DRUGS EXPTL. CLIN. RES. XXII(Suppl.) 43-50 (1996)

# COMPARISON OF CHEMOTHERAPY AND X-RAY THERAPY WITH UKRAIN MONOTHERAPY FOR COLORECTAL CANCER

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**Summary**: *Ninety six colorectal carcinoma patients were included in a randomised study. 48 were treated with Ukrain monotherapy (15 with metastatic and 33 with nonmetastatic colorectal carcinoma) and 48 with 5-fluorouracil (5-FU) and X-ray therapy (the same randomised groups). The results of therapy including clinical, haematological, immunological and biochemical parameters show that Ukrain has favourable properties in the treatment of colon and rectal cancer as a monotherapy because of its malignotoxic and immunomodulating action. Objective response rate in the group of metastatic colorectal cancer treated by Ukrain was 40% There was no registered tumour regression in the group treated by 5-FU. Operability is strongly facilitated by pretreatment with Ukrain. The survival rate (up to 21 months) in the Ukrain-treated patients with nonmetastatic colorectal cancer was 78.6% and 33.3% in a corresponding control group. Ukrain is a new effective drug in the therapy of colorectal cancer. It can be useful both for the therapy of metastatic colorectal cancer and for neoadjuvant therapy of nonmetastatic colorectal cancer.*

## Introduction

Ukraine is chemically a Chelidonine thiophosphoric acid derivative: Tris{2-{[5bS-(5ba,6b, 12ba)]-5b,6,7,12b,13,14-hexahydro-13-methyl[1,3]benzodioxolo[5,6-c-]-1,3-dioxolo[4,5-i]phenanthridinium-6-ol]-ethaneaminy}phosphine-sulfide 6HCl. (Patent No. 4.970.212. USA, 1990). (Fig.1)

High toxicity and unsatisfactory results of 5-fluorouracil require further investigation to find new agents for colorectal cancer treatment (1, 2).

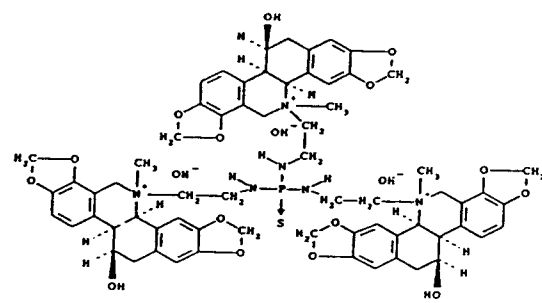

**Fig. 1** Formula of Ukrain.

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**Table I** *TNM and Dukes' Staging in colon carcinoma*

| | Patient group Ukrain | | | Patient group 5-Fluorouracil | | |
|---|---|---|---|---|---|---|
| | TNM-Staging | Dukes' | No. of pts. | TNM-Staging | Dukes' | No. of pts. |
| 1. Cancer of rectum | $T_1N_0M_0$ | a | 1 | $T_1N_0M_0$ | a | 1 |
| | $T_2N_xM_0$ | a | 4 | $T_2N_xM_0$ | a | 2 |
| | $T_2N_0M_0$ | $b_1$ | 2 | $T_2N_0M_0$ | $b_1$ | 3 |
| | $T_2N_1M_0$ | $b_1$ | 4 | $T_2N_1M_0$ | $b_1$ | 3 |
| | $T_3N_0M_0$ | $b_2$ | 4 | $T_3N_0M_0$ | $b_2$ | 3 |
| | $T_4N_0M_0$ | $b_2$ | 4 | $T_4N_0M_0$ | $b_2$ | 2 |
| | $T_3N_xM_1$ hep | c | 4 | $T_4N_xM_0$ | c | 2 |
| | $T_4N_1M_x$ | c | 1 | $T_4N_1M_0$ | c | 1 |
| | $T_4N_3M_1$ hep | c | 2 | $T_3N_xM_1$ hep | c | 4 |
| | | | | $T_4N_1M_x$ | c | 1 |
| | | | | $T_4N_3M_1$ hep | c | 2 |
| 2. Cancer of sigmoid | $T_2N_0M_0$ | a | 1 | $T_1N_0M_0$ | a | 1 |
| | $T_3N_0M_0$ | $b_1$ | 3 | $T_2N_0M_0$ | a | 1 |
| | $T_3N_1M_0$ | c | 2 | $T_3N_0M_0$ | $b_1$ | 4 |
| | $T_4N_1M_0$ | c | 2 | $T_3N_1M_0$ | c | 1 |
| | $T_4N_1M_1$ hep | c | 2 | $T_4N_1M_0$ | c | 2 |
| | | | | $T_4N_xM_1$ hep | c | 1 |
| | | | | $T_4N_1M_1$ hep | c | 1 |
| 3. Cancer of ascending colon | $T_3N_0M_0$ | $b_2$ | 2 | $T_3N_0M_0$ | $b_2$ | 1 |
| | $T_3N_xM_1$ hep | c | 1 | $T_3N_xM_1$ hep | c | 1 |
| | $T_4N_3M_1$ hep | c | 2 | $T_4N_3M_1$ hep | c | 3 |
| | $T_4N_3M_1$ hep pancreas | c | 1 | | | |
| 4. Cancer of caecum | $T_3N_0M_0$ | $b_2$ | 2 | $T_2N_xM_0$ | a | 1 |
| | $T_4N_xM_0$ | $b_2$ | 2 | $T_3N_0M_0$ | $b_2$ | 3 |
| | $T_4N_1M_1$ hep | c | 2 | $T_4N_xM_0$ | $b_2$ | 2 |
| | | | 48 | $T_4N_1M_1$ hep | c | 2 |
| | | | | | | 48 |

New properties of Ukrain are broadly shown (3-8) with special immunological activities *in vitro, in vivo* and clinically (9-13). The malignotoxic properties of Ukrain were evaluated on different cancer cell culture lines (EORTC, European Organisation of Research and Treatment of Cancer, The Netherlands: E90/029, W122, UKRS-222; NSC B238865; National Cancer Institute, Bethesda, Maryland, USA NSC: 63 1570-W/1) (14, 15). It was shown that Ukrain increased macrophage tumouricidal activity in murine adenocarcinomas (16).

Published results from the National Cancer Institute, Bethesda, Maryland, USA (17) showed that Ukrain (NSC 63 1570) had a more than 100-fold higher cytotoxic activity on human colon carcinoma cell culture lines (Colo 205, DLD-1, HCC-2998, HCT-116, HT29, KM12, KM20L2, SW620) than the traditionally broadly-used 5-fluorouracil (NSC 19893). In the EORTC study Ukrain was toxic to the colorectal cell line CXF. It was the aim of this study to show whether there is a correlation of the *in vitro* effects of Ukrain to clinical experience, and to evaluate the usefulness of Ukrain as a new drug in the treatment of colorectal cancer.

The toxic and immunosuppressive influence of cytostatic agents has adverse effects on homeostasis in colon cancer patients. Oncological therapy would require maximal toxicity against tumour cells and minimal toxicity to the organism

measured from the date of randomisation to death or to the date of last communication.

The criteria for treatment toxicity were defined by the World Health Organization (23).

**Results**

Group 1 patients with metastatic colorectal cancer, who had received Ukrain, showed after 5-6 injections in all cases (100%) from day 10 to 12 improvement of their general condition, decreased toxic signs, decreased fatigue and vomiting, reappearance of appetite, reduced subfebrility and improvement of sleep. Ten patients (66.7%) after treatment noticed a local effect such as decreased rectal bleeding, improvement of faecal movement and decreased local pain. Colostomy was postponed in five patients. After two courses of Ukrain treatment, the Karnofsky index increased from 60.7 to 72.9. Tumour nodes became softer and more movable. Objective decrease in the size of primary tumours or liver metastases in metastatic colorectal cancer after Ukrain treatment was noticed in 6 of 15 cases (response rate 40%). Of the four metastatic colorectal carcinoma patients started on Ukrain therapy in 1993, three had duration-of-life over 15 months and one patient is still alive after two years.

Group 2 Ukrain-treated patients with colorectal cancer showed in 30 cases (90.9%) notable improvement of general condition; in 13 cases (39.3%) there was a positive local effect, with decreased local pain; tenesmus and rectal bleeding stopped. Resectability was achieved in eight patients. No metastases were seen during operation. Decrease of bleeding from tumour tissue at mechanic contact and absence of ulceration were noted. After two courses of Ukrain treatment the Karnofsky index increased from 70.6 to 79.4. Of 14 patients in the second group treated by Ukrain in 1992-1993, 11 (78.6%) are still alive; two of them were not operated and nine had radical surgery. Only one patient has died from those operated radically.

In both Ukrain-treated groups, toxicity was 0 according to WHO criteria. No general or local negative responses (including allergic reactions) to administration of the preparation were reported. Three patients had an increase in body temperature up to 38°C during the first three injections but afterwards the temperature returned to normal.

Patients in the 1st control group with metastatic colorectal cancer showed after 5-FU therapy subjective deterioration in general condition in 14 cases (93.3%). We observed worsening of the general status, appetite, sleep and appearance of fatigue. Intoxication signs in these patients increased: nausea (toxicity 2), lethargy (toxicity 2), cardiac dysrhythmia (toxicity 1), and hand-foot syndrome (according to WHO criteria). The Karnofsky index decreased from 63.6 to 55.0 after courses of 5-FU therapy. Improvement of the local status (decrease of local pain and cessation of rectal bleeding) was observed in only one case (6.7%) in a patient receiving X-ray therapy. Objective regression of primary tumour or metastases was not observed. From the three patients treated by 5-FU in 1993, no one lived more than 11 months. In 12 cases (80%) we observed hepatotoxic or nephrotoxic effects of 5-FU and in five cases chemotherapy was stopped because of an increase of hepatotoxic effects manifested in two to three-fold increases in transaminase activity and an increase in bilirubin level above normal. Nephrotoxic effects were revealed by the appearance of protein in the urine and a rise of creatinine by more than 20%.

Patients in the 2nd control group of colorectal cancer showed a deterioration in their general condition in 29 cases (87.9%). Local effect was registered in only three cases (9.1%) receiving X-ray therapy. The Karnofsky index decreased from 70.3 to 65.6 after 5-FU therapy. Hepato-, nephro- and neurotoxicity were observed in 20 cases (60.6%); in three cases there was only one course of 5-FU because of toxicity. Of the 15 patients treated by 5-FU in 1992-1993, after the 21-months observation period five are still alive (33.3%); in three cases there was radical surgery and in two cases palliative surgery. Of 10 patients with fatal outcome, five were operated radically.

The median values of the haematological, bio-

Table II *Median values of haematological, biochemical and immunological parameters*

| | | UKRAIN Therapy | | Control: FU Therapy | |
|---|---|---|---|---|---|
| | | before | after | before | after |
| erythrocytes | $10^6$/mm³ | 4.01±0.22 | 4.02±0.24 | 3.98±0.36 | 3.31±0.24 |
| leukocytes | $10^3$/mm³ | 9.62±1.42 | 9.02±1.7 | 9.53±0.99 | 8.66±0.99 |
| lymphocytes | | 23.80±4.09 | 29.78±4.15 | 24.47±2.86 | 17.12±2.11 |
| rod-shaped | | 12.23±4.9 | 9 38±4.6 | 11.37±2.44 | 13.87±3.84 |
| segmented | | 55.35±3.7 | 53.76±3.6 | 56.31±2.31 | 58.55±2.89 |
| eosinophils | | 3.41±0.71 | 4.23±0.98 | 3.54±1.11 | 2.82±1.02 |
| monocytes | | 6.22±1.01 | 5.43±1.12 | 5.98±1.09 | 6.21±1.16 |
| l.per. lymph. | | 1.52±0.29 | 3.63±0.24 | 1.62±0.21 | 1.08±0.20 |
| T-lymphocytes | | 39.8±2.87 | 45.89±3.45 | 39.28±3.37 | 34.31±3.23 |
| B-lymphocytes | | 8.87±1.47 | 10.64±2.98 | 9.34±1.43 | 7.46±1.38 |
| T-helper | | 29.04±2.67 | 31.64±1.65 | 30.32±2.11 | 24.08±1.11 |
| T-suppressor | | 30.04±1.45 | 23.88±1.55 | 30.12±2.8 | 27.43±3.09 |
| H/S ratio | | 0.97±0.07 | 1.32±0.11 | 1.01±0.12 | 0.88±0.14 |
| granular lymphs. | % | 1.49±0.11 | 3.47±0.28 | 1.67±0.27 | 1.05±0.22 |
| NC-activity | | 25.43±3.33 | 37.96±4.12 | 24.98±3.02 | 27.7±4.53 |
| phag. activity | | 86.61±1.33 | 99.72±2.05 | 86.46±1.84 | 89.16±2.65 |
| phag. index | | 9.42±1.22 | 14.06±1.34 | 9.87±1.30 | 9.46±1.08 |
| SGOT | U/l | 0.29±0.08 | 0.21±0.03 | 0.33±0.07 | 0.92±0.4 |
| SGPT | U/l | 0.35±0.06 | 0.30±0.04 | 0.33±0.08 | 0.56±0.3 |
| IgA | mcg/ml | 2.88±1.12 | 4.07±1.02 | 2.91±0.82 | 3.01±0.56 |
| IgM | mcg/ml | 0.72±0.11 | 0.85±0.12 | 0.78±0.11 | 0.98±0.24 |
| IgG | mcg/ml | 12.23±1.42 | 18.54±2.21 | 11.98±1.66 | 14.84±1.74 |
| CIK | ng/ml | 271.84±17.3 | 202.33±15.82 | 287.41±20.2 | 305.43±21.8 |
| Interferon | IU | 15.3±1.8 | 21.8±2.0 | 15.9±2.6 | 10.7±2.3 |
| proteins | g/dl | 75.21±1.66 | 74.54±2.05 | 74.48±3.81 | 69±72±2.55 |
| bilirubin | mg/dl | 18.39±3.22 | 17.92±2.96 | 18.67±4.43 | 22.39±2.88 |
| sodium | m val | 142.5±5.22 | 136.8±7.88 | 142.3±4.96 | 133.8±6.42 |
| potassium | m val | 4.15±0.42 | 4.21±0.91 | 4.17±0.56 | 3.98±0.76 |

chemical and immunological parameters are shown in Table II. Positive changes in the Ukrain treated group were recognised in the following parameters:

1. Increase in lymphocytes, B-lymphocytes.
2. Decrease in erythrocyte sedimentation rate.
3. Tendency to increase of T-lymphocytes (Fig. 2); increase of T-helpers (Fig. 3).
4. Increase in killer cell activity (Fig. 4).
5. Increase in phagocytic activity and phagocytic index.
6. Normalization of the H/S ratio.
7. Increase in IgG.

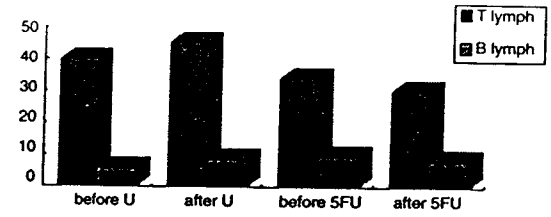


Fig. 2 Evaluation of T- and B-lymphocytes.

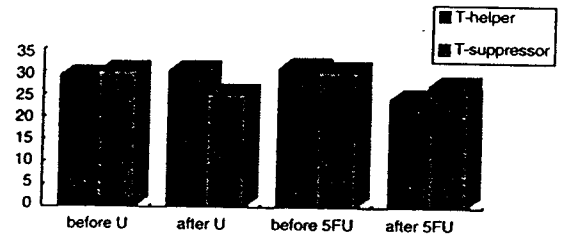


Fig. 3 Evaluation of T-helper and T-suppressor cells.

8. Decrease in circulating immune complexes.
9. Increase in large granular lymphocytes (Fig. 5).
10. Increase in peripheral blood lymphocytes (Fig. 6).
11. No negative changes in biochemical status.

Histological examination of tumour areas from biopsies showed a relative decrease of the adenocarcinoma mass, but an increase of tumour necrosis. Invading lymphocytes were found. Some cases showed sclerosis of the stroma of adenocarcinoma. Production of mature collagen was seen in the stroma of one case of rectal cancer. Necroses appeared in perivascular areas. Proliferation and development of metastases during the intervals between biopsies were not noticed.

The control group with 5-fluorouracil therapy showed in blood examinations:

1. Tendency to decrease in erythrocytes and lymphocytes.
2. Decrease in the immunological parameters.
3. Increase of the circulating immune complexes.
4. Decrease of the H/S ratio.
5. Tendency to decrease of the large granulated

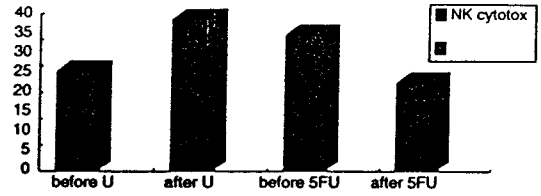


**Fig. 4** Evaluation of natural killer (NK) cytotoxicity.

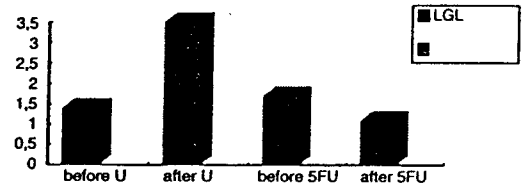


**Fig. 5** Evaluation of large granular lymphocytes (LGL).

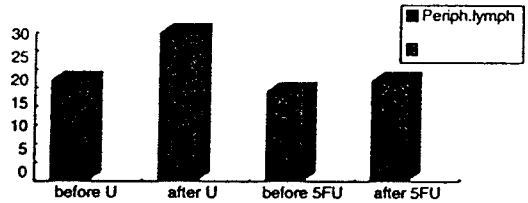


**Fig. 6** Evaluation of peripheral blood lymphocytes.

lymphocytes.

No positive changes in blood parameters and immunograms were observed in patients from the control groups.

The groups treated with Ukrain showed less bleeding during endoscopic procedures than the control groups. No wounds were seen after biopsy in Ukrain-treated patients in contrast to the control groups.

## Discussion

Results of treatment with 5-FU in our control group do not appear to differ from results in other clinical reports. Combination of 5-FU with interferon-alpha-2b for treatment of colorectal cancer has not been shown to be more effective (2, 24-26). No synergistic activity exists between the combination of 5-FU and alpha-interferon (24).

The present controlled clinical study shows clearly the major effects, atoxicity and tolerability of Ukrain in patients with colon carcinomas when compared with the traditional cytostatic therapy with 5-FU. The immunostimulating properties connected with the cancerostatic properties of Ukrain allow an improvement of the general status of advanced colorectal cancer patients whose other possible therapy modalities had already been exhausted. The most important result achieved by treatment with Ukrain was the possibility of changing an inoperable status to operability and resectability of tumours.

In respect to parameters of colorectal carcinoma patients before and after treatment with Ukrain or 5-fluorouracil our studies clearly show advantages of therapy with Ukrain, in contrast to 5-FU therapy, for all randomised groups. The objective response-rate in the group of metastatic colorectal cancers treated with Ukrain was 40%, while there was no tumour regression in the group treated with 5-FU. We observed improvement of general status, decrease of fatigue, restoration of appetite, decrease of toxic signs (10-12 days from start of treatment) in patients treated with Ukrain in 90.9-100% of cases. In patients of the control groups who received generally accepted therapy including 5-FU, we observed worsening of the general status, appetite and sleep, together with appearance of fatigue, in 87.9-93.3%. Local improvement was registered in 39.3-66.7% cases under Ukrain therapy and in 6.7-9.1% of 5-FU-treated patients only if they had concomitant X-ray therapy. In the group treated with Ukrain we observed disappearance of toxic signs: nausea, lethargy, cardiac dysrhythmia, with a toxicity of 0 according to WHO criteria. In the control group increased signs were observed: nausea (toxicity 2), lethargy (toxicity 2), cardiac dysrhythmia (toxicity 1) and hand-foot syndrome, according to WHO criteria.

The survival rate up to 21 months in the Ukrain group was 78.6%; in the corresponding control group it was 33.3%. The survival rate was analy-

sed from 14-15 patients in the 2nd and 4th groups from the years 1992-1993. The remaining 69 patients started their treatment only in 1994, so too short a time has elapsed for evaluation of the results, which is reasonable only after 12 months. For this reason these cases are not included in the survival rate control. Their results will be published later.

We have no doubt that Ukrain is a necessary component in treatment modalities of colorectal carcinomas. This study has shown that the high sensibility of human colorectal cancer in the clinic corresponds to the *in vitro* results of colon cancer cell lines (17). These results indicate the brood introduction of Ukrain in the treatment of human colorectal cancer.

**Conclusion**

This study shows the many advantages of Ukrain therapy, compared with standard therapies, in patients with colorectal cancer. Ukrain reduces the primary tumour and metastases in 40% of metastatic colorectal cancer patients and can be useful in these cases. With preoperative and postoperative application of Ukrain, the results of the treatment were improved so that we can recommend Ukrain for neoadjuvant therapy of colorectal cancer.

## References

(1) Nichols P.H. et al. *Peri-operative modulation of cellular immunization in patients with colorectal cancer.* Clin. Exp. Immunol., **94**, 4, 1993.

(2) Punt C.J. et al. *Continuous infusion of high-dose 5-fluorouracil in combination with leucovorin and recombinant interferon-alpha-2b in patients with advanced colorectal cancer. A Multicenter Phase 2 study.* Cancer, **72**, 2107, 1993.

(3) Chlopkiewicz B., Marczewska J., Ejchart A., Anusewska E., Koziorowska J. *Evaluation of mutagenic, genotoxic and transforming properties of Ukrain.* Durgs Exptl. Clin. Res., **XVIII** (Suppl.), 31-34, 1992.

(4) Juszkiewicz T., Minta M., Wlodarczyk B., Biernacki B. *Teratological evaluation of Ukrain in hamsters and rats.* Drugs Exptl. Clin. Res., **XVIII** (Suppl.), 23-29, 1992.

(5) Kleinrok Z., Jagiello-Wojtowicz E., Matuszek B., Chodkowska A. *Basic central pharmaological properties of thiophosphoric acid alkaloid derivatives from* Chelidonium Majus L. Pol. J. Pharmacol. Pharm., **44**, 227, 1992.

(6) Kleinrok Z., Jagiello-Wojtowicz E., Nowicky J.W., Chodkowska A., Feldo M., Matuszek B. *Some pharmacological properties of prolonged administration of Ukrain in rodents.* Drugs Exptl. Clin. Res., **XVIII** (Suppl.), 93-96, 1992.

(7) Remiszewska M., Wutkiewicz M., Jastrzebski Z., Czyzewska-Szafran H., Danysz A. *Pharmacological effects of Ukrain in rats and rabbits.* Acta Pol. Pharm., 49,43, 1992.

(8) Wyczolkowska J., Czuwaj M., Maslinski C. *The immunomodulating preparation Ukrain does not induce anaphylactic sensitization in mice and guinea pigs.* Drugs Exptl. Clin. Res., **XVIII** (Suppl.), 35-38, 1992.

(9) Danilos J., Zbroja-Sontag W., Baran E., Kutylcio L., Kondratowicz L., Jusiak L. *Preliminary studies on the effect of Ukrain (Tris {2-{5BS-(5BA,6B, 12BA)}-5B,6,7,12B,13,14-hexahydro-13-methyl[1,3] benzodioxolo[5,6-C]-1-3-dioxolo[4,5,i]phenanthridinium-6-OL}-ethaneaminyl)phosphinesulfide·6HCl) on the immunological response in patients with malignant tumours.* Drugs Exptl. Clin. Res., **XVIII** (Suppl.), 55-62, 1992.

(10) Liepins A. *Enhancement of cell mediated lysis on tumor cells by* Chelidonium Majus L. *alkaloids (Ukrain).* J. Cancer Res. Clin. Oncol., **116** (Suppl.), A3 118.10, 1990.

(11) Nowicky J.W., Staniszewski A., Zbroja-Sontag W., Slesak B., Nowicky W., Hiesmayr W. *Evaluation of thiophophoric acid alkaloid derivatives from* Chelidonium Majus L. *("Ukrain") as an immunostimulant in patients with various carcinomas.* Drugs Exptl. Clin. Res., **XVIII**, 139-143, 1991.

(12) Slesak B., Nowicky J.W., Harlozinska A. In vitro *effects of thiophosphoric acid derivatives from* Chelidonium Majus L. *on normal lymphocyte subpopulation.* J. Cancer Res. Clin. Oncol., **116** (Suppl.), A3.112.50, 1990.

(13) Slesak B., Nowicky J.W., Harlozinska A. In vitro *effects of* Chelidonium Majus L. *alkaloid thiophosphoric acid conjugates (Ukrain) on the phenotype of normal human lymphocytes.* Drugs Exptl. Clin. Res., **XVIII** (Suppl.), 17-21, 1992.

(14) Hohenwarter O., Strutzenberger K., Katinger H., Liepins A., Nowicky J.W. *Selective inhibition of* in vitro *cell growth by the anti-tumour drug Ukrain.* Drugs Exptl. Clin. Res., **XVIII** (Suppl.), 1-4, 1992.

(15) Lipiens A., Nowicky W. *Ukrain is selectively cytostatic and/or cytotoxic to human tumour and HIV-infected cells but not to human normal cells.* Recent Advances in Chemotherapy, Anticancer Section. Proceedings of the 17th International Congress of Chemotherapy, Berlin, 1991.

(16) Sotomayor E., Rad K., Lopez D.M., Liepins A. *Enhancement of macrophage tumouricidal activity by the alkaloid derivative Ukrain.* In vitro *and* in vivo *studies.* Drugs Exptl. Clin. Res., **XVIII** (Suppl.), 5-11, 1992.

(17) Nowicky J.W., Nowicky W., Liepins A. *Cytostatic and cytotoxic effects of Ukrain on malignant cells.* 8th Mediterranean Congress of Chemotherapy, Athens, Greece, May 1992. J. Chemioterapia, 5 (Suppl. 1), 797, 1993.

(18) Liepins A., Nowicky J.W. *Activation of spleen cell lytic*

*activity by the alkaloid thiophosphoric acid derivative: Ukrain.* Int. J. Immunopharmacol., **14**, 1437, 1992.

(19) Nowicky J.W. *New immuno-stimulating anti-cancer preparation "Ukrain".* 13th International Congress of Chemotherapy, Vienna 1983. PS 12 5 33/A-6, part. 288/57.

(20) Nowicky J.W., Liepins A., Staniszewski A., Slezak B., Nowicky W., Hiesmayr W. *The malignotoxic and immune modulating property of the alkaloid derivative Ukrain.* J. Cancer Res. Clin. Oncol., **118** (Suppl.), VI.09.05, 1992.

(21) Vatanasapt V. et al. *Preliminary report on clinical experience in the use of Ukrain.* Thai Cancer J., **17**, 20, 1991.

(22) Kampf M. et al. *Some problems involving* in vitro *cellular cytotoxicity assay.* Natl. Cancer Invest., **37**, 37, 1973.

(23) Miller A. et al. *Reporting results of cancer treatment.* Cancer, **47**, 207, 1981.

(24) Pittman K. et al. *Pharmacokinetcs of 5-fluorouracil in colorectal cancer patients receiving interferon.* Ann. Oncol., **4**, 515, 1993.

(25) Tsujimura T. et al. *Treatment of advanced gastric and colorectal cancer with 5-FU, leucovorin and interferon-alpha.* Gan.To. Kagaku. Ryoho, **20**, 493, 1993.

(26) Mitomi T. et al. *Randomized controlled study on adjuvant immunotherapy with PSK in curatively resected colorectal cancer.* The Cooperative Study Group of Surgical Adjuvant Immunochemotherapy for Cancer of Colon and Rectum. Gan. To. Kagaku Ryoho., **16**, 2241, 1989.