during Mitosis III. Quantitative analysis of ligand-receptor movement into the cleavage furrow: diffusion vs. flow. J Cell Biol 1982; 93:950-60.
5. Bramwell VHC, Crowther D, Gallagher J, Stoddart RW. Studies of lectin binding to normal and neoplastic lymphoid tissues. I. Normal nodes and Hodgkin's disease. Br J Cancer 1982; 46:568-81.
6. Wang Y, Silverman JD, Cao L. Single particle tracking of surface receptor movement during cell division. J Cell Biol 1994; 127:963-71.
7. Rosen, SD, Bertozzi CR. The selectins and their ligands. Curr Opin Cell Biol 1994; 3:701-10.
8. Pavalko FM, Walker DM, Graham L, Goheen M, Doerschuk CM, Kansas GS. The cytoplasmic domain of L-selectin interacts with cytoskeletal proteins via alpha-actinin: receptor positioning in microvilli does not require interaction with alpha-actinin. J Cell Biol 1995; 129:1155-64.
9. Bruehl RE, Springer TA, Bainton DF. Quantitation of L-selectin distribution on human leukocyte microvilli by immunogold labeling and electron microscopy. J Histochem Cytochem 1996; 44:835-44.
10. Borregaard N, Kjeldsen L, Sengelov H, et al. Changes in subcellular localization and surface expression of L-selectin, alkaline phosphatase, and MAC-1 in human neutrophils during stimulation with inflammatory mediators. J Leukoc Biol 1994; 56:80-7.
11. Brenner B, Glubins E, Busch GL, Koppenhoefer U, Lang F, Linderkamp O. L-selectin regulates actin polymerization via activation of the small G-protein Rac2. Biochem Biophys Res Commun 1997; 231:802-7.
12. Pilarski LM, Turley EA, Shaw AR, et al. FMC46, a cell protrusion-associated leukocyte adhesion molecule-1 epitope on human lymphocytes and thymocytes. J Immunol 1991; 147:136-43.
13. Lasky LA, Singer MS, Dowbenko D, et al. An endothelial ligand for L-selectin is a novel mucin-like molecule. Cell 1992; 69:927-38.
14. Baumhueter S, Dybdal N, Kyle C, Lasky LA. Global vascular expression of murine CD34, a sialomucin-like endothelial ligand for L-selectin. Blood 1994; 84: 2554-65.
15. Shailubhai K, Streeter PR, Smith CE, Jacob GS. Sulfation and sialylation requirements for a glycoform of CD34, a major endothelial ligand for L-selectin in porcine peripheral lymph nodes. Glycobiology 1997; 7:305-14.
16. Hemmerich S, Butcher EC, Rosen SD. Sulfation-dependent recognition of high endothelial venules (HEV)- ligands by L-selectin and MECA 79, and adhesion-blocking monoclonal antibody. J Exp Med 1994; 180:2219-26.
17. Hemmerich S, Bertozzi CR, Leffler H, Rosen SD. Identification of the sulfated monosaccharides of GlyCAM-1, an endothelial derived ligand for L-selectin. Biochemistry 1994; 33:4820-9.
18. Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. The role of alpha-4-integrins in lymphocyte homing to mucosal tissues in vivo. J Immunol. 1994; 52:3282-93.
19. True DD, Singer MS, Lasky, LA, Rosen SD. Requirement for sialic acid on the endothelial ligand of a lymphocyte homing receptor. J Cell Biol 1990; 111:2757-64.
20. Chandrasekaran EV, Rhodes JM, Jain RK, Bernack RJ, Matta KL. A biosynthetic control on structures serving as ligands for selectins: The precursor structures, 3-sialyl/sulfo Gal β1, 3/4GlcNAcβ-0-R, which are high affinity substrates for α1,3/4-l-fucosyltransferases, exhibit the phenomenon of substrate inhibition. Biochem Biophys Res Commun 1994; 201:78-89.
21. Varki A. Selectin ligands. Proc Natl Acad Sci USA 1994; 91:7390-7.
22. Spertini O, Kansas GS, Reimann KA, Mackay CR, Tedder TF. Function and evolutionary conservation of distinct epitopes of the leukocyte adhesion molecule-1 (TQ1, Leu8) that regulates leukocyte migration. J Immunol 1991; 147:942-9.
23. Kishimoto TK, Warnock RA, Jutila MA, et al. Antibodies against human neutrophil LECAM-1 (LAM-1/Leu-8/Dreg-56 antigen) and endothelial cell ELAM-1 inhibit a common CD18-independent adhesion pathway. Blood 1991; 78:805-11.
24. Allport JR, Ding HT, Ager A, Steeber DA, Tedder TF, Luscinskas FW. L-selectin shedding does not regulate human neutrophil attachment, rolling, or transmigration across human vascular endothelium in vitro. J Immunol 1997; 158:4365-72.
25. Konno A, Nunogami K, Wada T, et al. Inhibitory action of sulfatides, a putative ligand for L-selectin, on B cell proliferation and Ig production. Int Immunol 1996; 8:1905-13.
26. VanEden SF, Miyagashima R, Haley L, Hogg JC. A possible role for L-selectin in the release of polymorphonuclear leukocytes from bone marrow. Am J Physiol 1997; 272:H1717-24.
27. Sackstein R, Fu L, Allen KL. A hematopoietic cell L-selectin ligand exhibits sulfate-independent binding activity. Blood 1997; 89:2773-81.
28. Klein E, Klein G, Nadkarni JS, Nadkarni JJ, Wigzell H, Clifford P. Surface IgM-kappa specificity on a Burkitt lymphoma cell in vivo and in derived culture lines. Cancer Res 1968; 28:1300-10.
29. Spertini O, Callegari P, Cordey AS, et al. High levels of the shed form of L-selectin are present in patients with acute leukemia and inhibit blast cell adhesion to activated endothelium. Biochemistry 1994; 84:1249-56.
30. Po JL, Mazer B, Jensen GS. The L-selectin antibody FMC46 mediates rapid, transient increase in intracellular calcium in human peripheral blood mononuclear cells and Daudi lymphoma cells. Biochem Biophys Res Comm 1995; 217:1145-50.

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In vitro study of the combination gemcitabine + fludarabine on freshly isolated chronic lymphocytic leukemia cells

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Abstract

Background and Objective. **Fludarabine has shown a definite clinical activity in B-cell chronic lymphocytic leukemia (CLL). If the effects of this drug could be potentiated, it could be useful in order to obtain complete remissions. In this study we evaluated the effects of the combination of fludarabine and gemcitabine, a deoxycytidine analog that has shown both *in vitro* and *in vivo* activity against a variety of solid tumors.**

Design and Methods. **CLL cells from 10 patients were cultured *in vitro* in the presence of fludarabine (0.5-1,000 µg/mL) and gemcitabine (0.1-5,000 µg/mL), both alone and in different combinations. Cytotoxic activity was tested by the XTT colorimetric assay. Furthermore we evaluated BCL-2 protein expression and, subsequently, the induction of apoptosis at baseline and after exposing cells to different concentrations of fludarabine and gemcitabine.**

Results. **The IC_{50} of fludarabine and gemcitabine on CLL cells was 550 and 1,100 µg/mL, respectively, in our series of samples; the cytotoxicity of either drug was not influenced by the percentage of BCL-2 positive cells in the same sample. The addition of gemcitabine increased fludarabine-induced cytotoxicity; however, isobologram analysis of the data showed synergism only when lower doses of gemcitabine were combined to fludarabine. Induction of apoptosis reflected this pattern of activity.**

Interpretation and Conclusions. **Gemcitabine was able to increase the activity of fludarabine only when low doses of the former were employed. As both compounds incorporate into DNA blocking chain elongation, our results could be explained by the drugs interferring at that level. The possibility of potentiating the effects of fludarabine with low doses of gemcitabine renders this combination promising in view of an *in vivo* use.**

Key words: fludarabine, gemcitabine, CLL, apoptosis

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B-cell chronic lymphocytic leukemia (CLL) is a clonal hematologic disorder characterized by proliferation and accumulation of small lymphocytes, arrested at an intermediate stage of differentiation.[1] CLL the is most prevalent leukemia in western countries, affecting adults with a peak incidence between 60 and 80 years.[2] As mean survival of patients is 6 years,[3] the main goals of therapy have long been to improve quality of life and to prolong survival, rather than to eradicate the disease. Chlorambucil has been the most frequently used first-line drug in CLL therapy,[4] sometime substituted by cyclophosphamide or combination chemotherapy with COP or CVP[5,6] that without these producing a therapeutic advantage over single-drug schedules.

In the last decade fludarabine has been introduced in the treatment of CLL. It was initially demonstrated to be active in patients resistant to chlorambucil,[7-9] further studies have shown the superiority of fludarabine over chlorambucil even in untreated patients, with an increased percentage and duration of complete responses.[10] Furthermore, complete responses have been observed even at the molecular level,[11] rendering it feasible to eradicate the disease with more aggressive post-remission therapies, especially in younger patients.[12,13] It could thus be beneficial to enhance the activity of fludarabine by testing it in combination with other drugs.

Gemcitabine is a deoxycytidine analog that has shown significant antineoplastic activity both *in vitro* and *in vivo*.[14] This drug affects DNA synthesis by acting at different sites: incorporation into DNA and blockage of chain elongation,[15] incorporation into RNA with inhibition of synthesis,[16] and ribonucleotide reductase inhibition.[17] Furthermore it has been demonstrated that gemcitabine is able to induce apoptosis in chronic myeloid leukemia cells.[18] In this study we investigated the cytotoxic effects and the amount of apoptosis induced in CLL cells *in vitro* by the combination of gemcitabine and fludarabine.

Design and Methods

B-CLL cells

Heparinized peripheral blood was obtained, after informed consent, from 10 patients with B-CLL (Table 1). Mononuclear cells were collected after

Table 1. Patient characteristics.

Pt.	Sex/age	previous therapy	disease status	Lymph. (x10⁹/L)	Hb (g/dL)	Plt. (x10⁹/L)	BCL-2 (% cells)
1. MP	M/46	none	onset	11.6	13.9	183	72
2. ME	M/71	chlorambucil	stable	30.0	13.6	119	67
3. SD	F/75	none	onset	18.7	11.7	309	58
4. MI	F/78	chlorambucil	stable	15.0	8.6	104	63
5. TA	M/58	none	onset	53.5	14.3	281	85
6. TE	M/62	chlorambucil	progression	228.0	8.7	98	53
7. EL	M/67	chlorambucil	stable	32.0	14.1	150	73
8. GG	M/64	chlorambucil	progression	59.0	9.9	115	65
9. OE	M/69	chlorambucil	progression	40.3	13.5	117	81
10. GP	M/46	chlorambucil	progression	86.8	11.4	92	ND

sedimentation on Ficoll Hypaque (Lymphoprep, Nycomed Pharma, Oslo, Norway), adherent cells were eliminated by 1 hour adherence on plastic flasks. After purity testing with monoclonal antibodies (anti CD2, CD4, CD5, CD19, CD20), non-adherent cells were resuspended in RPMI1640 medium (Gibco Europe, Paisley, UK) supplemented with 10% fetal calf serum (FCS, Gibco).

Drugs

Fludarabine was purchased from Inveresk Clinical Research (Edinburgh, Scotland); the drug was dissolved in normal saline and subsequently diluted in RPMI1640 medium. Gemcitabine was kindly provided by Eli Lilly Italia SpA (Sesto Fiorentino, FI, Italy); the compound was first dissolved in normal saline, and then final dilutions made in RPMI1640 medium.

Evaluation of cytotoxicity

Two for 10^5 CLL cells were resuspended in 200 µL RPMI1640 + 10% FCS and were seeded in triplicate in 96 well microtiter plates. Drugs were added, either alone or in various combinations, at concentrations ranging from 0.5 to 1,000 µg/mL (fludarabine) and from 0.1 to 5,000 µg/mL (gemcitabine). These concentrations were chosen on the basis of previously reported data.[18,19] Control cultures were carried out with same amount of media but without drugs. After 72 hours of incubation at 37°C, 50 µg of 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-(phenylaminocarbonyl)-2H-tetrazolium hydroxide (XTT, Sigma Chemicals, St Louis, MO, USA) and 0.38 µg of phenazine methosulfate (PMS, Sigma), were added to each well, as previously described.[20,21] After 4 hours of incubation at 37°C the plates were mixed on a mechanical plate shaker and absorbance at 450 nM was measured by an EIA microwell reader (Sigma Diagnostics). Growth inhibition was calculated as percent of control; the cytotoxic activity of the drug combination was evaluated by isobologram analysis of the data.[22]

Evaluation of apoptosis

One million cells were seeded in 25 mm^2 sterile plastic flasks with gemcitabine 10 or 100 µg/mL±fludarabine 5 µg/mL. After 72 hours of incubation, apoptosis was evaluated by a cytofluorimetric method, as described elsewhere.[23] Briefly, the samples were fixed in ethanol 70% and subsequently treated with RNAse 0.5 mg/mL for 15' at 37°C. Cell pellets were resuspended in 50 µg/mL propidium iodide in PBS. Analysis was performed on a FACScan flow cytometer (Becton Dickinson) with the FL2 detection in logarithmic mode using Lysis II software. Apoptotic cells were located in the hypodyploid region of the histogram.

Evaluation of BCL-2 protein expression

Immunodetection of intracellular BCL-2 protein expression was performed as previously reported.[24] Briefly, 2×10^6 cells were fixed with paraformaldehyde/triton in PBS. Before labeling, cells were incubated for 10 minutes with 2% heat-inactivated human AB serum in order to prevent non-specific binding of monoclonal antibody (MoAb) to Fc receptors. Indirect immunofluorescence staining was then carried out using an anti-BCL-2 MoAb (Dako Italy, SpA, Milan, Italy) and the isotype-specific fluorescein conjugated goat anti-mouse antibody (Dako). Cells were then washed out and analyzed, using a FACscan flow cytometer equipped with Lysis II software. The results were expressed as the percentage of positive cells (compared to background fluorescence, for which mouse IgG was used in place of MoAb). Each experiment was performed in duplicate and repeated at least twice.

Results

Cytotoxic effect

Figure 1 shows the cytotoxic activity, as measured by XTT microculture assay, of gemcitabine on CLL cells of our series of patients. Under our experimental conditions, the IC_{50} of the drug was 1,100 µg/mL. The dose-

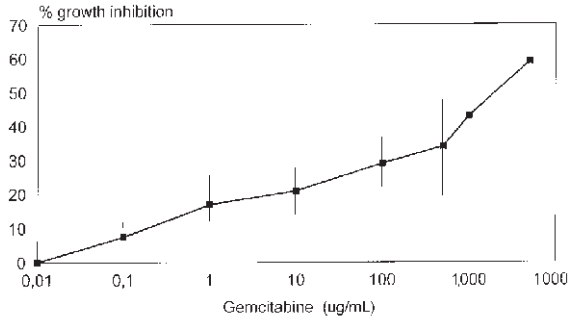

Figure 1. Cytotoxic effect of gemcitabine on CLL cells. Data are expressed as mean ± SD of 10 different cases.

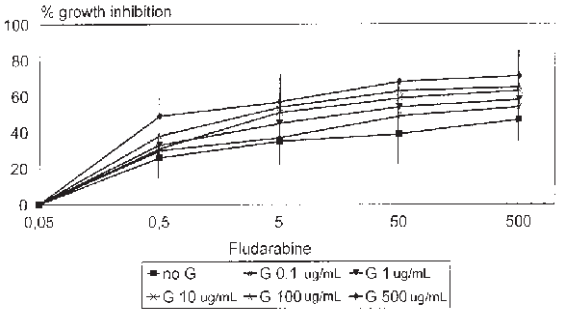

Figure 2. Cytotoxic effects of fludarabine ± gemcitabine on CLL cells. Data are expressed as mean ± SD of 10 different cases. *G = gemcitabine.*

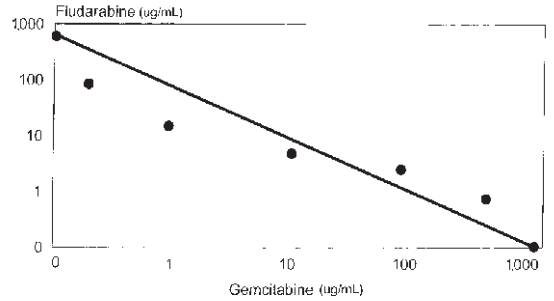

Figure 3. Isobologram analysis of the cytotoxic effect of the combination fludarabine + gemcitabine on CLL cells. Pooled data from 10 cases are represented.

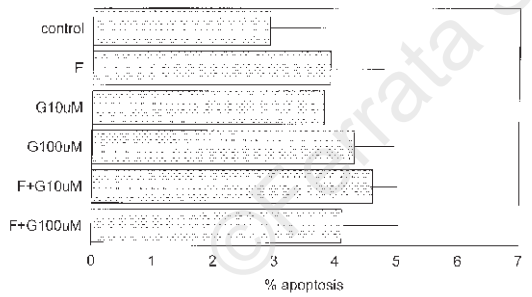

Figure 4. Induction of apoptosis on CLL cells after treatment with fludarabine (F, 5 µg/mL) and gemcitabine (G, 10 and 100 µg/mL) both alone and in combination. Data are expressed as mean ± SD of 10 different cases.

response curves of the combination of fludarabine (IC_{50} = 550 µg/mL) and gemcitabine are reported in Figure 2. Gemcitabine enhanced fludarabine-induced cytotoxicity on CLL cells (23% and 28% increase when gemcitabine 1 µg/mL was added to fludarabine 5 or 50 µg/mL, respectively). However, isobologram analysis of the data (Figure 3), demonstrated that the two drugs acted synergistically only when low doses of gemcitabine were combined with fludarabine, while with higher doses of gemcitabine the drug combination appeared to have merely additive effects.

Apoptosis

Both fludarabine[20] and gemcitabine[18] are known to induce apoptosis. Here we tested whether the combination of the two drugs could act synergistically on this phenomenon. As shown in Figure 4, a low percentage of apoptotic cells was detectable even in untreated samples, this percentage increased significantly upon incubation with either fludarabine or gemcitabine alone. The combination of fludarabine 5 µg/mL with a low dose of gemcitabine (10 µg/mL) induced a significantly higher amount of apoptosis than that induced by each drug alone (p=0.02 and p=0.007 compared to fludarabine 5 µg/mL and to gemcitabine 10 µg/mL, respectively). Increasing the concentration of gemcitabine, however, apoptosis failed to increase by more than the amount observed after exposure to the single drug.

BCL-2 protein expression

BCL-2 protein was detected in all the samples in which the assay was performed (Table 1). The mean percentage of positive cells was 68.5±10.3 (range 53-85%). There was no relationship between the percentage of BCL-2 cells and different disease status, or sensitivity to Fludarabine and to gemcitabine.

Discussion

In recent years, the use of fludarabine has significantly changed the therapeutic options that can be offered to CLL patients. In fact, the possibility of achieving complete remissions even at the molecular level renders it feasible to employ intensive consolidation therapies, such as autologous stem cell transplantation, at least in younger patients. With the aim of eradicating the disease, it could be useful to potentiate the effects of Fludarabine by adding drugs that could exert a synergistic activity. The addition of cyclophosphamide ± mitoxantrone improved the activity of fludarabine *in vitro*[25] and *in vivo*, in low-grade non-Hodgkin's lymphomas;[26] fludarabine has shown to inhibit cellular repair of cisplatin-induced DNA damage *in vitro*;[27] combination with Ara-C resulted in a synergistic effect as fludarabine enhances Ara-C metabolism and incorporation into DNA.[28]

In this study we evaluated the effects of the combination of fludarabine with gemcitabine, another pyrimidine deoxyribonucleoside analog, that has shown activity, both *in vitro* in a variety of solid tumors and leukemia models,[14] and *in vivo* as monotherapy for non-small cell lung cancer,[29] pancreatic cancer,[30] peripheral T-cell lymphomas,[31] Hodgkin's disease and non-Hodgkin's lymphomas.[32] In accordance with results reported by Morabito *et al.*,[33] our data show that BCL-2 protein expression is not correlated to *in vitro* sensitivity to fludarabine and no relation-

ship was found between BCL-2 and the efficacy of gemcitabine. Our results demonstrated a synergistic activity of the drug combination when low doses of gemcitabine were used, while higher concentrations of gemcitabine had only an additive effect. The evaluation of apoptosis reflected this pattern of activity. These data are not, however, unexpected. Both fludarabine and gemcitabine exert their actions by incorporating into DNA, so one can hypothesize interference of the two drugs at that level when both drugs are used in high doses. This was indirectly demonstrated by Iwasaki *et al.*,[34] who examined the different patterns of incorporation into DNA of various nucleoside analogs. At variance to Ara-C, which incorporates preferentially into repairing DNA, both fludarabine and gemcitabine incorporate within replicating DNA. So fludarabine and Ara-C could cooperate at different DNA sites, while fludarabine and gemcitabine, when both are used in high doses, could cause an intracellular accumulation of *false* substrates to replicating DNA that cells are not able to use. This effect would be further enhanced in CLL by the slow proliferating activity of the cells.

Although studies would be useful in order to clarify the relationship between fludarabine and gemcitabine at the level of DNA, the possibility of enhancing the efficacy of fludarabine with a low dose gemcitabine is worth testing *in vivo* in CLL patients.

Contributions and Acknowledgments

PT and PLZ designed the study, analyzed the data and wrote the paper with the collaboration of MM; AP performed all the experiments; GV and ST revised the manuscript and gave their final approval.

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Disclosures

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References

1. Gale RP, Foon KA. Chronic lymphocytic leukemia: recent advances in biology and treatment. Ann Intern Med 1985; 103:101-20.
2. Li FP. Epidemiology of chronic leukemias. In: Wiernik PH, Canellos GP, Kyle RA, Schiffer CA, eds. Neoplastic diseases of tha blood, 2nd ed. New York:Churchill and Livingstone 1991, p. 7-14.
3. Lee JS, Dixon DO, Kantarjian HM, Keating MJ, Talpaz M. Prognosis of chronic lymphocytic leukemia: a multivariate regression analysis of 325 untreated patients. Blood 1987; 63:929-36.
4. Galton DAG, Wiltshaw E, Szur E, Dacie JV. The use of chlorambucil and steroids in the treatment of chronic lymphocytic leukemia. Br J Hematol 1961; 7:73-98.
5. Liepman M, Votaw ML. The treatment of chronic lymphocytic leukemia with COP chemotherapy. Cancer 1978; 41:1664-9.
6. Oken MM, Kaplan ME. Combination chemotherapy with cyclophosphamide, vincristine and prednisone in the treatment of refractory chronic lymphocytic leukemia. Cancer Treat Rep 1979; 63:441-7.
7. Keating MJ, Kantarjian H, Talpaz M. Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. Blood 1988; 74:19-25.
8. O'Brien S, Kantarjian H, Beran M, et al. Results of fludarabine and prednisone therapy in 264 patients with chronic lymphocytic leukemia with multivariate analysis-derived prognostic model for response to treatment. Blood 1993; 82:1695-700.
9. Zinzani PL, Lauria F, Rondelli D, et al. Fludarabine in patients with advanced and/or resistant B-chronic lymphocytic leukemia. Eur J Haematol 1993; 51:93-7.
10. Keating MJ, Kantarjian H, O'Brien S, et al. Fludarabine: a new agent with marked cytoreductive activity in untreated chronic lymphocytic leukemia. J Clin Oncol 1991; 9:44-9.
11. Spriano M, Clavio M, Carrara P, et al. Fludarabine in untreated and previously treated B-CLL patients: a report on efficacy and toxicity. Haematologica 1994; 79:218-26.
12. Bastion Y, Felman P, Dumontet C, Espinouse D, Coiffier B. Intensive radiochemotherapy with peripheral blood stem cell transplantation in young patients with chronic lymphocytic leukemia. Bone Marrow Transplant 1992; 10:467-8.
13. Khouri I, Keating MJ, Vriesendorp HM, et al. Autologous and allogeneic bone marrow transplantation for chronic lymphocytic leukemia: preliminary results. J Clin Oncol 1994; 12:748-58.
14. Hertel LW, Border JB, Kroin JS, et al. Evaluation of antitumor activity of gemcitabine (2'-2'-difluoro-2'-deoxycytidine). Cancer Res 1990; 50:4417-9.
15. Plunkett W, Huang P, Xu YZ, Heinemann V, Grunewald R, Gandhi V. Gemcitabine:metabolism, mechanism of action and self-potentiation. Semin Oncol 1995; 22(Suppl 11):3-10.
16. Heinemann V, Schultz L, Issels RD, Plunkett W. Gemcitabine: a modulator of intracellular nucleotide and deoxynucleotide metabolism. Semin Oncol 1995;22 (Suppl 11):11-8
17. Heinemann V, Xu YZ, Chubb S, et al. Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2'2'-difluorodeoxycytidine. Mol Pharmacol 1990; 38:567-72.
18. Santini V, Bernabei PA, Gozzini A, et al. Apoptotic and antiproliferative effects of gemcitabine and gemcitabine plus Ara-C on blast cells from patients with blast crisis chronic myeloproliferative disorders. Haematologica 1997; 82:11-5.
19. Santini V, D'Ippolito G, Bernabei PA, Zoccolante A, Ermini A, Rossi-Ferrini P. Effects of fludarabine and gemcitabine on human acute myeloid leukemia cell line HL-60. Direct comparison of cytotoxicity and cellular Ara-C uptake enhancement. Leuk Res 1996; 20:37-45.
20. Zinzani PL, Buzzi M, Farabegoli P, et al. Apoptosis induction with fludarabine on freshly isolated chronic myeloid leukemia cells. Haematologica 1994; 79: 127-31.
21. Zinzani PL, Tosi P, Visani G, et al. Apoptosis induction with three nucleoside analogs on freshly isolated B-chronic lymphocytic leukemia cells. Am J Hematol 1994; 47:301-6.
22. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple

drugs and enzyme inhibitors. Adv Enzyme Regul 1984; 22:27-55.
23. Tosi P, Visani G, Ottaviani E, Manfroi S, Zinzani PL, Tura S. Fludarabine-Ara-C+G-CSF:cytotoxic effects and induction of apoptosis on fresh acute leukemia cells. Leukemia 1994; 8:2076-82.
24. Tosi P, Pellacani A, Visani G, et al. In vitro treatment with retinoids decreases BCL-2 protein expression and enhances dexamethasone-induced cytotoxicity and apoptosis in multiple myeloma cells. Eur J Haematol 1998; 61:1-6.
25. Morabito F, Callea I, Console G, et al. The in vitro cytotoxic effect of mitoxantrone in combination with fludarabine or pentostatin in B-cell chronic lymphocytic leukemia. Haematologica 1997; 82:560-5.
26. Mc Laughlin P, Hagemeister FB, Romaguera JE, et al: Fludarabine, mitoxantrone and dexamethasone: an effective new regimen for indolent lymphoma. J Clin Oncol 1996; 14:1262-8.
27. Yang LY, Li L, Keating MJ, et al. Arabinosyl-2-fluoroadenine augments cisplatin cytotoxicity and inhibits cisplatin-DNA cross-link repair. Mol Pharmacol 1995; 47:1072-9.
28. Gandhi V, Novak B, Keating MJ, Plunkett W. Modulation of arabinosylcytosine metabolism by arabinosyl-2-fluoroadenine in lymphocytes from patients with chronic lymphocytic leukemia: implications for combination therapy. Blood 1989; 74:2070-5.
29. Postmus PE, Schranel FM, Smit EF, et al. Evaluation of new drugs in small cell lung cancer: the activity of gemcitabine. Semin Oncol 1998; 25 (suppl 9):79-82.
30. Brand R, Capadano M, Tempero M. A phase I trial of weekly gemcitabine administered as a prolonged infusion in patients with pancreatic cancer and solid tumors. Invest New Drugs 1997; 15:331-41.
31. Zinzani PL, Magagnoli M, Bendandi M, et al. Therapy with gemcitabine in pretreated peripheral T-cell lymphoma patients. Annal Oncol 1998; 9:1351-3.
32. Santoro A, Devizzi L, Bonfante V, et al. Phase II study with gemcitabine in pretreated patients with Hodgkin's (HD) and non-Hodgkin's lymphomas (NHL): results of a multicentric study. Proc ASCO 1997; 16:A71.
33. Morabito F, Filangeri M, Callea I, et al. BCL-2 protein expression and p53 gene mutation in CLL: correlations with in vitro sensitivity to chlorambucil and purine analogs. Haematologica 1997; 82:16-20.
34. Iwasaki H, Huang P, Plunkett W. Differential incorporation of Ara-C, gemcitabine and fludarabine into replicating and repairing DNA in proliferating human leukemia cells. Blood 1997; 90:270-8.