in vivo *19*: 85-92 (2005)

Review

Potential Role of Cell Cycle Synchronizing Agents in Combination Treatment Modalities of Malignant Tumors

ATTILA ZALATNAI

First Department of Pathology and Experimental Cancer Research, Semmelweis University, Faculty of Medicine, Budapest, Hungary

Abstract. *Malignant neoplasms consist of heterogeneous cell populations and their cellular elements proliferate asynchronously. Since the tumor cells of various cell cycle phases respond differently to many chemotherapeutic drugs, attempts at synchronization seemed to be a promising way to achieve a more powerful antineoplastic effect. Mainly based on in vitro data, it was shown that numerous compounds, including hormones, were able to arrest the cell cycle in different phases, and some of them also induced apoptotic cell death. The better understanding of the molecular mechanisms of cell cycle control has brought the cyclin-dependent kinases into focus and hundreds of compounds have been synthesized in order to regulate malignant cells at their checkpoints, especially at G1 progression. Some of these compounds have been found to be effective not only in vitro, but also in in vivo experiments, and they were further evaluated in Phase I – II clinical trials. Generally speaking, these studies have yielded modest, although potentially promising, results, but the adverse effects sometimes restricted the applicability of the products. Nevertheless, extended studies in cancer patients are under way. Moreover, after encouraging preclinical investigations, the combination of cell cycle regulators with different cytostatic drugs may offer a novel therapeutic alternative in the field of oncology.*

During the evolution of malignant neoplasms, genetic instability, random mutations, abnormal mitoses and micro-environmental effects result in a profoundly heterogeneous cell population within the individual tumor. Significant intratumoral DNA heterogeneity has long been recognized in different malignancies, *e.g.* in colorectal, renal, prostatic, breast, ovarian, head and neck cancers, or gliomas (1-10). Moreover, the degree of heterogeneity is increased with the tumor stage and grade (2, 10). Regarding ploidy analysis, different regions of a given tumor may display evenly DNA diploid, DNA diploid + aneuploid or various aneuploid stemlines (3, 4, 6). Similarly, the proportion of the S-phase fraction (SPF) is also inhomogeneous: in breast cancer samples it can vary between 2 – 32 % (5, 7) and in ovarian carcinomas between 0 – 28 % (8), depending on which part of the tumor is analyzed. In a Swedish study, SPF heterogeneity reached as high as 71% in breast carcinomas (6). Proliferation rate, as measured immunohistochemically by means of PCNA, has also proved to be considerably variable, ranging from 0.7 to 45% in different areas of breast cancer samples, the PCNA score being higher in peripheral regions than in the central areas (5). In xenotransplanted human tumors, substantial heterogeneity in the cell cycle phases could also be observed (11, 12). Thus, genetic and kinetic heterogeneities may cause significant errors in an attempt to predict the clinical prognosis of a given tumor when it is based on a single sample. In addition, there may be a clinical impact because malignant cells in various cell cycle phases may respond differently to antineoplastic treatments and a number of chemotherapeutic drugs exhibit a phase-specificity.

Early studies with hydroxyurea revealed that it is active as a tumor-inhibiting drug at the G1-S boundary of the cell cycle (13), and its phase-specificity was also demonstrated in animal experiments (14). Using CEM lymphoma cells, flow cytometric studies have shown a cell cycle arrest in early S-phase, but later than the aphidicolin arrest point (15). Doxorubicin (adriamycin) predominantly induces G2 arrest (16, 17), while 5-fluorouracil (5-FU) results in an increase in G1-S-phase tumor cells accompanied by augmentation of the p21 expression and a striking cyclin E up-regulation (18). Etoposide, cisplatin or phleomycin D1 were found to block the S-phase entry, which was sustained for up to two weeks

Correspondence to: Dr. med. habil. Attila Zalatnai, First Department of Pathology and Experimental Cancer Research, Semmelweis University, Faculty of Medicine, H-1085 Budapest, Ülloi ut 26, Hungary. Tel: +(36-1) 266-1638, 215-6920, Fax: +(36-1) 317-1074, e-mail: zalatnai@korb1.sote.hu

Key Words: Cell cycle synchronization, cyclin-dependent kinase inhibitors, malignant tumors, mimosine, review.

(19, 20). Incubation of human prostatic carcinoma cells with taxol yielded an 87-90% accumulation in G2-M-phase (similar to the effects of vincristine or vinorelbine) (20). In addition, taxol resulted in a sensitization of small cell lung cancer cells to cisplatin or chlorambucil in a dose-dependent manner, but this sensitization effect was not due to cell cycle arrest (21). The cell cycle effects of taxol, however, seem to be more complex, because *in vitro* the G2-M block is observable immediately, the G1 block becomes active later and the S-phase delay is seen after 24 hours (22). Interestingly, after gamma-irradiation there was no difference in the susceptibility to DNA damage in G0-G1-S-G2-or M-phases (16).

Not only chemotherapeutic drugs, but also natural or seminatural compounds, are able to induce cell cycle blockade. For example, several flavonoids may also induce arrest in cultured cell lines, but the site of checkpoints varies with the flavonoids applied or with the cell types (23). Carotenoids, especially lycopene, were also reported to inhibit the cell growth of human prostatic carcinoma *in vitro* and, during this process, the number of cells in G2-M-phase accumulated and there was a decrease of the S-phase cells (24).

All these data indicate that various antitumor agents have points of attack at different cell cycle phases. Theoretically, if the cells in a malignant tumor displaying various stages of the cycle were in the same phase, selective, phase-specific cytostatics could be administered and their therapeutic effect would be more marked. Attempts at synchronization of tumor cells are based on this assumption.

Synchronization of proliferating cells

The process by which proliferating cells are arrested in specific phases of the cell cycle is termed *synchronization*. It can easily be achieved and studied in cultured cells, therefore most of the studies have been performed *in vitro*. Many different pharmacological methods have been established. The classical method is serum deprivation but, of course, it has no clinical connection. Similarly, dimethyl sulfoxide (DMSO) is also a powerful inducer of G1 synchronization with no genotoxic or cytotoxic effects (25), but this feature can be exploited only *in vitro*. Butyrolactone-I was shown to arrest not only porcine fetal fibroblasts (26), but also human pancreatic cell lines (27). Aphidicolin, a potent mycotoxin, proved to synchronize the cell cycle both *in vitro* and *in vivo* (28), the majority of cells being accumulated at the G1-S transition (26). Staurosporine also reversibly inhibits lymphoma cells acting in early G1 (15).

Among the cell cycle regulators mimosine, a nonprotein amino acid derived from Mimosa and Leucaena plants, has long been recognized as a synchronizing agent. On addition to asynchronously growing cultures (human lymphoblastoid, erythroleukemia, promyeloid leukemia, HeLa, EJ30 cells), it inhibited the cell cycle at late G1, time- and dose-dependently (29-33). The exact mechanism by which mimosine exerts its blocking effect is still incompletely understood, but multiple targets seem to be important: interference with the synthesis of histone H1 kinase, a key enzyme that is required for cell cycle progression (34), up-regulation of p27(Kip1) protein levels (35), specific inhibition of cyclin D1 expression (36), as well as its iron chelating effect have been reported (37).

It is of special interest that the synchronization effect can also be achieved by hormonal manipulations. It was reported, more than a decade ago, that glycocorticosteroids reversibly induced an early G1 block in a hepatoma cell line, preventing the cells from entering the S-phase, accompanied by the elevated expression of c-jun transcripts (38). Antiestrogens can also modulate the cell cycle. Tamoxifen (and related compounds) gives rise to an accumulation of human breast cancer cells in the G1-phase; more than 90 % of cells were in G1 72-96 hours after adding Tamoxifen to the culture and the arrested cells retained their viability and responsiveness to estrogen (39-41). These effects of antiestrogens depend strongly on the presence of specific estrogen receptors (42). Somatostatin (SS) and its analogs also initiate antiproliferative signals in various endocrine and non-endocrine tumors, and the ligand-activated SS-receptors led to G1 arrest *in vitro* through up-regulation of the cyclin-dependent kinase inhibitor p27(Kip1) with concomitant accumulation and hypophosphorylation of Rb-protein (43, 44). The synthetic SS-analog octreotide was shown to exert a strong inhibitory action on cerulein-induced cyclin E expression (45). A new, synthetic heptapeptide SS-analog (TT-232) irreversibly blocks the cell cycle G1-S transition in human epidermoid carcinoma (A431) cells (46).

From synchronization to the checkpoint-regulators

Cell cycle regulators have attracted much attention, both in the field of cancer research and in treatment modalities. The cell cycle is mainly regulated through the cyclin-dependent kinases (CDKs) and cyclin complexes. Their deregulation often leads to development of malignant tumors, therefore it is not surprising that profound alterations are demonstrated in most human cancers. Hence, in the last decade, the "checkpoint-regulator" CDK-inhibitors have attracted particular interest and many new, promising molecules have been synthesized and tested. These studies have led to a better understanding of the molecular mechanisms of cell cycle control in malignant tumors and raised the prospect of specific molecule-targeted therapies.

On treatment of human pancreatic cancer cells with butyrolactone-I, the phosphorylation of RB-protein and the cyclin A expressions were significantly inhibited, and apoptotic cell death was detected accompanied by bcl-2 down-regulation (27). As for staurosporine, the primary cellular response to administration of this compound was found to be the accumulation of $p27^{kip1}$ (47), while CDK2

activity was suppressed. The latter effect was mainly due to rapidly increased levels of p21 and p27 proteins that inhibited CDK2 activity upon binding (48). Although the precise molecular mechanisms by which mimosine induces G1 arrest is still being unfolded, it seems to act at multiple points by regulating the expression of cyclins and the activities of CDKs. It inhibited cyclin D1-associated kinase activities without affecting gene transcription directly, and induced $p21^{cip1}$ expression (independently of p53 status) and $p27^{kip1}$ in non-small cell lung cancer cell lines. Unfortunately, one cannot generalize about these effects, because the results were cell line-dependent (36). The effect on the p21 pathway, however, seems to be more complex, because there are experimental data that continuous elevation in both p53 and p21 protein levels were detected over 48 hours after cells re-entered the cycle following synchronization (49).

Some compounds have been found effective not only *in vitro* but also *in vivo*. Olomoucine, a purine analog with a CDK1 inhibitory action (50), induced rapid and marked apoptosis in a dog with metastatic malignant melanoma after *i.v.* treatment for a week (51). Mimosine was also reported to significantly suppress human lung cancer growth xenografted into nude mice: 45-55% reduction of tumor volume was observed, accompanied by reduction of immunohistochemically-detected cyclin D1 and by induction of $p21^{WAF1}$. Moreover, TUNEL-analysis has revealed an increase in the apoptotic index of these xenografts (52). Our results also have shown that this plant amino acid can be applied *in vivo* without any measurable toxicity: when immunosuppressed mice bearing human pancreatic carcinoma xenografts were treated with *i.p.* mimosine a decreased tumor growth was achieved, and flow cytometry revealed a highly significant apoptosis (53). The Ki-67 index was also found to be significantly ($p<0.0001$) decreased indicating that, in addition to the *in vitro* effects, mimosine also acts as an antiproliferative agent *in vivo*.

Clinical applications

Without relevant clinical applicability, cell cycle blocking molecules would just remain within the confines of laboratories. Promising preclinical studies, however, have raised the possibility that various checkpoint regulators that affect G1 progression and/or G1-S transition might be suitable targets for cancer therapy (54, 55). Particularly the inhibition of CDKs seems to be the most productive strategy and hundreds of such compounds have been synthesized. Unfortunately, there is no individually selective CDK-inhibitor, but semi-selective agents (such as olomoucine), pan-CDK inhibitors (*e.g.* flavopiridol) or non-specific protein kinase groups (*e.g.* staurosporine) are worth investigating. Several Phase I and II clinical trials are currently under way, suggesting that this therapeutic approach could be justifiable in the treatment of various malignant tumors. Excellent reviews are available with these results (56, 57).

Flavopiridol, a synthetic flavonoid which inhibits several CDKs by competing with ATP, decreases the levels of cyclin D1 and D3, shows anti-angiogenic effect and induces apoptosis *in vitro* and in xenograft systems (58, 59), was the first such a compound to enter the clinic. Accumulated data show that this compound does have an antitumor effect on different malignancies with various therapeutic protocols; disease stabilization, minor response, partial response or even (occasionally) complete response could be achieved with mostly reversible side-effects (diarrhea, hypotension, proinflammatory syndrome, neutropenia, arterial and venous thromboses) (see 56, 57). Conversely, however, no clinical response was demonstrated in refractory mantle zone lymphoma patients who had received a prior chemotherapy regimen and 1 out of 10 patients developed grade III-IV non-hematological toxicity (60).

Staurosporine, which is a promiscuous protein kinase C inhibitor, *per se* has not been applied in clinical trials, but its derivate, 7-hydroxystaurosporine (UCN-01), is being tested with modest results (reviewed in 56, 57). In one patient suffering from malignant melanoma partial response was observed, and an anaplastic large cell lymphoma patient is disease-free after 4 years of introduction of the therapy (57). Side-effects were vomiting, hypoglycemia, hypotension and pulmonary toxicity. In another Phase II study, 21 renal cell carcinoma patients were treated with UCN-01, but no objective response was demonstrated. Although the therapy was well-tolerated, the time to disease progression proved to be about 3 months (61).

Proteosome inhibitors interfere with the degradation of CKIs (p21, p27) and may lead to stabilization of both CKI and cyclins. To date, only a single agent (bortezomib, Velcade) has been approved to enter clinical trials. Bortezomib showed antitumor activity in various hematological malignancies (62) and solid tumors (63) with reversible, but sometimes serious, side-effects. In a multiple myeloma patient, a complete response and in patients with mantle zone or follicular lymphoma, shrinkage of the tumor was noted (64). Phase III trials of this compound are under way.

E7070 is a sulfonamide-related compound that induces arrest at the G1-S boundary and inactivates the cyclin E/cdk2 complex. Administration of this drug to tumorous patients has yielded a limited antineoplastic effect: less than one-third of patients displayed temporary stabilization of their diseases, occasional partial response was observed and the dose-limiting toxicities were mainly hematological (65, 66).

Depsipeptide (FR901228) is a histone deacetylase inhibitor that was shown to produce mitotic arrest with G2-M accumulation in different cell lines (67). Phase I trials have demonstrated some antitumor activity of this

compound: in 1 out of 37 patients with refractory neoplasms a partial response was observed (68), while the therapeutic results were more promising in T-cell lymphomas; one patient with peripheral T-cell lymphoma having a complete response and 3 cutaneous lymphomas exhibiting a partial response (69). In chronic lymphoid leukemia or acute myeloid leukemia patients, however, no objective responses were noted (70). At present, Phase II studies are under way.

Another histone deacetylase inhibitor, a synthetic benzamide derivate (*MS-27-275*), was also tested *in vitro* and *in vivo* systems and Phase I studies are currently under way. MS-27-275 resulted in a shift in cell cycle (decrease of S-phase cells, increase of G1), induced $p21^{WAF1/cip1}$, and it had a marked antineoplastic activity in xenografts of various tumors (71, 72).

Combination treatment modalities

Unfortunately, malignant neoplasms frequently acquire resistance to the administered chemotherapeutic drugs during treatment. Multiple points of attack may result in a more powerful tumor-killing effect, may overcome the resistance and the individual doses could be decreased, minimizing the – sometimes serious – toxicities. Therefore, the combination of different drugs is a well established regimen in oncology. Accumulating data suggest that a combination of cell cycle regulators with different cytostatic drugs may be an alternative of choice.

In vitro studies have revealed that butyrolactone-I enhanced human colorectal carcinoma cell (DLD1) survival after doxorubicin treatment, but, unfortunately, it also blocks the process of apoptosis (73). Aphidicolin was also checked *in vitro*, and advantageous effects were detected: it enhanced etoposide cytotoxicity on human ovarian carcinoma cells (74), increased significantly the sensitivity of leukemia cells to cytosine arabinoside (particularly in acute myeloid leukemia) (75), and it was found to act synergistically with vincristine and doxorubicin against neuroblastoma cell lines (76). However, no potentiating effect was observed on cisplatin-resistant colonic or lung cancer cell lines (77) that might suggest a tumor-specificity.

Staurosporine and its derivates have also been studied in combination regimens. After pretreatment with staurosporine, breast cancer cells were selectively killed by doxorubicin and camptothecin, whereas normal mammary epithel cells resumed their proliferation after the cytostatic drugs were washed out (78). 7-Hydroxystaurosporine (UCN-01) proved to have a beneficial effect in different experimental model systems: combination with perifosine led to virtually complete growth inhibition *in vitro* (79), while enhanced radiosensitivity was observed in human lung adenocarcinoma cell lines (80). Hormonal treatments can also be augmented by this compound as was evidenced by Koh *et al.* (81): when MCF-7 human breast cancer cells were co-treated with UCN-01 plus Tamoxifen, this combination inhibited the cell growth synergistically and in xenografts exhibited superior antitumor effects. The results of ongoing clinical trials will show the real place of UCN-01 in practice.

Experimental and clinical studies have also been performed applying flavopiridol in combination. In addition to its radiosensitivity-enhancing effect alone or with docetaxel (82, 83), it enhanced the apoptotic rate of docetaxel in human gastric cancer *in vitro*, and markedly interfered with tumor growth in gastric cancer xenografts (84). The latter results seem to be meaningful, because carcinomas of the stomach are rarely sensitive to docetaxel. However, combination therapy in patients with metastatic breast cancer (n=6) failed, because dose-limiting neutropenia developed, although two disease stabilizations and one partial response were observed (85). Flavopiridol was also evaluated in a Phase I study in patients with advanced solid tumors (esophagus, lung, prostate carcinomas) following paclitaxel infusions, and clinical activities were observed. Despite neutropenia and pulmonary toxicity at higher doses, the authors recommended Phase II trials with lower dosage (86).

Earlier, it was mentioned that the proteosome inhibitor bortezomib given alone showed antitumor activity in various malignancies (62, 64). Experimental data have also shown its effectiveness when combined with cytostatic drugs. Using A549 non-small cell lung cancer cells, bortezomib plus a gemcitabine/carboplatin combination proved to be schedule-dependent: when the proteosome inhibitor was applied first, apoptotic activity was diminished, but simultaneous administration resulted in an increased apoptotic ratio (87). In another study, bortezomib in combination with gemcitabine enhanced p53 and p21 expression, induced apoptosis, destroyed 80% of tumor cells, and the A549 xenografts grew significantly slower in the combination group than in animals given gemcitabine alone (88). The same group has reported the results of the combination of two cell cycle regulators in lung cancer cells: bortezomib + a histone deacetylase inhibitor synergistically increased apoptosis (89). A similar synergistic effect was also observed *in vitro* and *in vivo* using bladder carcinoma cells and xenografts grown in nude mice (90).

Conclusion

Theoretically, chemotherapy should be more effective if the tumor cells (or the majority of them) were synchronized, because many chemotherapeutic drugs exhibit a phase-specificity. Recognition of the molecular checkpoints of the cell cycle has led to a novel antineoplastic strategy: inhibition of the cyclin- dependent kinases. Many cell cycle regulators have been synthesized and some of them are effective not only *in vitro* but also in *in vivo* experiments. Based on encouraging experimental studies, clinical trials are currently under way with these compounds, mainly in

Phase I or II. Administration of CDK inhibitors alone has rarely yielded complete remission of malignant tumors, though it is possible that combination with traditional cytostatic drugs may enhance their tumor-killing effects.

Acknowledgements

This work was financially supported by the Hungarian Scientific Research Fund (OTKA T 34899).

References

1 Lindmark G, Glimelius B, Pahlman L and Enblad P: Heterogeneity in ploidy and S-phase fraction in colorectal adenocarcinomas. Int J Colorectal Dis *6*: 115-120, 1991.
2 Fukunari H, Iwama T, Sugihara K and Miyaki M: Intratumoral heterogeneity of genetic changes in primary colorectal carcinomas with metastasis. Surg Today *33*: 408-413, 2003.
3 Baretton G, Kuhlmann B, Krech R and Lohrs U: Intratumoral heterogeneity of nuclear DNA-content and proliferation in clear cell type carcinomas of the kidney. Virchows Arch B Cell Pathol *61*: 57-63, 1991.
4 O'Malley FP, Grignon DJ, Keeney M, Kerkvliet N and McLean C: DNA heterogeneity in prostatic adenocarcinoma. A DNA flow cytometric mapping study with whole organ sections of prostate. Cancer *71*: 2797-2802, 1993.
5 Siitonen SM, Isola JJ, Rantala IS and Helin HJ: Intratumor variation in cell proliferation in breast carcinoma as determined by antiproliferating cell nuclear antigen monoclonal antibody and automated image analysis. Am J Clin Pathol *99*: 226-231, 1993.
6 Arnerlov C, Emdin SO, Cajander S, Bengtsson NO, Tavelin B and Roos G: Intratumoral variations in DNA ploidy and S-phase fraction in human breast cancer. Anal Cell Pathol *23*: 21-28, 2001.
7 Bergers E, vanDiest PJ and Baak JP: Tumor heterogeneity of DNA cell cycle variables in breast cancer measured by flow cytometry. J Clin Pathol *49*: 931-937, 1996.
8 Kaern J, Trope CG, Kristensen GB and Pettersen EO: Flow cytometric DNA ploidy and S-phase heterogeneity in advanced ovarian carcinoma. Cancer *73*: 1870-1877, 1994.
9 Tremmel SC, Gotte K, Popp S, Weber S, Hormann K, Bartram CR and Jauch A: Intratumoral genomic heterogeneity in advanced head and neck cancer detected by comparative genomic hybridization. Cancer Genet Cytogenet *144*: 165-174, 2003.
10 Coons SW and Johnson PC: Regional heterogeneity in the DNA content of human gliomas. Cancer *72*: 3052-3060, 1993.
11 Baisch H and Otto U: Intratumoral heterogeneity of S phase transition in solid tumours determined by bromodeoxyuridine labelling and flow cytometry. Cell Prolif *26*: 439-448, 1993.
12 Bertuzzi A, Faretta M, Gandolfi A, Sinisgalli C, Starace G, Valoti G and Ubezio P: Kinetic heterogeneity of an experimental tumour revealed by BrdUrd incorporation and mathematical modelling. Bull Math Biol *64*: 355-384, 2002.
13 Timson J: Hydroxyurea. Mutat Res *32*: 115-132, 1975.
14 Grdina DJ: Phase-specific cytotoxicity *in vivo* of hydroxyurea on murine fibrosarcoma pulmonary nodules. Br J Cancer *45*: 438-446, 1982.
15 Yerly-Motta V, Pavy JJ and Herve P: Screening of five specific cell cycle inhibitors using T cell lymphoma cell line synchrony/asynchrony assay. Biotech Histochem *74*: 119-128, 1999.
16 Siu WY, Yam CH and Poon RY: G1 *versus* G2 cell cycle arrest after adriamycin-induced damage in mouse Swiss3T3 cells. FEBS Lett *461*: 299-305, 1999.
17 Potter AJ, Gollahon KA, Palanca BJ, Harbert MJ, Choi YM, Moskovitz AH, Potter JD and Rabinovitch PS: Flow cytometric analysis of the cell cycle phase specificity of DNA damage induced by radiation, hydrogen peroxide and doxorubicin. Carcinogenesis *23*: 389-401, 2002.
18 Li MH, Ito D, Sanada M, Odani T, Hatori M, Iwase M and Nagumo M: Effect of 5-fluorouracil on G1 phase cell regulation in oral cancer cell lines. Oral Oncol *40*: 63-70, 2004.
19 Robles SJ, Buehler PW, Negrusz A and Adami GR: Permanent cell cycle arrest in asynchronously proliferating normal human fibroblasts treated with doxorubicin or etoposide but not camptothecin. Biochem Pharmacol *58*: 675-685, 1999.
20 Mastbergen SC, Duivenvoorden I, Versteegh RT and Geldof AA: Cell cycle arrest and clonogenic tumor cell kill by divergent chemotherapeutic drugs. Anticancer Res *20*: 1833-1838, 2000.
21 Locke V, Davey R and Davey M: Paclitaxel sensitization of multidrug-resistant cells to chemotherapy is independent of the cell cycle. Cytometry *43*: 170-174, 2001.
22 Sena G, Onado C, Cappella P, Montaleni F and Ubezio P: Measuring the complexity of cell cycle arrest and killing of drugs: kinetics of phase-specific effects induced by taxol. Cytometry *37*: 113-124, 1999.
23 Reiners JJ jr, Clift R and Mathieu P: Suppression of cell cycle progression by flavonoids: dependence on the aryl hydrocarbon receptor. Carcinogenesis *20*: 1561-1566, 1999.
24 Hwang S and Bowen PE: Cell cycle arrest and induction of apoptosis by lycopene in LNCaP human prostate cancer cells. J Med Food *7*: 284-289, 2004.
25 Fiore M, Zanier R and Degrassi F: Reversible G1 arrest by dimethyl sulfoxide as a new method to synchronize Chinese hamster cells. Mutagenesis *17*: 419-424, 2002.
26 Kues WA, Anger M, Carnwath JW, Paul D, Motlik J and Niemann H: Cell cycle synchronization of porcine fetal fibroblasts: effect of serum deprivation and reversible cell cycle inhibitors. Biol Reprod *62*: 412-419, 2000.
27 Wada M, Hosotani R, Lee JU, Doi R, Koshiba T, Fujimoto K, Miyamoto Y, Tsuji S, Nakajima S, Okuyama A and Imamura M: An exogenous cdk inhibitor, butyrolactone-I, induces apoptosis with increased Bax/Bcl2 ratio in p53-mutated pancreatic cancer cells. Anticancer Res *18*: 2559-2566, 1998.
28 Spadari S, Focher F, Sala F, Ciarrocchi G, Koch G, Falaschi A and Pedrali-Noy G: Control of cell division by aphidicolin without adverse effects upon resting cells. Arzneimittelforschung *35*: 1108-1116, 1985.
29 Lalande M: A reversible arrest point in the late G1 phase of the mammalian cell cycle. Exp Cell Res *186*: 332-339, 1990.
30 Watson PA, Hanauske-Abel HH, Flint A and Lalande M: Mimosine reversibly arrests cell cycle progression at the G1-S phase border. Cytometry *12:* 242-246, 1991.
31 Tsvetkov LM, Russev GC and Anachkova BB: Effect of mimosine on DNA synthesis in mammalian cells. Cancer Res *57*: 2252-2255, 1997.
32 Krude T: Mimosine arrests proliferating human cells before onset of DNA replication in a dose-dependent manner. Exp Cell Res *247*: 148-159, 1999.
33 Hoffman BD, Hanauske-Abel HM, Flint A and Lalande M: A new class of reversible cell cycle inhibitors. Cytometry *12*: 26-32, 1991.

34 Feldman ST and Schönthal A: Negative regulation of histone H1 kinase expression by mimosine, a plant amino acid. Cancer Res *54*: 494-498, 1994.
35 Wang G, Miskimins R and Miskimins WK: Mimosine arrests cells in G1 by enhancing the levels of p27(Kip1). Exp Cell Res *254*: 64-71, 2000.
36 Chang HC, Lee TH, Chuang LY, Yen MH and Hung WC: Inhibitory effect of mimosine on proliferation of human lung cancer cells is mediated by multiple mechanisms. Cancer Lett *145*: 1-8, 1999.
37 Kulp KS and Vulliet PR: Mimosine blocks cell cycle progression by chelating iron in asynchronous human breast cancer cells. Toxicol Appl Pharmacol *139*: 356-364, 1996.
38 Sanchez I, Goya L, Vallerga AK and Firestone GL: Glucocorticoids reversibly arrest rat hepatoma cell growth by inducing an early G1 block in cell cycle progression. Cell Growth Differ *4*: 215-225, 1993.
39 Osborne CK, Boldt DH, Clark GM and Trent JM: Effects of tamoxifen on human breast cancer cell cycle kinetics: accumulation of cells in early G1 phase. Cancer Res *43*: 3583-3585, 1983.
40 Osborne CK, Boldt DH and Estrada P: Human breast cancer cell cycle synchronization by estrogens and antiestrogens in culture. Cancer Res *44*: 1433-1439, 1984.
41 Bruno S, Di Vinci A, Geido E and Giaretti W: Cell cycle synchronization induced by tamoxifen and 17 beta-estradiol on MCF-7 cells using flow cytometry and a monoclonal antibody against bromodeoxyuridine. Breast Cancer Res Treat *11*: 221-229, 1988.
42 Dong XF, Berthois Y, Colomb E and Martin PM: Cell cycle phase dependence of estrogen and epidermal growth factor (EGF) receptor expression in MCF-7 cells: implications in antiestrogen and EGF cell responsiveness. Endocrinol *129*: 2719-2728, 1991.
43 Sharma K, Patel YC and Srikant CB: C-terminal region of human somatostatin receptor 5 is required for induction of Rb and G1 cell cycle arrest. Mol Endocrinol *13*: 82-90, 1999.
44 Pages P, Benali N, Saint-Laurent N, Esteve JP, Schally AV, Tkaczuk J, Vaysse N, Susini C and Buscail L: Sst2 somatostatin receptor mediates cell cycle arrest and induction of p27(Kip1). Evidence for the role of SHP-1. J Biol Chem *274*: 15186-15193, 1999.
45 Charland S, Boucher MJ, Houde M and Rivard N: Somatostatin inhibits Akt phosphorylation and cell cycle entry, but not p42/p44 mitogen-activated protein (MAP) kinase activation in normal and tumoral pancreatic acinar cells. Endocrinol *142*: 121-128, 2001.
46 Stetak A, Lankenau A, Vantus T, Csermely P, Ullrich A and Keri G: The antitumor somatostatin analogue TT-232 induces cell cycle arrest through PKCdelta and c-Src. Biochem Biophys Res Commun *285*: 483-488, 2001.
47 Nishi K, Schnier JB and Bradbury EM: The accumulation of cyclin-dependent kinase inhibitor p27kip1 is a primary response to staurosporine and independent of G1 cell cycle arrest. Exp Cell Res *243*: 222-231, 1998.
48 Shimizu T, Takahashi N, Tachibana K and Takeda K: Complex regulation of CDK2 and G1 arrest during neuronal differentiation of human prostatic cancer TSU-Pr1 cells by staurosporine. Anticancer Res *21*: 893-898, 2001.
49 Ji C, Marnett LJ and Pietenpol JA: Cell cycle re-entry following chemically-induced cell cycle synchronization leads to elevated p53 and p21 protein levels. Oncogene *15*: 2749-2753, 1997.
50 Schutte B, Nieland L, van Engeland M, Henfling ME, Meijer L and Ramaekers FC: The effect of the cyclin-dependent kinase inhibitor olomoucine on cell cycle kinetics. Exp Cell Res *236*: 4-15, 1997.
51 Hajduch M, Kolar Z, Novotny R, Hanus J, Mihal V, Hlobilkova A, Noskova V and Strnad M: Induction of apoptosis and regression of spontaneous dog melanoma following *in vivo* application of synthetic cyclin-dependent kinase inhibitor olomoucine. Anticancer Drugs *8*: 1007-1013, 1997.
52 Chang HC, Weng CF, Yen MH, Chuang LY and Hung WC: Modulation of cell cycle regulatory protein expression and suppression of tumor growth by mimosine in nude mice. Int J Oncol *17*: 659-665, 2000.
53 Zalatnai A and Bocsi J: Mimosine, a plant-derived amino acid induces apoptosis in human pancreatic cancer xenografts. Anticancer Res *23*: 4007-4010, 2003.
54 Buolamwini JK: Cell cycle molecular targets in novel anticancer drug discovery. Curr Pharm Des *6*: 379-392, 2000.
55 Owa T, Yoshino H, Yoshimatsu K and Nagasu T: Cell cycle regulation in the G1 phase: a promising target for the development of new chemotherapeutic anticancer agents. Curr Med Chem *8*: 1487-1503, 2001.
56 Senderowicz AM: The cell cycle as a target for cancer therapy: basic and clinical findings with the small molecule inhibitors flavopiridol and UCN-01. Oncologist *7* (Suppl. 3): 12-19, 2002.
57 Swanton C: Cell-cycle targeted therapies. Lancet Oncol *5*: 27-36, 2004.
58 Patel V, Senderowicz AM, Pinto D jr, Igishi T, Raffeld M, Quintanilla-Martinez L, Ensley JF, Sausville EA and Gutkind JS: Flavopiridol, a novel cyclin-dependent kinase-inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis. J Clin Invest *102*: 1674-1681, 1998.
59 Shapiro GI, Koestner DA, Matranga CB and Rollins BJ: Flavopiridol induces cell cycle arrest and p53-independent apoptosis in non-small cell lung cancer cell lines. Clin Cancer Res *5*: 2925-2938, 1999.
60 Lin TS, Howard OM, Neuberg DS, Kim HH and Shipp MA: Seventy-two continuous infusion flavopiridol in relapsed and refractory mantle cell lymphoma. Leuk Lymphoma *43*: 793-797, 2002.
61 Rini BI, Weinberg V, Shaw V, Scott J, Bok R, Park JW and Small EJ: Time to disease progression to evaluate a novel protein kinase C inhibitor, UCN-01, in renal cell carcinoma. Cancer *101*: 90-95, 2004.
62 Richardson PG, Hideshima T, Mitsiades C and Anderson K: Proteosome inhibition in hematologic malignancies. Ann Med *36*: 304-314, 2004.
63 Park DJ and Lenz HJ: The role of proteosome inhibitors in solid tumors. Ann Med *36*: 296-303, 2004.
64 Orlowski RZ, Stinchcombe TE, Mitchell BS, Shea TC, Baldwin AS, Stahl S, Adams J, Esseltine DL, Elliott PJ, Pien CS, Guerciolini R, Anderson JK, Depcik-Smith ND, Bhagat R, Lehman MJ, Novick SC, O'Connor OA and Soignet SL: Phase I trial of the proteosome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol *20*: 4420-4427, 2002.
65 Dittrich C, Dumez H, Calvert H, Hanauske A, Faber M, Wanders J, Yule M, Ravic M and Fomoleau P: Phase I and pharmacokinetic study of E7070, a chloroindolyl-sulfonamide anticancer agent, administered on a weekly schedule to patients with solid tumors. Clin Cancer Res *9*: 5195-5204, 2003.

66 Van Kesteren C, Beijnen JH and Schellens JH: E7070: a novel synthetic sulfonamide targeting the cell cycle progression of the treatment of cancer. Anticancer Drugs *13*: 989-997, 2002.
67 Sandor V, Robbins AR, Robey R, Myers T, Sausville E, Bates SE and Sackett DL: FR901228 causes mitotic arrest but does not alter microtubule polymerization. Anticancer Drugs *11*: 445-545, 2000.
68 Sandor V, Bakke S, Robey RW, Kang MH, Blagosklonny MV, Bender J, Brooks R, Piekarz RL, Tucker E, Figg WD, Chan KK, Goldspiel B, Fojo AT, Balcerzak SP and Bates SE: Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. Clin Cancer Res *8*: 718-728, 2002.
69 Piekarz RL, Robey R, Sandor V, Bakke S, Wilson WH, Dahmoush L, Kingma DM, Turner ML, Altemus R and Bates SE: Inhibitor of histone-deacetlyation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. Blood *98*: 2865-2868, 2001.
70 Byrd JC, Marcucci G, Parthun MR, Xiao JJ, Klisovic RB, Moran M, Lin TS, Liu S, Sklenar AR, Davis ME, Lucas DM, Fischer B, Shank R, Tejaswi SL, Binkley P, Wright J, Chan KK and Grever MR: A phase I and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia. Blood (prepublished online, Oct 5, 2004).
71 Saito A, Yamashita T, Maariko Y, Nosaka Y, Tsuchiya K, Ando T, Suzuki T, Tsuruo T and Nakanishi O: A synthetic inhibitor of histone deacetylase, MS-27-275, with marked *in vivo* antitumor activity against human tumors. Proc Natl Acad Sci USA *96*: 4592-4597, 1999.
72 Jaboin J, Wild J, Hamidi H, Khanna C, Kim CJ, Robey R, Bates SE and Thiele CJ: MS-27-275, an inhibitor of histone deacetylase, has marked *in vitro* and *in vivo* antitumor activity against pediatric solid tumors. Cancer Res *62*: 6108-6115, 2002.
73 Lu Y, Takebe H and Yagi T: Inhibition of X-ray and doxorubicin-induced apoptosis by butyrolactone I, a CDK-specific inhibitor, in human tumor cells. J Radiat Res (Tokyo) *41*: 341-348, 2000.
74 Erba E, Sen S, Lorico A and D'Incalci M: Potentiation of etoposide cytotoxicity against a human ovarian cancer cell line by pretreatment with non-toxic concentrations of methotrexate or aphidicolin. Eur J Cancer *38*: 66-71, 1992.
75 Sargent JM, Williamson CJ, Hubeek I, Elgie AW, Taylor CG, Den Boer ML, Peters GJ and Kaspers GL: Aphidicolin decreases *ex vivo* resistance to cytosine arabinoside in childhood acute leukemia. Oncol Rep *10*: 2027-2031, 2003.
76 Michaelis M, Vogel JU, Cinatl J, Langer K, Kreuter J, Schwabe D, Driever PH and Cinatl J: Cytotoxicity of aphidicolin and its derivatives against neuroblastoma cells *in vitro*: synergism with doxorubicin and vincristine. Anticancer Drugs *11*: 479-485, 2000.
77 Heim MM, Eberhardt W, Seeber S and Muller MR: Differential modulation of chemosensitivity to alkylating agents and platinum compounds by DNA repair modulators in human lung cancer cell lines. J Cancer Res Clin Oncol *126*: 198-204, 2000.
78 Chen X, Lowe M, Herliczek T, Hall MJ, Danes C, Lawrence DA and Keyomarsi K: Protection of normal proliferating cells against chemotherapy by staurosporine-mediated, selective, and reversible G1 arrest. J Natl Cancer Inst *92*: 1999-2008, 2000.
79 Dasmahapatra GP, Didolkar P, Alley MC, Ghosh S, Sausville EA and Roy KK: *In vitro* combination treatment with perifosine and UCN-01 demonstrates synergism against prostate (PC-3) and lung (A549) epithelial adenocarcinoma cell lines. Clin Cancer Res *10*: 5242-5252, 2004.
80 Xiao HH, Makeyev Y, Butler J, Vikram B and Franklin WA: 7-Hydroxystaurosporine (UCN-01) preferentially sensitizes cells with a disrupted TP53 to gamma radiation in lung cancer cell lines. Radiat Res *158*: 84-93, 2002.
81 Koh J, Kubota T, Koyama T, Migita T, Hashimoto M, Hosoda Y and Kitajima M: Combined antitumor activity of 7-hydroxystaurosporine (UCN-01) and tamoxifen against human breast carcinoma *in vitro* and *in vivo*. Breast Cancer *10*: 260-267, 2003.
82 Raju U, Nakata E, Mason KA, Ang KK and Milas L: Flavopiridol, a cyclin-dependent kinase inhibitor, enhances radiosensitivity of ovarian carcinoma cells. Cancer Res *63*: 3263-3267, 2003.
83 Kim JC, Saha D, Cao Q and Choy H: Enhancement of radiation effects by combined docetaxel and flavopiridol treatment in lung cancer cells. Radiother Oncol *71*: 213-221, 2004.
84 Motwani M, Rizzo C, Sirotnak F, She Y and Schwartz GK: Flavopiridol enhances the effect of docetaxel *in vitro* and *in vivo* in human gastric cancer cells. Mol Cancer Ther *2*: 549-555, 2003.
85 Tan AR, Yang X, Berman A, Zhai S, Sparreboom A, Parr AL, Chow C, Brahim JS, Steinberg SM, Figg WD and Swain SM: Phase I trial of the cyclin-dependent kinase inhibitor flavopiridol in combination with docetaxel in patients with metastatic breast cancer. Clin Cancer Res *10*: 5038-5047, 2004.
86 Schwartz GK, O'Reilly E, Ilson D, Saltz L, Sharma S, Tong W, Maslak P, Stoltz M, Eden L, Perkins P, Endres S, Barazzoul J, Spriggs D and Kelsen D: Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. J Clin Oncol *20*: 2157-2170, 2002.
87 Mortenson MM, Schlieman MG, Virudachalam S and Bold RJ: Effect of the proteosome inhibitor bortezomib alone and in combination with chemotherapy in the A549 non-small-cell lung cancer cell line. Cancer Chemother Pharmacol *54*: 343.353, 2004.
88 Denlinger CE, Rundall BK, Keller MD and Jones DR: Proteosome inhibition sensitizes non-small-cell lung cancer to gemcitabine-induced apoptosis. Ann Thorac Surg *78*: 1207-1214, 2004.
89 Denlinger CE, Keller MD, Mayo MW, Broad RM and Jones DR: Combined proteosome and histone deacetylase inhibition in non-small cell lung cancer. J Thorac Cardiovasc Surg *127*: 1078-1086, 2004.
90 Kamat AM, Karashima T, Davis DW, Lashinger L, Bar-Eli M, Millikan R, Shen Y, Dinney CP and McConkey DJ: The proteosome inhibitor bortezomib synergizes with gemcitabine to block the growth of human 253JB-V bladder tumors *in vivo*. Mol Cancer Ther *3*: 279-290, 2004.

Received November 9, 2004
Revised December 27, 2004
Accepted January 7, 2005