replication and two years later Flavo was shown to inhibit HIV replication. Such antiviral effects of PCIs had not been commonly anticipated because both HSV and HIV can replicate in non-dividing cells. It is now known that cellular proteins that are normally regulated in a cell-cycle specific manner are induced in HSV- and HIV-infected cells and that PCIs also inhibit cellular proteins that are regulated independently of the cell-cycle [reviewed in 14,15,101,102,103]. In fact, it was recently demonstrated that the purified (*R*)-isomer of Rosco (Cyc202, seliciclib), inhibits HIV replication independently of the cell-cycle status of the treated cells [104]. Thus, the ability of PCIs to inhibit replication of viruses that do not require cell-cycle progression does not appear as paradoxical now as it was when first identified.

Regarding their antiviral mechanisms, PCIs were first shown to inhibit viral DNA replication [105], as expected from the then commonly accepted requirement for CDK2 in nuclear DNA replication. More surprisingly, PCIs were soon shown to inhibit viral transcription, first for HSV-1 [26,106] then for HIV [13,17] and more recently for HTLV [46,93], Adenovirus [92], VZV [96], EBV [95], KSHV [94] and animal retroviruses [107,108]. The effects of PCIs on transcription had not been anticipated, but were promptly ascribed to their ability to inhibit CDK9 [13], or perhaps CDK7 [90]. However, recent results, discussed later, suggest that PCI inhibition of HSV-1 transcription may not be mediated by these two CDKs [90,109,110].

PCIs have now been shown to inhibit many viral functions. They inhibit activation of viral proteins of VZV and HSV-1 [111-113], subcellular localization of proteins of VZV and HSV [96,110], reactivation from latency of HSV-1, HIV, EBV and KSHV [17,94,95,114], DNA replication of HCMV, HSV-1, VZV and EBV [96,105,115,116], specific alternative splicing of HCMV [117] and, as discussed, transcription of HSV-1, HIV, HTLV, KSHV, VZV, EBV, adenovirus and animal retroviruses [13,17,26,46,92,94,96,106-108].

It is currently accepted that inhibition of viral transcription is the most important antiviral activity of PCIs. Surprisingly, the precise mechanisms whereby PCIs preferentially inhibit viral, but not cellular, transcription

remain incompletely characterized. Earlier work focused on Flavo and other non-purine PCIs that inhibit CDK9 at least as efficiently as they inhibit CDK1 or 2. These “CDK9-specific” PCIs were shown to inhibit HIV transcription elongation, mostly by targeting CDK9 [13,97,98,118]. More recent work has focused on purine PCIs such as Rosco, which preferentially inhibit CDK1, 2, 5 and 7. Since these PCIs inhibit accumulation of viral transcripts as efficiently as Flavo, they were also hypothesized to primarily act on CDK9 [17]. In support of this hypothesis, Rosco inhibited immunoprecipitated CDK9 of non-assessed purity [17]. In more recent experiments, however, highly purified recombinant CDK9 was not inhibited by 0.5 μ M Rosco [119], a concentration that inhibits highly purified recombinant or native CDK1, 2, 5, or 7 by approximately 50% [6,90,119,120]. In three other recent reports, the purified (*R*)-isomer of Rosco (Cyc202, seliciclib) inhibited HIV replication as efficiently as the racemic mixture of (*R*)- and (*S*)-Rosco. However, Cyc202 inhibited CDK9 with IC_{50} 10 fold higher than its IC_{50} toward CDK2 [104,121]. The purine PCI purvalanol, which is structurally related to Rosco, inhibits viral transcription under conditions in which it binds to CDK1, 2, 5 and 7, but not to CDK9 [90]. Furthermore, high concentrations of Flavo and other “CDK9-specific” PCIs inhibit global cellular transcription *in vitro*, as expected from inhibition of CDK9, whereas purine PCIs such as Rosco do not [58,109]. Thus, we have proposed that inhibition of viral transcription by Rosco and related purine-type PCIs may not be mediated by inhibition of CDK9, as discussed later.

Although inhibition of viral transcription by Flavo is clearly mediated in part by CDK9 [for example, 13,46,122], several published results raise questions about whether CDK9 is the exclusive mediator. For example, although high concentrations of Flavo inhibit cellular transcription *in vitro* and in cultured cells [58,87,109], as expected from CDK9 inhibition, antiviral concentrations of Flavo failed to inhibit global cellular transcription in kidneys of treated mice [123]. Moreover, concentrations of Flavo and other “CDK9-specific” PCIs that have antiviral activities on cultured cells have been repeatedly reported to be non-cytotoxic [for examples 13,98,99,108,118,124]. Such lack of cellular toxicity would be surprising if these drugs acted primarily on a protein believed essential for cellular transcription, like CDK9. It is thus possible that even the “CDK9-specific” PCIs may inhibit viral transcription in part as a consequence of inhibition of kinases other than CDK9. We have, therefore, recently re-examined the mechanisms of inhibition of viral transcription by purine and non-purine type PCIs.

CDK9 is required for early transcription elongation, but not for transcription initiation [125]. We thus tested whether Rosco inhibited initiation or elongation of viral transcription, using run-on transcription assays in nuclei of infected cells [109]. This approach allows us to evaluate the potential effects of chromatin or subnuclear localization in regulation of viral transcription. Rosco prevented initiation of HSV-1 transcription in “run-on” transcription assays, but had no effect on HSV-1 transcription elongation [109]. Therefore, Rosco inhibits viral transcription at a step in which CDK9 is not known to play a limiting role. We concluded from these

results that inhibition of viral transcription by Rosco is not likely consequence of inhibition of CDK9. Consistent with this conclusion, Rosco inhibits transcription of HIV mutants in the *tat* gene or TAR sequences [17], which are not responsive to CDK9 activation. Furthermore, Rosco inhibits transcription driven by HIV LTR promoters in the absence of transactivation by *tat*/TAR [17]. Rosco also inhibits transcription of other retroviruses such as HTLV, MLV, or MMTV, and herpesviruses such as KSHV or EBV, neither of which encodes true *tat* homologues or is known to require CDK9. Considered together, these results argue that inhibition of viral transcription by Rosco does not result primarily from inhibition of CDK9. We are currently pursuing the identification of the kinases that mediate the effects of Rosco on viral transcription.

We have also tested the effects of Flavo on initiation or elongation of run-on transcription. Confirming previous results, we found that concentrations of Flavo that inhibit viral transcription in cultured cells (but which do not result in major cytotoxic effects) inhibit elongation of viral transcription, as expected for a CDK9 inhibitor [110]. Surprisingly, however, we also found that these concentrations prevent initiation of HSV-1 transcription far more efficiently than they inhibit transcription elongation [110]. These results were even more surprising considering the recent finding that Flavo does not inhibit CDK7 *in vivo* [46]. We concluded from our experiments that inhibition of HSV-1 transcription by Flavo is probably mediated by CDK9 and other Flavo-sensitive kinases required for initiation of viral transcription.

The “run on” transcription assays that identified the transcription function inhibited by PCIs also indicated that Rosco inhibited with similar efficiency transcription driven by a number of HSV-1 promoters [109]. Because these promoters are activated by a variety of transcription factors, we postulated that the effects of Rosco on viral transcription be specific for viral genomes, as opposed to viral promoters. To test this hypothesis, we generated stably transfected cells in which multiple copies of an HSV-1 promoter driving expression of a reporter red-fluorescent protein (RFP) gene were recombined into the cellular genome. These cells express no RFP unless they are infected with HSV-1, as expected because a virion protein, VP16, must transactivate the promoter used. We then infected these cells with HSV-1 in the presence or absence of Rosco. Infection in the absence of Rosco activated transcription of the RFP reporter gene, as expected, and Rosco inhibited transcription driven by the HSV-1 promoters in the viral genome, also as expected. Surprisingly, Rosco failed to inhibit transcription driven by the copies of the same promoter recombined into the cellular genome [109]. Therefore, the effects of Rosco on viral transcription are not specific for viral promoters, but rather for viral genomes. In yet unpublished experiments we have found that, conversely, Rosco inhibits promoters that are otherwise insensitive to it when these promoters are recombined in HSV-1 genomes. From these results, we conclude that at least one PCI, Rosco, inhibits activation of transcription of viral genomes. Such activity would be highly desirable in an antiviral drug, as it would require no specific viral proteins or genome sequences. Consequently, selection

of drug-resistant viral strains would be far more difficult using drugs with such activities than using drugs that target viral proteins or genome sequences.

ANTIVIRAL ACTIVITIES OF PCIs *IN VIVO*

We have proposed before that PCIs could be tested as antivirals in clinical trials against proliferative diseases [15,101]. The pathogenesis of several proliferative diseases, such as Kaposi's sarcoma, cervical carcinoma and HIV-associated nephropathy (HIVAN), require cellular proliferation and expression of viral genes. A PCI could inhibit at once the pathogenic mechanism (i.e., unrestricted cell proliferation), the triggering signal (i.e., expression of the viral genes that induce cell proliferation) and the multiplication of the etiological agent (i.e., the virus). The potential antiviral activities of PCIs could be evaluated in patients suffering from these types of diseases, who would benefit from the anti-proliferative activities of these drugs. Encouragingly, two recent publications suggest that PCIs may indeed have therapeutic effects on virus-induced proliferative diseases. As predicted by their effects *in vitro* [6,13,17,90,126,127], Flavo and Rosco ameliorated pathogenesis in a transgenic mouse model of HIVAN [121,123]. Pathogenesis in this model is mediated by unrestricted cell proliferation in the kidneys. Both drugs corrected the expression of the vast majority of genes upregulated in HIVAN, without inhibiting general cellular transcription [121,123]. The lack of effect of Flavo on cellular transcription *in vivo* was in stark contrast to its ability to inhibit cellular transcription in cultured cells [87]. However, Flavo inhibited cellular transcription in cultured cells only at 1 μ M, much higher concentration than those necessary to inhibit HIV-1 replication (200 nM) and unlikely to be reached *in vivo*. Flavo or Rosco appeared to be well tolerated in this transgenic mouse model, which further supports the hypothesis that these drugs can have antiviral activities *in vivo* at doses that produce no major negative side effects. The apparent safety of PCIs in this transgenic model was most remarkable considering that the animals are growing (from 21 days old at the beginning of the experiments to 41 days old at the end), when they would be expected to be the most sensitive to inhibition of proteins required for cell proliferation, such as CDKs. Only a non-statistically significant tendency to lymphopenia was identified after 21 days of treatment [121,123]. As a word of caution, the therapeutic effects of PCIs in this model may be mediated solely by their anti-proliferative effects. In fact, the tg26 model of HIVAN used in these experiments does not involve viral replication, although it requires viral gene expression from the integrated proviruses [121,123]. Consequently, the actual antiviral properties of PCIs *in vivo* remain to be evaluated.

Perhaps fortuitously, the purified (*R*)-isomer of Rosco (Cyc202, seliciclib) had just entered clinical trials against glomerulonephritis, a (non-viral) proliferative kidney disease when the beneficial effects of PCIs on HIVAN were first reported. We could thus expect that the possible use of PCIs against virally induced proliferative diseases is likely to be tested in clinical trials in the relatively near future.

FUTURE DIRECTIONS

Although much progress has been made in the few years since PCIs were first shown to have antiviral properties in cultured cells [13,26,105], several important questions remain unanswered. For example, the ability of PCIs to inhibit viral replication *in vivo* at non-toxic concentrations is still unknown. Several avenues could be explored to address this important question. As already discussed, the antiviral activities of PCIs could be evaluated in virus-induced proliferative diseases.

As a second approach, the antiviral properties of PCIs *in vivo* could be evaluated in patients already enrolled in clinical trials against cancer or other proliferative diseases. Like the general population, these patients are likely to be infected with HSV-1 or HCMV. The number, intensity and duration of HSV-1 or HCMV reactivation events in patients receiving PCIs, placebo, or control drugs could be used to evaluate the potential antiviral effects of PCIs in humans.

As a third approach, the potential antiviral effects of PCIs could also be tested in humans by adding PCIs to current antiviral treatments. The antiviral effects of PCIs and standard antiviral drugs are expected to be additive or synergistic, because PCIs act through different mechanisms than standard antiviral drugs. In support of this model, we have shown that the antiviral effects of Rosco and purvalanol A are indeed additive to those of acyclovir in cultured cells [90]. This approach would allow the evaluation of the antiviral effects of PCIs without discontinuing (partially) effective antiviral treatments.

The proteins that mediate the antiviral effects of PCIs are cellular, not viral, in origin [13-15,17,26,90,91,105,106,114,115]. However, the identities of the specific kinases that mediate their antiviral effects remain uncertain. All antiviral PCIs inhibit several protein kinases that could mediate their antiviral activities. The inhibitory effects of different PCIs on different viral functions could all result from inhibition of a single protein, or from inhibition of many different proteins. Studies to address these outstanding but technically challenging questions are currently underway.

The mechanisms whereby purine PCIs prevent initiation of transcription in a genome-dependent manner also remain unknown. Many groups, including ours, are currently working toward addressing this issue. Regardless of the specifics of the mechanisms, however, genome-specific inhibition of viral transcription may be a major advantage for antiviral drugs. First, a drug using such mechanisms is likely to be active against a variety of viruses and, consequently, could be promptly tested against novel viral pathogens the genes of which are transcribed in the nucleus. Second, such mechanisms would be independent of viral gene and genome sequences. It would thus be expected that selection for viral resistance against such a drug would be more difficult than against conventional antiviral drugs that target specific viral proteins. At present, no PCI-resistant strain of any virus has been reported, although extensive efforts to select for such a mutant of HIV-1, HSV-1, or HCMV have been reported [17,26]. Drugs that prevent initiation of transcription from viral genomes should also be active against viral strains that are resistant to conventional antiviral drugs, which target

viral proteins. In support of this hypothesis, we have shown that PCIs are indeed fully active against HIV and HSV-1 strains resistant to several current antiviral drugs, including reverse-transcriptase or protease inhibitors [90]. These results have been recently confirmed and extended using (*R*)-Rosco (Cyc202; seliciclib) [104].

CONCLUSIONS

In conclusion, CDKs appear to be promising targets for novel antiviral drugs. Among other advantages, CDKs are well characterized and at least a subgroup of CDKs is (surprisingly) non-essential for mammalian cells. A considerable body of expertise on protein kinase inhibitors has been developed in the last ten years, which should facilitate further development of specific PCIs. Moreover, PCIs have been demonstrated to be apparently well tolerated and to be active against a variety of unrelated viruses, including strains resistant to current antiviral drugs. Lastly, CDKs are required for replication of a variety of unrelated viruses and resistance to PCIs has not been easily selected for to present.

We can expect that the antiviral mechanisms of PCIs will be fully characterized in the near future. The antiviral activities of PCIs *in vivo* at non-toxic doses also appear likely to be fully evaluated in the coming years. In conclusion, this is an exciting time in this area of research, as we expect that the full antiviral potential of PCIs will soon be known. Such excitement is clearly reflected in the increasing number of studies on the antiviral properties of PCIs being published during this last year.

ACKNOWLEDGEMENTS

Our lab is supported by the Canadian Institute of Health Research (CIHR MOP 49551). LMS is a Medical Scholar of the Alberta Heritage Foundation for Medical Research (AHFMR) and a New Investigator of the CIHR. Our laboratory was equipped with funds provided by the AHFMR and the Faculty of Medicine and Dentistry of the University of Alberta.

ABBREVIATIONS

CDK	= Cyclin-dependent kinase
DYRK1a	= Drosophila YAK-related kinase 1a
EBV	= Epstein-Barr virus
ERK	= Extra-cellular receptor activated kinase
Flavo	= Flavopiridol
HCMV	= Human cytomegalovirus
HSV	= Herpes simplex virus
HSV-1	= Herpes simplex virus type 1
HSV-2	= Herpes simplex virus type 2
HTLV	= Human T-lymphotropic virus
KSHV	= Kaposi's sarcoma herpes virus
LTR	= Long-terminal repeat

MLV	=	Murine leukemia viruses
MMTV	=	Mouse mammary gland tumour virus
Olo	=	Olomoucine
Rosco	=	Roscovitine
PCI	=	Pharmacological CDK inhibitor
PKA	=	Protein kinase A
ZVZ	=	Varicella-zoster virus

REFERENCES

- Meyerson, M., Enders, G. H., Wu, C. L., Su, L. K., Gorka, C., Nelson, C., Harlow, E. and Tsai, L. H. *EMBO J.* **1992**, *11*, 2909.
- Andrew W. Murray and Debora Marks. *Nature* **2001**, *409*, 844.
- Knockaert, M., Greengard, P. and Meijer, L. *TRENDS in Pharmacological Sciences* **2002**, *23*, 417.
- Vesely, J., Havlicek, L., Strnad, M., Blow, J. J., Donella-Deana, A., Pinna, L., Letham, D. S., Kato, J., Detivquad, L., Leclerc, S. and Meijer, L. *Eur. J. Biochem.* **1994**, *224*, 771.
- Parker, C. W., Entsch, B. and Letham, D. S. *Phytochemistry Oxford, Eng.* **1986**, *25*, 303.
- Meijer, L., Borgne, A., Mulner, O., Chong, J. P., Blow, J. J., Inagaki, N., Inagaki, M., Delcros, J. G. and Moulinoux, J. P. *Euro. J. Biochem.* **1997**, *243*, 527.
- Gray, N. S., Wodicka, L., Thunnissen, A. M., Norman, T. C., Kwon, S., Espinoza, F. H., Morgan, D. O., Barnes, G., LeClerc, S., Meijer, L., Kim, S. H., Lockhart, D. J. and Schultz, P. G. *Science* **1998**, *281*, 533.
- Losiewicz, M. D., Carlson, B. A., Kaur, G., Sausville, E. A. and Worland, P. J. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 589.
- Zaharevitz, D. W., Gussio, R., Leost, M., Senderowicz, A. M., Lahusen, T., Kunick, C., Meijer, L. and Sausville, E. A. *Cancer Res.* **1999**, *59*, 2566.
- Mettey, Y., Gompel, M., Thomas, V., Garnier, M., Leost, M., Ceballos-Picot, I., Noble, M., Endicott, J., Vierfond, J. M. and Meijer, L. *J. Med. Chem.* **2003**, *46*, 222.
- Hoessel, R., Leclerc, S., Endicott, J. A., Nobel, M. E., Lawrie, A., Tunnah, P., Leost, M., Damiens, E., Marie, D., Marko, D., Niederberger, E., Tang, W., Eisenbrand, G. and Meijer, L. *Nat. Cell Biol.* **1999**, *1*, 60.
- Kim, K. S., Sack, J. S., Tokarski, J. S., Qian, L., Chao, S. T., Leith, L., Kelly, Y. F., Misra, R. N., Hunt, J. T., Kimball, S. D., Humphreys, W. G., Wautlet, B. S., Mulheron, J. G. and Webster, K. R. *J. Med. Chem.* **2000**, *43*, 4126.
- Chao, S. H., Fujinaga, K., Marion, J. E., Taube, R., Sausville, E. A., Senderowicz, A. M., Peterlin, B. M. and Price, D. H. *J. Biol. Chem.* **2000**, *275*, 28345.
- Schang, L. M. *Antivir. Chem. Chemother.* **2001**, *12* Suppl. 1, 157.
- Schang, L. M. *J. Antimicrob. Chemo.* **2002**, *50*, 779.
- Fischer, P. M. and Gianella-Borradori, A. *Expert. Opin. Investig. Drugs* **2003**, *12*, 955.
- Wang, D., de la Fuente, C., Deng, L., Wang, L., Zilberman, I., Eadie, C., Healey, M., Stein, D., Denny, T., Harrison, L. E., Meijer, L. and Kashanchi, F. *J. Virol.* **2001**, *75*, 7266.
- Meijer, L., Borgne, A., Mulner, O., Chong, J. P., Blow, J. J., Inagaki, N., Inagaki, M., Delcros, J. G. and Moulinoux, J. P. *Eur. J. Biochem.* **1997**, *243*, 527.
- Gray, N. S., Wodicka, L., Thunnissen, A. M., Norman, T. C., Kwon, S., Espinoza, F. H., Morgan, D. O., Barnes, G., LeClerc, S., Meijer, L., Kim, S. H., Lockhart, D. J. and Schultz, P. G. *Science* **1998**, *281*, 533.
- Leclerc, S., Garnier, M., Hoessel, R., Marko, D., Bibb, J. A., Snyder, G. L., Greengard, P., Biernat, J., Wu, Y. Z., Mandelkow, E. M., Eisenbrand, G. and Meijer, L. *J. Biol. Chem.* **2001**, *276*, 251.
- Alessi, F., Quarta, S., Savio, M., Riva, F., Rossi, L., Stivala, L. A., Scovassi, A. I., Meijer, L. and Prosperi, E. *Exp. Cell Res.* **1998**, *245*, 8.
- Iseki, H., Ko, T. C., Xue, X. Y., Seapan, A., Hellmich, M. R. and Townsend, C. M. Jr. *Surgery*, **1997**, *122*, 187.
- Zhang, G. J., Safran, M., Wei, W., Sorensen, E., Lassota, P., Zhelev, N., Neuberg, D. S., Shapiro, G. and Kaelin, W. G. Jr. *Nat. Med.* **2004**, *10*, 643.
- Glab, N., Labidi, B., Qin, L. X., Trehin, C., Bergounioux, C. and Meijer, L. *FEBS Lett.* **1994**, *353*, 207.
- Planchais, S., Glab, N., Trehin, C., Perennes, C., Bureau, J. M., Meijer, L. and Bergounioux, C. *Plant J.* **1997**, *12*, 191.
- Schang, L. M., Phillips, J. and Schaffer, P. A. *J. Virol.* **1998**, *72*, 5626.
- Abraham, R. T., Acquarone, M., Andersen, A., Asensi, A., Belle, R., Berger, F., Bergounioux, C., Brunn, G., Buquet-Fagot, C., Fagot, D. and et al. *Biol. Cell* **1995**, *83*, 105.
- Yakisich, J. S., Siden, A., Idoyaga Vargas, V., Eneroth, P. and Cruz, M. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 674.
- Yakisich, J. S., Boethius, J., Lindblom, I. O., Wallstedt, L., Vargas, V. I., Siden, A. and Cruz, M. H. *Neuroreport* **1999**, *10*, 2563.
- Alevizopoulos, K., Catarin, B., Vlach, J. and Amati, B. *EMBO J.* **1998**, *17*, 5987.
- Patrick, G. N., Zhou, P., Kwon, Y. T., Howley, P. M. and Tsai, L. H. *J. Biol. Chem.* **1998**, *273*, 24057.
- Bibb, J. A., Snyder, G. L., Nishi, A., Yan, Z., Meijer, L., Fienberg, A. A., Tsai, L. H., Kwon, Y. T., Girault, J. A., Czernik, A. J., Haganir, R. L., Hemmings, H. C. Jr., Nairn, A. C. and Greengard, P. *Nature* **1999**, *402*, 669.
- Matsumoto, Y., Hayashi, K. and Nishida, E. *Curr. Biol.* **1999**, *9*, 429.
- Mermillod, P., Tomanek, M., Marchal, R. and Meijer, L. *Mol. Reprod. Dev.* **2000**, *55*, 89.
- Corellou, F., Bisgrove, S. R., Kropf, D. L., Meijer, L., Kloareg, B. and Bouget, F. Y. *Development* **2000**, *127*, 1651.
- Kwon, Y. G., Lee, S. Y., Choi, Y., Greengard, P. and Nairn, A. C. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2168.
- Choi, K. S., Eom, Y. W., Kang, Y., Ha, M. J., Rhee, H., Yoon, J. W. and Kim, S. J. *J. Biol. Chem.* **1999**, *274*, 31775.
- Harmon, A. D., Weiss, U. and Silverton, J. V. *Tetrahedron Letters* **1979**, *20*, 721.
- Sedlacek, H., Czech, J., Naik, R., Kaur, G., Worland, P., Losiewicz, M., Parker, B., Carlson, B., Smith, A., Senderowicz, A. M. and Sausville, E. A. *Int. J. Oncol.* **1996**, *9*, 1143.
- Mans, D. R., da Rocha, A. B. and Schwartzmann, G. *Oncologist* **2000**, *5*, 185.
- Senderowicz, A. M. *Oncologist* **2002**, *7* Suppl. 3, 12.
- Carlson, B. A., Dubay, M. M., Sausville, E. A., Brizuela, L. and Worland, P. J. *Cancer Res.* **1996**, *56*, 2973.
- Losiewicz, M. D., Carlson, B. A., Kaur, G., Sausville, E. A. and Worland, P. J. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 589.
- Chao, S. H., Fujinaga, K., Marion, J. E., Taube, R., Sausville, E. A., Senderowicz, A. M., Peterlin, B. M. and Price, D. H. *J. Biol. Chem.* **2000**, *275*, 28345.
- Carlson, B., Pearlstein, R., Naik, R., Sedlacek, H., Sausville, E. and Worland, P. *Proc. Am. Assoc. Cancer Res.* **1996**, *Vol. 37*, pp. 424.
- Zhou, M., Deng, L., Lacoste, V., Park, H. U., Pumfery, A., Kashanchi, F., Brady, J. N. and Kumar, A. *J. Virol.* **2004**, *78*, 13522.
- De Azevedo, W. F. Jr., Mueller-Dieckmann, H. J., Schulze-Gahmen, U., Worland, P. J., Sausville, E. and Kim, S. H. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 2735.
- Kaiser, A., Nishi, K., Gorin, F. A., Walsh, D. A., Bradbury, E. M. and Schnier, J. B. *Arch. Biochem. Biophys.* **2001**, *386*, 179.
- Oikonomakos, N. G., Schnier, J. B., Zographos, S. E., Skamnaki, V. T., Tsitsanou, K. E. and Johnson, L. N. *J. Biol. Chem.* **2000**, *275*, 34566.
- Hooijberg, J. H., Broxterman, H. J., Scheffer, G. L., Vrasdonk, C., Heijn, M., de Jong, M. C., Scheper, R. J., Lankelma, J. and Pinedo, H. M. *Br. J. Cancer* **1999**, *81*, 269.
- Hooijberg, J. H., Broxterman, H. J., Heijn, M., Fles, D. L., Lankelma, J. and Pinedo, H. M. *FEBS Lett.* **1997**, *413*, 344.
- Bible, K. C., Bible, R. H. Jr., Kottke, T. J., Svigen, P. A., Xu, K., Pang, Y. P., Hajdu, E. and Kaufmann, S. H. *Cancer Res.* **2000**, *60*, 2419.
- Schnier, J. B., Kaur, G., Kaiser, A., Stinson, S. F., Sausville, E. A., Gardner, J., Nishi, K., Bradbury, E. M. and Senderowicz, A. M. *FEBS Lett.* **1999**, *454*, 100.

- [54] Kaur, G., Stetler-Stevenson, M., Sebers, S., Worland, P., Sedlacek, H., Myers, C., Czech, J., Naik, R. and Sausville, E. *J. Natl. Cancer Inst.* **1992**, *84*, 1736.
- [55] Sausville, E. A., Zaharevitz, D., Gussio, R., Meijer, L., Louarn-Leost, M., Kunick, C., Schultz, R., Lahusen, T., Headlee, D., Stinson, S., Arbuck, S. G. and Senderowicz, A. *Pharmacol. Ther.* **1999**, *82*, 285.
- [56] Foskett, S. M., Ghose, R., Tang, D. N., Lewis, D. E. and Rice, A. P. *J. Virol.* **2001**, *75*, 1220.
- [57] Chao, S. H. and Price, D. H. *J. Biol. Chem.* **2001**, *276*, 31793.
- [58] Lam, L. T., Pickeral, O. K., Peng, A. C., Rosenwald, A., Hurt, E. M., Giltneane, J. M., Averett, L. M., Zhao, H., Davis, R. E., Sathyamoorthy, M., Wahl, L. M., Harris, E. D., Mikovits, J. A., Monks, A. P., Hollingshead, M. G., Sausville, E. A. and Staudt, L. M. *Genome Biol.* **2001**, *2*, RESEARCH0041.
- [59] Ortega, S., Prieto, I., Odajima, J., Martin, A., Dubus, P., Sotillo, R., Barbero, J. L., Malumbres, M. and Barbacid, M. *Nat. Genet.* **2003**, *35*, 25.
- [60] Berthet, C., Aleem, E., Coppola, V., Tessarollo, L. and Kaldis, P., *Curr. Biol.* **2003**, *13*, 1775.
- [61] Zou, X., Ray, D., Aziyu, A., Christov, K., Boiko, A. D., Gudkov, A. V. and Kiyokawa, H. *Genes and Development* **2002**, *16*, 2923.
- [62] Parisi, T., Beck, A. R., Rougier, N., McNeil, T., Lucian, L., Werb, Z. and Amati, B. *EMBO J.* **2003**, *22*, 4794.
- [63] Drees, M., Dengler, W. A., Roth, T., Labonte, H., Mayo, J., Malspeis, L., Grever, M., Sausville, E. A. and Fiebig, H. H. *Clin. Cancer Res.* **1997**, *3*, 273.
- [64] Arguello, F., Alexander, M., Sterry, J. A., Tudor, G., Smith, E. M., Kalavar, N. T., Greene, J. F. Jr., Koss, W., Morgan, C. D., Stinson, S. F., Siford, T. J., Alford, W. G., Klabansky, R. L. and Sausville, E. A. *Blood* **1998**, *91*, 2482.
- [65] Patel, V., Senderowicz, A. M., Pinto, D. Jr., Igishi, T., Raffeld, M., Quintanilla-Martinez, L., Ensley, J. F., Sausville, E. A. and Gutkind, J. S. *J. Clin. Invest.* **1998**, *102*, 1674.
- [66] Pippin, J. W., Qu, Q., Meijer, L. and Shankland, S. J. *J. Clin. Invest.* **1997**, *100*, 2512.
- [67] Hoessel, R., Leclerc, S., Endicott, J. A., Nobel, M. E., Lawrie, A., Tunnah, P., Leost, M., Damiens, E., Marie, D., Marko, D., Niederberger, E., Tang, W., Eisenbrand, G. and Meijer, L. *Nat. Cell Biol.* **1999**, *1*, 60.
- [68] Innocenti, F., Stadler, W. M., Iyer, L., Ramirez, J., Vokes, E. E. and Ratain, M. J. *Clin. Cancer Res.* **2000**, *6*, 3400.
- [69] Stadler, W. M., Vogelzang, N. J., Amato, R., Sosman, J., Taber, D., Liebowitz, D. and Vokes, E. E. *J. Clin. Oncol.* **2000**, *18*, 371.
- [70] Stinson, S. F., Hill, K., Siford, T. J., Phillips, L. R. and Daw, T. W. *Cancer Chemother Pharmacol.* **1998**, *42*, 261.
- [71] Senderowicz, A. M., Headlee, D., Stinson, S. F., Lush, R. M., Kalil, N., Villalba, L., Hill, K., Steinberg, S. M., Figg, W. D., Tompkins, A., Arbuck, S. G. and Sausville, E. A. *J. Clin. Oncol.* **1998**, *16*, 2986.
- [72] Schwartz, G. K., Ilson, D., Saltz, L., O'Reilly, E., Tong, W., Maslak, P., Werner, J., Perkins, P., Stoltz, M. and Kelsen, D. *J. Clin. Oncol.* **2001**, *19*, 1985.
- [73] Shapiro, G. I., Supko, J. G., Patterson, A., Lynch, C., Lucca, J., Zaccarola, P. F., Muzikansky, A., Wright, J. J., Lynch, T. J. Jr. and Rollins, B. J. *Clin. Cancer Res.* **2001**, *7*, 1590.
- [74] Brooks, E. E., Gray, N. S., Joly, A., Kerwar, S. S., Lum, R., Mackman, R. L., Norman, T. C., Rosete, J., Rowe, M., Schow, S. R., Schultz, P. G., Wang, X., Wick, M. M. and Shiffman, D. J. *Biol. Chem.* **1997**, *272*, 29207.
- [75] Hsu, B. *Am. J. Chin. Med.* **1980**, *8*, 301.
- [76] Ma, M. Z. and Yao, B. Y. *J. Tradit. Chin. Med.* **1983**, *3*, 245.
- [77] Bennett, P., Mani, S., O'Reilly, S., Wright, J., Schilsky, R. L., Vokes, E. E. and Grochow, L. **1999**, in *1999 American Society of Clinical Oncology Meeting*, Oncology, A. S. o. C., ed, Vol. 18, *Proc. Am. Soc. Clin. Oncol.* pp. 277, Abstract 1065.
- [78] Benson, C., White, J., Twelves, A., O'Donnell, A., Cruickshank, C., Tan, S., Gianella-Borradori, A. and Judson, I. **2003**, in *2003 American Society of Clinical Oncology Annual Meeting*, Oncology, A. S. o. C., ed, Vol. 22, *Proc. Am. Soc. Clin. Oncol.* pp. 209, Abstract 838.
- [79] Pierga, J., Faivre, S., Vera, K., Laurence, V., Delbaldo, C., Bekradda, M., Armand, J., Gianella-Borradori, A., Dieras, V. and Raymond, E. *Proc. Am. Soc. Clin. Oncol.* **2003**, *22*, 210, Abstract 840.
- [80] Laurence, V., Faivre, S., Vera, K., Pierga, J., Delbaldo, C., Bekradda, M., Armand, J., Gianella-Borradori, A., Dieras, V. and Raymond, E. *Euro. J. Cancer* **2002**, *38*, Suppl. 7, 49.
- [81] Benson, C., Raynaud, D., O'Donnell, A., Gianella-Borradori, A., Westwood, R., McClue, S. J., Workman, P. and Judson, I. **2002**, in *92nd Annual Meeting of the American Association for Cancer Research*, American Association for Cancer Research, San Francisco, California.
- [82] Senderowicz, A. M. *Invest New Drugs* **1999**, *17*, 313.
- [83] Thomas, J. P., Tutsch, K. D., Cleary, J. F., Bailey, H. H., Arzoomanian, R., Alberti, D., Simon, K., Feierabend, C., Binger, K., Marnocha, R., Dresen, A. and Wilding, G. *Cancer Chemother Pharmacol.* **2002**, *50*, 465.
- [84] Mingzhu, M. and Banguan, Y. *J. Tradit. Chin. Med.* **1983**, *3*, 245.
- [85] McClue, S. J., Blake, D., Clarke, R., Cowan, A., Cummings, L., Fischer, P. M., MacKenzie, M., Melville, J., Stewart, K., Wang, S., Zhelev, N., Zheleva, D. and Lane, D. P. *Int. J. Cancer* **2002**, *102*, 463.
- [86] Zaharevitz, D. W., Gussio, R., Leost, M., Senderowicz, A. M., Lahusen, T., Kunick, C., Meijer, L. and Sausville, E. A. *Cancer Res.* **1999**, *59*, 2566.
- [87] Lam, L., Pickeral, O., Peng, A., Rosenwald, A., Hurt, E., Giltneane, J., Averett, L., Zhao, H., Davis, R., Sathyamoorthy, M., Wahl, L., Harris, E., Mikovits, J., Monks, A., Hollingshead, M., Sausville, E. and Staudt, L. *Genome Biol.* **2001**, *2*, 0041.1.
- [88] Schwartz, G. K., Ilson, D., Saltz, L., O'Reilly, E., Tong, W., Maslak, P., Werner, J., Perkins, P., Stoltz, M. and Kelsen, D. *J. Clin. Oncol.* **2001**, *19*, 1985.
- [89] Kouroukis, C. T., Belch, A., Crump, M., Eisenhauer, E., Gascoyne, R. D., Meyer, R., Lohmann, R., Lopez, P., Powers, J., Turner, R. and Connors, J. M. *J. Clin. Oncol.* **2003**, *21*, 1740.
- [90] Schang, L. M., Bantly, A., Knockaert, M., Shaheen, F., Meijer, L., Malim, M. H., Gray, N. S. and Schaffer, P. A. *J. Virol.* **2002**, *76*, 7874.
- [91] Bresnahan, W. A., Thompson, E. A. and Albrecht, T. J. *Gen. Virol.* **1997**, *78*, 1993.
- [92] Fax, P., Lehmkuhler, O., Kuhn, C., Esche, H. and Brockmann, D., *J. Biol. Chem.* **275**, **2000**, 40554–40560.
- [93] Wang, L., Deng, L., Wu, K., de la Fuente, C., Wang, D., Kehn, K., Maddukuri, A., Baylor, S., Santiago, F., Agbottah, E., Trigon, S., Morange, M., Mahieux, R. and Kashanchi, F. *Mol. Cell Biochem.* **2002**, *237*, 137.
- [94] Ghedin, E., Pumfery, A., De La Fuente, C., Yao, K., Miller, N., Lacoste, V., Quackenbush, J., Jacobson, S. and Kashanchi, F. *Retrovirology* **2004**, *1*, 10.
- [95] Kudoh, A., Daikoku, T., Sugaya, Y., Isomura, H., Fujita, M., Kiyono, T., Nishiyama, Y. and Tsurumi, T. *J. Virol.* **2004**, *78*, 104.
- [96] Taylor, S. L., Kinchington, P. R., Brooks, A. and Moffat, J. F. *J. Virol.* **2004**, *78*, 2853–2862.
- [97] Mancebo, H. S., Lee, G., Flygare, J., Tomassini, J., Luu, P., Zhu, Y., Peng, J., Blau, C., Hazuda, D., Price, D. and Flores, O. *Genes Dev.* **1997**, *11*, 2633.
- [98] Flores, O., Lee, G., Kessler, J., Miller, M., Schlieff, W., Tomassini, J. and Hazuda, D. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 7208.
- [99] Pisell, T. L., Ho, O., Lee, G. and Butera, S. T. *Antivir. Chem. Chemother.* **2001**, *12* Suppl. 1, 33.
- [100] Bresnahan, W. A., Boldogh, I., Chi, P., Thompson, E. A. and Albrecht, T. *Virology* **1997**, *231*, 239.
- [101] Schang, L. M. *Biochim. Biophys. Acta-Proteins and Proteom.* **2004**, *1697*, 197.
- [102] Schang, L. M. **2003**, in *Progress in cell cycle research volume 5: Cell cycle regulators as therapeutic targets*, Meijer, L., Jézéquel, A. and Roberge, M., eds, Vol. 50, pp. 103, Life in progress, Roscoff, France.
- [103] Provencher, V. M. I., Coccaro, E., Lacasse, J. J. and Schang, L. M. *Curr. Pharm. Des.* **2004**, *10*, 4081.
- [104] Agbottah, E., de la Fuente, C., Nekhai, S., Barnett, A., Gianella-Borradori, A., Pumfery, A. and Kashanchi, F. *J. Biol. Chem.* **2005**, in press.
- [105] Bresnahan, W. A., Boldogh, I., Chi, P., Thompson, E. A. and Albrecht, T. *Virology* **1997**, *231*, 239.
- [106] Schang, L. M., Rosenberg, A. and Schaffer, P. A. *J. Virol.* **1999**, *73*, 2161.
- [107] Bhattacharjee, R. N., Banks, G. C., Trotter, K. W., Lee, H. L. and Archer, T. K. *Mol. Cell Biol.* **2001**, *21*, 5417.

- [108] Chao, S. H., Walker, J. R., Chanda, S. K., Gray, N. S. and Caldwell, J. S. *Mol. Cell Biol.* **2003**, 23, 831.
- [109] Diwan, P., Lacasse, J. J. and Schang, L. M. *Journal of Virology* **2004**, 78, 9352.
- [110] Lacasse, J. J., Provencher, V. M. I., Urbanowski, M. D. and Schang, L. M. *Therapy* **2004**, 2, 1.
- [111] Ye, M., Duus, K. M., Peng, J. M., Price, D. H. and Grose, C. *J. Virol.* **1999**, 73, 1320.
- [112] Advani, S. J., Hagglund, R., Weichselbaum, R. R. and Roizman, B. *J. Virol.* **2001**, 75, 7904.
- [113] Davido, D. J., Leib, D. A. and Schaffer, P. A. *J. Virol.* **2002**, 76, 1077.
- [114] Schang, L. M., Bantly, A. and Schaffer, P. A. *J. Virol.* **2002**, 76, 7724.
- [115] Schang, L. M., Rosenberg, A. and Schaffer, P. A. *J. Virol.* **2000**, 74, 2107.
- [116] Kudoh, A., Daikoku, T., Sugaya, Y., Isomura, H., Fujita, M., Kiyono, T., Nishiyama, Y. and Tsurumi, T. *J. Virol.* **2004**, 78, 104.
- [117] Sanchez, V., McElroy, A. K., Yen, J., Tamrakar, S., Clark, C. L., Schwartz, R. A. and Spector, D. H. *J. Virol.* **2004**, 78, 11219.
- [118] Mancebo, H. S., Lee, G., Flygare, J., Tomassini, J., Luu, P., Zhu, Y., Peng, J., Blau, C., Hazuda, D., Price, D. and Flores, O. *Genes Dev.* **1997**, 11, 2633.
- [119] Pinhero, R., Liaw, P. and Yankulov, K. *Biol. Proced Online* **2004**, 6, 163.
- [120] Schulze-Gahmen, U., Brandsen, J., Jones, H. D., Morgan, D. O., Meijer, L., Vesely, J. and Kim, S. H. *Proteins* **1995**, 22, 378.
- [121] Gherardi, D., D'Agati, V., Chu, T.-H. T., Barnett, A., Gianella-Borradori, A., Gelman, I. H. and Nelson, P. J. *J. Am. Soc. Nephrol.* **2004**, 15, 1212.
- [122] Chao, S. H. and Price, D. H. *J. Biol. Chem.* **2001**, 276, 31793.
- [123] Nelson, P. J., D'Agati, V. D., Gries, J.-M., Suarez, J.-R. and Gelman, I. H. *J. Antimicrob. Chemo.* **2003**, 51, 921.
- [124] Nelson, P. J., Gelman, I. H. and Klotman, P. E. *J. Am. Soc. Nephrol.* **2001**, 12, 2827.
- [125] Price, D. H. *Mol. Cell Biol.* **2000**, 20, 2629.
- [126] Nelson, P. J., Gelman, I. H. and Klotman, P. E. *J. Am. Soc. Nephrol.* **2001**, 12, 2827.
- [127] Carlson, B. A., Dubay, M. M., Sausville, E. A., Brizuela, L. and Worland, P. J. *Cancer Res.* **1996**, 56, 2973.