Expert Report

On the

CLINICAL DOCUMENTATION

of

Ukrain

(Chelidonium majus L. – alkaloid thiophosphoric acid complex)

BY

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Vienna, 08. August 2002

AUTHOR´S SIGNATURE

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Abbreviations

AFP	alpha-fetal protein
b.w.	body weight
CEA	carcino-embryonal antigen
CR	complete remission
CRP	C-reactive protein
DAB	Deutsches Arzneimittelbuch
EORTC	European Organisation for Research and Treatment of Cancer
ESR	erythrocyte sedimentation rate
5-FU	5-fluorouracil
MCA	mucin-cancer antigen
NCI	National Cancer Institute
NK	natural killer (cells)
PD	progressive disease
PR	partial remission
SD	stable disease
TEPA	triethylene-thiophosphoric acid
TME	total mesorectal excision
TNM	tumor -node-metastasis classification

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1. Problem statement

1.1 Current treatment

Cancer ranks among the most frequent reasons for death. Despite of enormous efforts to improve results in the treatment of cancer patients, most drugs that are currently marketed are rather toxic. Their use in order to reduce tumor burden and to improve survival has to be balanced against their (at least transient) impact on the quality of life.

Among cancers, carcinoma of the gastrointestinal tract and of breast rank among the most frequent tumors observed.

Pancreas cancer makes up 2-3% of all malignant tumors and is the 6th most common oncological disease and the 5th most common cause of cancer death with an incidence of approximately 9 per 100,000 (1). At the time of diagnosis most patients show progression of the disease beyond the pancreas, either through direct invasion of surrounding structures or metastases formation in regional lymph nodes, liver, peritoneum, lungs, bones or brain. Median survival time is approximately 4-6 months after diagnosis, with five-year survival rates <5 %. Prognosis is slightly better only in those few cases where pancreatic resection is possible but the impact of existing chemotherapy is still negligible.

Fluorouracil (5-FU) has been studied most extensively, with no consistent effect on survival. More recently, gemcitabine alone or in combination, has been shown to increase marginally survival by a few months to a median of 6 to 8 months (2, 3).

Colorectal cancer is the second leading cause of cancer death (after lung cancer) and the third leading cause of cancer in men and women. Incidence rates vary from 2 to 50/100,000 with highest rates in the United States and the lowest rates in parts of Southeast Asia and the middle East. In the United States, in 1998, there were approximately 95,600 new cases and 47,700 deaths from colon cancer and 36,000 new cases from rectal cancer with 8,800 deaths respectively (4). The majority (60%) of rectal carcinoma are diagnosed in the age group of 60-79 years. About 58% present at stage I or II, 27% at stage III and 15% at stage IV (5). Apart from cancer stage, prognostic risk factors for rectal carcinoma are obstructing tumors, mucin-producing tumors, cancers arising less than 6 cm from the anal verge, aneuploid tumors, perforated tumors (serosal and venous invasion), age, and perioperative transfusions. Low serum albumin, high gGT and high CEA predict also for poor survival (after 6).

Surgical treatment is the primary approach of colorectal carcinoma. Resection of rectal carcinoma however is usually limited by the proximity of the tumor to adjacent structures in the pelvis. Even when a resection is considered curative (R0), the 5-year survival is only around 50%. Relative survival for colon cancer according to various sources is given below.

1: 1-7

2: 7-18

3: 19-24

4: 25-36

5: 37-47

6: 48-54

Survival of Patients with Rectal Adenocarcinoma (treated with resection only

AJCC/ UICC-Stage	Dukes-Stage	TNM-Group	Median 5y Disease-free Survival (95% CI)	
I	A, B1	pT1/pT2, pN0, M0	81%[K]; 84.4%[T] (73.8-95),	T=**7**:55-60
II	B2, B3	pT3/pT4, pN0, M0	63.8%[K]; 62.7%[T] (53.7-71.7)	K=**8**:61-70
III	C1-3	pT1-4, pN1/pN2/pN3, M0	20-50%[K]; 39.3%[T], (30.3-48.3)	
IV	D	(+ resection of liver metastases)	<30%	

AJCC = American Joint Committee of Cancer; UICC = Union Internationale contre le Cancer;
percentages after [T] Tominaga et al., 1996; [K] Kane 1991);

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Colorectal carcinoma tend to disseminate micro-metastases early during disease. Distant relapse is the underlaying cause of mortality in most patients of stage II and III. Relapse is more frequent in rectal carcinoma; the first sites are local recurrence, the liver and the lung, followed by bone and other sites. Most recurrences of colorectal carcinoma after surgery are reported to occur within 2-3 years of surgery. Dissemination is the main rationale for an adjuvant chemotherapy for Dukes stage C, but also stage B. However, apart from tumor resection, there is no universally accepted drug treatment: In 1995, it was estimated that less than 40% of Dutch and British oncologists recommended 5-FU after surgery whereas the majority of German and US oncologists practised adjuvant chemotherapy (after 9).

9: 71-78

Pre-operative radiotherapy can significantly reduce local recurrence rates. Five-years survival with surgery alone is between 40 to 69% (local recurrence: 24 to 30%), but 10 to 20% (relative percentages) higher after adjuvant preoperative radiotherapy for stage II to III carcinoma (15 to 34.5 Gy; after 9). Combination of radiation with chemotherapy also improves treatment results in comparison to surgery alone with 65% 5-year survival and 11% local recurrence rates (after 7); total mesorectal excision (TME) and additional radiotherapy may also substantially lower recurrence rates. Adjuvant chemotherapy considered to be the actual standard consists of 5-FU combined with Leucovorin or Levamisole and shows improved 5-years survival rates around 65% and local recurrence rates around 15%. However, follow-up shorter than 5 years may result in false-positive differences as advantages of chemotherapy seem to disappear with time (8). Chemotherapy, in addition to surgical treatment with resection of liver metastases, improves survival also in advanced, colorectal carcinoma by approximately 6 months, from a median between 5-11 months to 7.5-15 months. It affects also positively the quality of remaining life (6).

7:55-60

8: 61-70

6: 48-54

From these observations it must be concluded that prognosis of patients with colorectal cancer is still poor and that the therapeutic gain of current adjuvant (radio) chemotherapy is small, being statistically significant only for disease-free survival but rarely also for overall survival, whatever the treatment will be.

Apart from colorectal cancer, breast cancer ranks also among the most frequent reasons for death. Breast cancer is currently the primary cause for tumor-induced death among women, especially those aged between 35 and 55 years. According to statistics of the WHO, annual death rates/100.000 were in Austria (1998): 39.0, in Germany (1997): 43.7. The estimated incidence is about twice as high.

The tumor-inducing properties of most drugs used for treatment of breast cancer preclude their (neoadjuvant or prophylactic) use in non-metastatic disease, in particular in young women.

1.2 The contribution of UKRAIN

Since the first therapeutic use in 1978, Ukrain (administered either as neoadjuvant treatment before surgery or as combination therapy or alone) has been the subject of numerous experimental and clinical tests.

According to the manufacturer, Ukrain (NSC-631570) is a Chelidonium majus L. -thiophosphoric acid derivative, a complex of Chelidonium majus L.-alkaloids with

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triethylene-thiophosphoric acid triamide (Thio-TEPA). The molecular structure of Ukrain is unknown. The injection solution contains Ukrain in concentration of 1 mg/ml (at least 90% Chelidonium majus alkaloid-thiophosphoric acid derivative and a maximum of 10% of free Chelidonium majus alkaloids). No Thio-TEPA or free Aziridine ring compounds can be detected.
Chelidonium majus (Greater celandine) extracts are well known in herbal medicine since more than 3,000 years, in particular for the treatment of inflammatory and gastrointestinal diseases. Chelidonium herba (Schöllkraut) is listed in the "Deutsches Arzneimittelbuch" (DAB) 10, 2nd Appendix 1993.

Ukrain: European Patent No. 0083600,
US Patent No. 2,670,347.

Ukrain has received marketing authorisation in numerous Eastern-European and Asian countries such as Belarus (White Russia, 8.1.1995, reg. No. 1330/95), Ukraine (15.10.1998, reg. No. 3641) Georgia (5.8.1999, reg. No. 002861), Turkmenistan (13.4.2000, reg. No. 0001707), Azerbaijan Republic (5.9.2000, reg. No. 00267), and Tadchikistan (7.9.2000, reg. No. 000568) for the treatment of various cancer.
The substance is a bright yellow crystalline powder. The injection solution is a transparent, bright yellow liquid with the odour of freshly cut grass and a bitter taste. The preparation comes as a sterile 0.1% (1 mg/ml) aqueous injection solution (pH: 3.5 to 6.5) in amber-coloured ampoules of 5 ml, without any excipients.
Ukrain is stable against UV radiation; under UV light Ukrain shows a yellow-orange autofluorescence. Due to this autofluorescence Ukrain can also be easily detected in tissues.
Thio-TEPA is listed in many pharmacopoeia (e.g. Great Britain, Japan, France, USA) and is approved as a cytostatic in Austria. It is worth mentioning that Thio-TEPA was ineffective in previous trials with colorectal carcinoma (after 8); this is, accordingly, also not a registered indication.

8: 61-70

The exact mode of action of Ukrain is largely unknown. In a recent in-vitro study where various pancreatic cancer cell lines have been incubated with different concenctrations of Ukrain and its pure components, Thio-TEPA and chelidonine, it was found that Ukrain and chelidonine lead to a significant accumulation of cells in G2/M phase in all investigated cell lines in concentrations of 0.6 µg/ml chelidonine or above and 5 µg/ml Ukrain or above. At the same concentrations also a significant reduction of proliferation rates after 48 hours could be observed (all cell lines: $p < 0.05$). In Giemsa stains, a significant accumulation of cells in the prophase was found; fluorescence immuno-histochemistry with antibodies against a-tubulin revealed that Ukrain and chelidonine lead to a disruption of the microtubule network in the investigated cell lines. Furthermore, it was shown that in in-vitro polymerisation assays Ukrain and chelidonine stabilize monomeric tubulin (10).

10: 79-103

In another experiment with pancreas cancer cell lines, Ukrain (10µg/mL) showed a high accumulation of treated cells in the G2/M phase, whereas apoptosis rate of peripheral mononuclear cells did not show any differences between treated and untreated cells; mitogen-stimulated lymphocytes showed even an increased blastogenic response (11).

11: 104-109

In another experiment using cancer cell lines A431 and ME180 as well as normal human keratinocytes as control, it was demonstrated that, at concentrations of 7µM Ukrain, cancer cells but not human keratinocytes accumulate in G2/M-phase over a 24-h period. In addition, apoptosis was detected following 48 h treatment (12).

12: 110-114

Other investigations on the possible mechanism of action of Ukrain on malignant cells (K562 leukemia cells) showed similar results, suggesting that Ukrain induces bimodal cell death programmes: First, apoptosis, mediated by quinidine sensitive

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Ca++ dependent K+ channels and second, blister cell death, by preventing microtubule formation, thus inducing polyploidy (13).
From these experiments it can be concluded that Ukrain inhibits cell cycle progression of pancreatic and other cancer cells in M-phase by stabilizing monomeric tubulin, thus being an "anti-tubulin-drug".
Ukrain seems to inhibit (reversibly) also angiogenesis at relatively low concentrations (10-50μM, approx. 15-75μg/mL, 14).

13: 115-121

14: 122-126

In vitro tests of the National Cancer Institute (NCI), Bethesda, USA, demonstrated an inhibitory effect of Ukrain against all of 8 colon cancer cell lines tested, at molar concentrations between Log $10^{-4.5}$ and Log $10^{-5.5}$ (corresponding to concentrations between ≈7.6μg/mL and 76.0μg/mL). In contrast, 5-FU showed barely an inhibition of the same cell lines at 100 to 1,000 folds higher concentrations, not achieving lethal effects even at the highest concentration (Log $10^{-2.5}$) in contrast to Ukrain which is lethal at Log $10^{-3.5}$ concentration, i.e. ≈760μg/mL (15).
Dose dependency of in vitro cytotoxic effects against tumor cell lines has also been confirmed independently by other research groups: The European Organisation for Research and Treatment of Cancer (EORTC) found that Ukrain was cytotoxic against 5 of 6 colorectal cell lines (human xenografts) at concentrations of 100μg/mL (communication of the EORTC, New Drug Development Office, 9. June 1991; 16).

15: 127-131

16: 132-133

Fluoroscopic examinations on malignant cells show that Ukrain has a strong affinity to elements of the nuclei of cancer cells but not to normal cells. In a series of experiments with 14 different cell lines of human and animal origin, including normal and cancer cell lines, effects of 4 different doses of Ukrain (0.1, 1.0, 10, 100mcg/ml) on the DNA, RNA and protein synthesis was investigated by measuring the incorporation of 3-H labelled thymidine, uridine and leucine (17). Usually, a dose-dependent inhibition of all anabolic processes, the DNA-, RNA and protein synthesis was found that was more pronounced in malignant cells than in normal cells, even in those normal cell lines known for fast replication rates. According to the authors, no toxic effects were seen in normal cells treated in doses that are 100% growth inhibitory to cancer cell lines.

17: 134-144

Tumor tissues from human breast cancer, treated before surgery with Ukrain (5mg i.v. every 2nd day for 20 days, followed by surgery 7-10 days later) show a number of striking changes compared to the untreated tumor of control patients (18): Histopathological examinations demonstrate that the tumor is surrounded / encapsulated by connective tissue with massive infiltrations by mononuclear cells (mostly lymphocytes and plasma cells). Many neoplastic cells surrounded by inflammatory infiltrates are degenerated, enlarged with vacuolated cytoplasm, undergoing necrosis or already necrotic. Immunfluorescence examinations show connective tissues within the tumor heavily embedded in IgG and the predominance of IgM-positive cells. Mononuclears surrounding and infiltrating the tumor are B-lymphocytes and T-lymphocytes that are almost exclusively CD8-positive. IgM can be found in the cytoplasm and in the nucleus of tumor cells, but also on the surface of the cell membrane; it is particular abundant in necrotic foci, covering all disrupted cell fragments. Due to its autofluorescence, Ukrain can also be detected within neoplastic cells.
When tissues of ten patients treated with Ukrain were examined under the electron microscope, again massive changes were found in comparison to an untreated control group (19): Under the influence of Ukrain the endoplasmatic reticulum underwent fragmentation, and mitochondria became swollen with the cristae

18: 145-151

19: 152-158

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damaged. In addition, the cytoplasm was also swollen with an increased number of lysosomes, phagolysosomes and myelin bodies indicating destruction of the cancer cells. Ultrastructures of other cells however were not affected. Treatment with Ukrain resulted also in a markedly higher number of fibroblasts and extracellular connective fibres as compared to controls. Histochemical examinations demonstrated quantitative changes of the enzyme content, in particular of those enzymes that are key factors in the Krebs-cycle (tricarboxylic acid cycle) and therefore in the flow of cell-respiration; these enzymes are responsible for the generation and transfer of energy in form of ATP, e.g., NADH, SDH, LDH. On the other hand, the activity of glucose-6-phosphate-dehydrogenase and acid phosphatase was increased, indicating an enhanced process of destruction of cancer cells.
From these observations it may be concluded that Ukrain has direct effects on cancer cells in man as it can be found within the cytoplasma but also indirect cytotoxic activity via immunological processes, possibly changing the antigenic expression of tumor cells.

Ukrain has a low toxicity. The LD_{50} in rats after i.v. application is 43 and 76mg/kg b.w. (males and females respectively), in mice 80 and 68 mg/kg b.w. (unpublished report of the Öst. Forschungszentrum Seibersdorf, Internal study code A-4483, Oct. 1998 and L-0400, May 2000; see Expert Report on the Toxicological and Pharmacological Documentation for details).
Ukrain has no cumulative toxicity and is - in cases where no tumor is present – rapidly excreted.
In a 6-month i.v. toxicity study with rabbits (0-negative control, 0 -negative control recovery, 0.07 -low dose, 0.30 -mid dose, 0.70 -high dose and 0.70 mg Ukrain /kg -high dose recovery, groups of 6 animals each), statistically significant differences between dosed groups and the control group have been observed concerning bone marrow (sternum) with hypocellularity (mid dosed males and females, high dosed males), karyorrhexis (mid dosed males and females), inactive megakaryocytes (high dosed males), pyknosis (mid dosed females), cytolysis (mid dosed males) and concerning the kidneys with proximal tubuli and epithelium degeneration (high dosed males and females). Differences occurred also about white blood cells, with a slight increase of leucocytes, lymphocytes and bands in the high dose group (both sexes) after 4 months. Haematocrit and reticulocytes were also slightly elevated in the high dose group. Occasionally, other differences between the groups have been observed but can be considered as medically not relevant (unpublished report of the Öst. Forschungszentrum Seibersdorf, Internal study code NO10, Dec. 2001; see Expert Report on the Toxicological and Pharmacological Documentation for details).

Reproduction studies have given no indications of teratogenic, mutagenic or cancerogenic properties of the preparation, even in doses, which were 100 times larger than the therapeutic dose.

Ukrain induces no sensitisation and is also not genotoxic.

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2. Clinical pharmacology, physiological, pharmacokinetic properties

2.1. Pharmacodynamics

Pharmacodynamic effects have been investigated in a group of 19 healthy volunteers (10 female, 9 male), aged 25-65 years (20). Subjects were allocated to 5 subgroups that received Ukrain according to the following scheme: subgroup 1 (7 subjects): 10mg i.v. daily for 7-17 days; subgroup 2 (6 subjects): 5mg i.m. or i.v. daily for 20 days; subgroup 3 (4 subjects): 20mg i.v. every 2nd day for 40 days; subgroup 4 and 5 (1 subject each): 10mg i.v. daily for 5 days followed by 15 injections every 2nd day, or 5-50mg i.v. or i.m. daily/every 2nd or 3rd day respectively, in courses of 20-40 days up to 3 years, total of 3,500 mg. **20**: 159-163

All subjects have been regularly examined physically, with routine laboratory tests, as well as chest X-ray, ECG, before and 7 days after application of Ukrain, in addition to urinalysis, and immunological parameters (e.g., T4/T8 ratio, NK-cells); in some of the subjects, blood pressure, pulse rate, body temperature (twice daily) and neopterin was checked in addition.

No significant changes in vital parameters have been reported. This is in line with animal experiments where only doses of ≥0.7mg Ukrain/kg b.w. induced a dose-dependant significant decrease of the mean arterial blood pressure and increase of the breathing rate in rabbits, the most sensitive species. Similar effects have been observed in rats only at 10-times higher doses of ≥7.0mg Ukrain/kg b.w. (21). There were also no significant changes of blood cells resulting from the administration of Ukrain. All parameters remained within their normes. Only the T4/T8 ratio as well as the IgM level and the level of NK cells increased slightly (T4/T8 ratio by ~10%, IgM ~20% and NK cells ~40% respectively). In an other study (20 healthy volunteers) the T4/T8 ratio remained unchanged (22). **21**: 164-166 **22**: 167- 170

Similar, biochemical parameters such as liver- or renal function tests as well as concerning glucose- or lipid metabolism, were also not affected by Ukrain. These findings are consistent with results of clinical trials with patients performed later on.

Coagulation parameters of patients were also not significantly affected by Ukrain (23, 24). The same is true for various hormone levels that stay, apart from influences due to the disease or surgical procedures, within the ranges expected (25). **23**: 171-179 **24**: 180-183 **25**: 184-186

The preparation was well tolerated in all cases. Localized pain for 2 minutes with a burning sensation was reported after i.m. injection. Transient unwanted reactions that appeared after therapy were increased thirst during the day, poliuria, a slight increase in body temperature and a slight fatigue. Similar reactions have also been observed in most of the clinical trials with patients. In one case – a long-term observation – 3,500 mg Ukrain was administered over a period of 3 years - without negative effects. No allergic, toxic or cumulative effects were observed.

It is concluded that repeated administration of Ukrain does not induce clinically significant changes in haematological, biochemical and immunological parameters, organ functions or vital parameters.

2.2. Interaction with the immune system

Ukrain had a normalizing effect on the immune system known to be more or less imbalanced by the underlaying neoplastic process.

A number of studies could show that Ukrain, injected in doses of 5-10mg every 2nd

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day, increases, in patients more than in healthy volunteers, the number of T-lymphocytes, whereby the number of T-helper and NK cells were increased (by ~10% and ~25% respectively), and the number of T-suppressors reduced (by ~13 to 20%), thus normalising the T_H/T_S ratio (23, 26, 27, 28, 29, 31). A similar effect – although less pronounced - can be observed in healthy volunteers (20, 32).

23: 171-179
26: 187-190
27: 191-195
28: 196-203
29: 204-206
31: 212-223

These effects of Ukrain on normal human lymphocytes have also been confirmed by an ex-vivo experiment (30). Incubation of lymphocytes with Ukrain had a direct immunomodulatory effect with an increase of lymphocytes expressing T-helper phenotype and a decrease of lymphocytes expressing T-suppressor phenotype, resulting in a more or less pronounced increase of the T-helper / T-suppressor ratio in 9 of the 12 individual lymphocyte preparations.

20: 159-163
32: 224-227

In addition, an increase of the levels of interferon by ~35% was also observed, as well as an increase of the phagocytic index by ~50% and, occasionally, of immunoglobulin levels (28, 31). The rosette forming ability of the T-lymphocytes remained normal, even after radiotherapy or chemotherapy. Thus, Ukrain seems to have no significant influence on humoral immunity: The number of B-lymphocytes, serum immunoglobulin levels, complement components and acute phase proteins were not significantly enhanced.

Other ex vivo experiments with human peripheral blood mononuclear cells of healthy donors demonstrated that Ukrain (in therapeutically relevant concentrations of 0.001 to 10.0 μg/ml) stimulates the activity of a known T-cell mitogen such as phyto-haemagglutinin (PHA) that induces polyclonal proliferation of lymphocytes (33).

33: 228-230

The increase in tumor size and hardness observed in histopathologic examinations during clinical trials may correspond to a diffuse, intensive inflammatory (possibly rejection) reaction that can also be demonstrated by an increase of ESR and CRP (24).

24: 180-183

2.3. Pharmacokinetics

In a pilot study, Ukrain was administered to 6 healthy men at a dose of 20 mg / 20 ml, undiluted, as slow intravenous injection; plasma concentration have been determined 5, 15, 30, 45, 60, 90, 120, 150, and 180 min after administration, urine has been collected over 24 hours. In this study, half life of Ukrain, $t_{1/2\ \beta}$ was 27.55±2.45 minutes and the apparent Volume of distribution (V) was 27.93±1.38 l. Around 47% of Ukrain was found in the urine, more than half of the amount being eliminated during the first 6 hours. Further data can be found in the report (34). No significant changes concerning results of physical examination, laboratory parameters and ECG have been reported.

34:231-250

Binding to human plasma proteins seems to be insignificant and around 2% (35).

35: 251-258

From animal experiments it may be concluded that Ukrain concentrations are highest in tumor tissues (2.84-fold higher than in plasma) followed by normal liver and kidney tissues; the lowest concentration was found in muscles and the brain. Ukrain does not significantly cross the blood-brain barrier (36).

36: 259-265

Ukrain can be detected in tumor tissues within minutes after i.v. injection and concentrates in the nucleoli of tumor cells; healthy cells remain unaffected. The

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presence of Ukrain in tumor tissues can be demonstrated up to 19 days after injection by means of its autofluorescence under UV light. From healthy tissues however Ukrain is rapidly excreted.

During repeated injections of Ukrain no dose-limiting signs of accumulation have been observed.

2.4. Drug interactions

From experimental studies with mice it must be concluded that Ukrain (injected i.p. in relatively high doses of 9.5 and 19mg/kg/day for 10 days) has an antinociceptive effect in the writhing syndrome and hot-plate test which – after 10 injections - is more pronounced than the effect of morphine, as compared to control animals treated with the vehicle only. On the other hand, the effect of morphine (0.1mg s.c./kg) administered 1 hour after the last dose of Ukrain, was significantly decreased in animals treated with Ukrain. The effect in the writhing syndrome disappeared completely at a lower dose of 2.37mg Ukrain/kg (which is still more than 10 times the therapeutic dose in men). (37). The simultaneous administration of Ukrain + morphine has no antinociceptive effect in the writhing syndrome test over a wide range of doses of Ukrain, as compared to control animals treated with the vehicle only. (38).

37: 266-268

38: 269-270

Other experimental studies with mice and rats that received Ukrain only once, shortly before the test (in contrast to the experiment mentioned above), and aminophenazone (50 or 100mg i.p./kg) as analgetic gave mixed results depending on the test: Ukrain alone showed an antinociceptive effect only in the hot-plate test and only in a dose of 19mg/kg but decreased the effect of aminophenazone at both dose levels. In the writhing syndrome test, Ukrain alone was ineffective under these test conditions but potentiated the effect of aminophenazone. Similar, Ukrain potentiated the effect of aminophenazone in the tail-flick tests of rats (39).

39: 271-274

Interestingly, in clinical trials some patients reported reduced pain after receiving Ukrain, with a morphine-saving effect (40, 28).

40: 275-280
28: 196-203

Although doses in these animal experiments have been considerably above usual therapeutic doses in men (0.1-0.2mg/kg b.w.), it cannot be ruled out from these observations that Ukrain can interact with nociceptive receptors of the brain and concomitant analgetic treatment.

Only very few patients received Ukrain combined with chemotherapy or radiation (40). From these data it may be concluded that Ukrain does not antagonise other therapeutic approaches.

40: 275-280

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3. Clinical trials
3.1. Controlled clinical trials

Pancreas cancer

A total of 2x21 patients with pancreas cancer received, after palliative surgery, either Ukrain (10mg i.v. every 2nd day for 20 days) combined with vitamin C (3g i.v. + 0.8g p.o. every 8 h, every 2nd day for 20 days) or vitamin C alone. Median survival was significantly prolonged (p <0.001) and more than twice as long in the group treated with Ukrain than in the control group (574 versus 197 days, corresponding to 18.8 vs. 6.4 months); 5 of 21 patients have survived 3 years, 1 patient is still alive after 5 years (31, 59 and internal study report). The Karnofsky index was also significantly better after therapy with Ukrain (unblinded assessment). Patients of the Ukrain-group had also a significantly higher ratio of T-helper-/suppressor cells as well as of the phagocytic activity.

31: 212-223
59: 355-360

A total of 17 of 21 patients of the group receiving Ukrain and 11 of 21 receiving the control treatment had adverse events, in particular increase of body temperature (9 of 21 patients treated with Ukrain, 1/21 control) and thirst. Nausea and vomiting were more frequently reported in the control group (11/21 versus 2/21). As the treating physicians were not blinded it cannot be excluded that this has resulted in bias, e.g., underreporting of adverse events. Serious adverse events observed were two cases of cholangitis in the group treated with Ukrain and seem to be related to the disease. Liver enzymes did not show clinically significant changes as a result of Ukrain-therapy and no WHO-grade 3 toxicity reaction was observed.

Survival	Ukrain + Vitamin C N of 21 Patients (%)	Vitamin C N of 21 Patients (%)
1 year – 365d	16 (76%)	2 (10%)
2 years – 730d	8 (38%)	0
3 years – 1,095d	5 (24%)	-
4 years – 1,460d	1 (5%)	
5 years – 1,825d	1 (5%)	

In an other controlled clinical trial, a total of 3x30 patients with histologically proven unresectable adenocarcinoma of the pancreas have been treated either with gemcitabine (group A, 1000mg gemcitabine / sqm weekly, 7 weeks therapy, one week rest), Ukrain (group B, 20mg / week, 7 weeks therapy, one week rest), or a combination of Ukrain+ gemcitabine (group C, 1000mg gemcitabine / sqm followed by 20mg Ukrain weekly). Median survival was significantly longer after Ukrain alone or Ukraine combined with gemcitabine (7.9 months, 5.2 months, and 10.4 months respectively; p <0.01); the 12-months survival rate in group A (Ukrain alone), B (gemcitabine) and C (Ukraine + gemcitabine) was 13%, 29%, and 32% respectively (41).

41: 281-288

In all three arms therapy was well tolerated and no severe side effects occured. In non of the patients therapy had to be stopped due to side effects. In arm A, nausea seemed to be more frequent than in arm B and arm C (total of 53% versus 22% versus 27% p < 0.05), whereas in arm B and arm C fever was observed more frequently (22% versus 42% versus 24%, p < 0.05). In arm C (Gemcitabine plus NSC-631570) hematological toxicities WHO II occured significantly more frequent than in arm A and arm B (85% versus 71% and 43%). Raises in liver enzymes occured in all three arms in the same frequency and were related to stent occlusion

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or disease progression of hepatic metastases. In four patients tumor bleeding occured (2 patients arm B, 2 patients arm C), which were treated by angiographically intervention. Other adverse events such as obstipation or diarrhea were approximately equally distributed. WHO-grade III reactions were rare (3 patients in each group with haematological reactions).

Response (after 3 months)	Patients treated with **Ukrain**	Patients treated with **gemcitabine**	Patients treated with **Ukrain+gemcitabine**
CR	0/20 (0%)	0/28 (0%)	0/28 (0%)
PR	2/20 (10%)	1/28 (4%)	6/28 (21%)
SD	13/20 (65%)	8/28 (29%)	17/28 (61%)
PD	5/20 (25%)	19/28 (68%)	5/28 (18%)
CR+PR+SD / PD	15/5	9/19	23/5

Colorectal carcinoma

In a controlled clinical trial with a total of 96 patients suffering from colorectal carcinoma, Ukrain was given to patients before and after surgical intervention (28). The control group received 5-fluorouracil (600mg 5-FU/qm/day before surgery, up to a total dose of 5.5 –6.0g); 48 patients were allocated to each of the two treatment arms (method not described but probably according to the "matched pair" technique). Patients with metastatic and non-metastatic colorectal carcinomas (stages T1-4N0-3M0-1) were eligible. All patients had histologically verified adenocarcinoma. Karnofsky index at entry was between 50 and 90%. Data have been presented for patients with metastatic and non-metastatic carcinomas separately for both treatment arms (15 and 33 patients respectively in each treatment arm).

28: 196-203

In the Ukrain group the general condition as measured by the Karnofsky index improved after surgery (increase from 60.7 to 72.9 and from 70.6 to 79.4 in patients with and without metastases respectively). In contrast, the Karnofsky index decreased in the control group receiving 5-FU (from 63.6 to 55.0 and from 70.3 to 65.6 in patients with / without metastases respectively). Similar, Ukrain increased, in contrast to 5-FU, the T4/T8 ratio by ~30%, the NK cell activity by ~50%, the phagocytic index (as measured by the average number of staphylococci lysed by one neutrophilic cell, observed under the microscope) by ~50% and circulating interferon levels by ~40%. It is however not mentioned whether the Karnofsky index was evaluated by a "blinded" rater in order to be objective.

Regression of tumor masses were observed in the Ukrain group only, with a tumor response in 6/15 patients with metastatic disease (see table below for details). No response was reported in the control group.

Response (colorectal ca)	Metastatic patients **Ukrain** (n=15)	Non-Metastatic patients **Ukrain** (n=33)	Metastatic patients **5-FU** (n=15)	Non-Metastatic patients **5-FU** (n=33)
CR	0	0	0	0
PR	6 (40%)	8 (24%)	0 (0%)	3 (9%)
SD	5 (33%)	22 (67%)	4 (27%)	15 (46%)
PD	4 (27%)	3 (9%)	11 (73%)	15 (46%)

After 21 months, overall survival was 11/14 (78.6%) in the group that received Ukrain in contrast to 5/15 (33.3%) in the control group. According to further, unpublished data, survival is 2 to 3 times longer in all groups treated with Ukrain as can be seen

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from the tables below:

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Survival (colorectal ca)	Metastatic patients **Ukrain** (n=15)	Non-Metastatic patients **Ukrain** (n=33)	Metastatic patients **5-FU** (n=15)	Non-Metastatic patients **5-FU** (n=33)
2-years	7 (47%)	22 (67%)	3 (20%)	8 (24%)
5-years	4 (27%)	14 (42%)	2 (13%)	5 (15%)

A transient increase of body temperature around 37°C occurred in 18/48 patients, temperature up to 38°C in 9/48 patients. Other adverse events were less frequent: thirst (14), mild insomnia (4) and fatigue (2); there is no mentionning of post-operative complications such as infections.

In a further, controlled clinical trial with a total of 48 patients suffering from rectal carcinoma (stage T3-4N0M0, T3-4N1-3M0), either Ukrain or a combined radio-chemotherapy was given (to 24 patients in each group). Patients with distant metastases have been excluded (42). A 1st course of Ukrain was given before surgical intervention (10mg i.v. every 2nd day up to a total dose of 60mg before surgery and 10mg i.v. every 2nd day up to a total dose of 40mg, started 8 days after surgery); a 2nd course of 20 days (100mg) was repeated 6 months after surgery. The control group received high fractional X-ray therapy (cumulative dose up to 25 Gy, 5Gy/d) and 5-fluorouracil (600mg/qm/day, up to a total dose of 5g) before surgery; a similar course was repeated after surgery. **42:** 289-294

In the Ukrain group, the general condition was improved after surgery as measured by the Karnofsky index; it increased in the Ukrain group from 70.8 to 78.3, but decreased in the control group from 71.3 to 66.4. Tumor markers such as AFP, CEA and MCA decreased by a factor of 4 (decrease of AFP: ~80%, CEA: ~75%, MCA: ~77%) in the group treated with Ukrain but remained almost stable in the control group. Tumor size decreased in 5/24 (22%) and 4/24 (18%) respectively after the pre-surgical treatment.

Fourteen months after surgery, only 2 patients have died in the group that has received Ukrain, but 6 of the control group. After 24 months, cancer had reoccurred in only 4/24 patients who had received Ukrain but in 8/24 of the control group.

Post-operative complications occurred in only 2/24 of the Ukrain group (2 patients treated with Ukrain developped urinary bladder atony, lasting for 5-7 days according to personal communication) but in 7/24 patients of the control group. Three patients had fever up to 38°C during the first three injections. No patient in the Ukrain group experienced toxic reactions in contrast to the control group (nausea and lethargy WHO-toxicity grade 2, cardiac dysrythmia and hand-foot syndrom grade 1).

Breast cancer

In a controlled clinical trial with a total of 75 breast cancer patients, either 5mg or 10mg Ukrain was administered i.v. every 2nd day, for 20 days, and stopped 7-10 days before surgery (23). The control group was not treated before surgery. Each group included 25 women, patients were matched for tumor stage and age. Breast cancer was confirmed by clinical examination, ultrasonography, mammography and histological examination of tumors obtained during surgery (clinical stage I, II, III, T1-3N0-2M0, age <70 years). **23:** 171-179

After treatment with Ukrain, hardening of the tumor, a slight increase in the tumor size (5-10%) and proliferation of connective tissues was observed. The ratio of T4/T8

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lymphocytes increased after treatment with a total dose of 50mg Ukrain (by ~30%), but decreased in the group receiving 100mg (~25%); it remained unchanged in the control group. Seven to 10 days after surgery however, this ratio was about 3 times higher in (both) Ukrain-groups compared to the control group, mostly due to a decrease of the ratio in the latter; Ukrain in the dose of 50mg had a slightly greater effect (increase compared to pre-treatment level ~64% compared to ~25% in the group receiving a total dose of 100mg Ukrain). Similar, 7-10 days after surgery, the percentage of total lymphocytes was less than 50% of pre-treatment levels in untreated control, whereas it remained almost constant in both groups receiving Ukrain. The higher dose of Ukrain resulted in a slight increase 7-10 days after surgery. Immediately after Ukrain treatment, only marginally changes of the percentages of total lymphocytes could be observed.

Immediately after treatment with Ukrain, the ESR was slightly more elevated; this was more pronounced in the group receiving the higher dose (+10% and +21% respectively) but remained unchanged in the control group. Eight to 10 weeks after surgery however, the ESR, known to increase regularly after an operation, was nearly twice as high in the control group than in the groups receiving Ukrain indicating a faster normalisation in the latter.

Complications such as prolonged lymphorrhea, skin necrosis, suppuration of the wound and pneumonia were twice as frequent in the control group than in the groups receiving Ukrain (5/25 patients versus 5/50). Ukrain-treated patients reported slight pain in the tumor area, and a slightly increased temperature with the higher dose.

The same centre published already earlier data on 18 patients with breast cancer, all histologically verified (several publications exist on the same group of patients); 10 patients (T2-3N0-1M0) have been treated with Ukrain (5mg i.v., every 2nd day, for 20 days; treatment stopped 7-10 days before mastectomy); 8 patients matching in tumor stage, age and other characteristics (T1-3N0-1M0) served as control group (untreated before surgery), (24). Laboratory parameters showed no major changes after treatment with Ukrain compared to the control, except of a slight increase of the sedimentation rate (approximately 10%). However, 8-10 weeks after surgery, the ESR, that regularly increases after surgery, was almost half the value of the control group. In addition, the ratio T4/T8 that had already increased slightly after treatment with Ukrain was - after surgery - almost 3 times higher in Ukrain-treated patients than in the control group (29). The tumors appeared harder and slightly enlarged after Ukrain therapy, and were easier to detect by ultrasound or radiological examination. Metastatic lymph nodes were also hardened and sclerosed. Through hardening under the influence of Ukrain, tumors and metastatic lymph nodes were clearly demarcated from healthy tissue and therefore easier to remove. Only 2/10 patients complained of adverse events (pain in the breast at the site of the tumor); 8/10 women were without adverse events.

24: 180-184

29: 204-206

Bladder cancer

A total of 28 patients with superficial bladder carcinoma (confirmed by biopsy, either highly-, moderately - or un-differentiated, tumor size 0.5x0.5 to 3x4 cm, T1N0M0) were subject to a prospective controlled clinical trial with either one cycle of Ukrain (10mg i.v./day, 10 days), 2 or 3 cycles with a 2-weeks intervall between cycles (32). The tumor was assessed by cystoscopy and ultrasound before and 2 weeks after end of treatment and results evaluated according to common response criteria. A response to therapy (CR+PR) was observed in 4/9 patients receiving one cycle, in 5/10 receiving two and in 8/9 receiving three cycles. No progression was observed.

32: 224-227

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Cases of CR were proportional to the number of cycles / total dose of Ukrain. The 3 patients with a CR had no relapse during 5-6 months of follow-up. No adverse events were reported. The authors conclude that better results are obtained with a longer treatment, i.e. 3 cycles.

Unfortunately, in none of the above-mentioned publications details of randomisation have been described.

3.2. Clinical trials without control group

In an open clinical trial, Ukrain was given to 70 patients of various end-stage carcinoma (including 17 patients with colorectal carcinoma, 26 with breast cancer and various other carcinoma e.g., of the lung, pancreas, stomach) either as monotherapy (n=36) or combined with chemo- and radiotherapy (n=34) (40). **40:** 275-280
Doses were between 2.5mg and 25mg, either i.v. or i.m., increasing but also decreasing, injected daily or every 2nd, 3rd, 4th or 5th day, up to three months. In 47/70 patients, a partial remission and in 20/70 a stable disease was achieved. Progression occurred in 3 patients (details communicated personally). Cancer-related laboratory parameters such as tumor markers improved also (e.g., CEA levels decreased by ~35% from a mean level of 28.0 ng/ml to 18.3ng/ml).
In some patients a slight increase in body temperature by 1 – 2°C, thirst, pain and feeling of heat in the tumor area, increased pulse rate, excessive urine excretion and a reduction in blood pressure occurred. Slight, transient pain after i.m. injection was also reported.

In another open clinical trial with 36 patients suffering from various carcinoma of stage III (including 13 patients with rectal cancer and 8 with breast cancer), Ukrain was given in a dose of 10 mg, i.v. every 2nd day up to two months (total dose of 300 mg) (22). Ukrain decreased the mean level of CEA in patients with rectal cancer from 48 to 27ng/ml (-55%) and the level of CA-125 from 3,200 to 1,100 U/ml (- 66%) in patients with ovarian cancer. Ukrain increased subsets of T lymphocytes such as CD2, CD4 and CD8 in these patients with various cancers. In contrast, a control group of 20 healthy individuals, which received a similar treatment with 10mg Ukrain i.v. showed no or only minimal changes of their lymphocyte subpopulations. A partial response was observed in 26/36 patients, a stable disease in 8; in 2 patients tumor progression was observed (personal communication). An improvement in the clinical condition, decrease of tumor - / metastatic- lesion size as measured by ultrasound and / or computer tomography was also reported. Some patients with rectal carcinoma experienced short lasting bleeding from the tumor undergoing necrobiosis. **22:** 167-170

Other adverse events were rare: Pain and feeling of warmth in the tumor area occurred in 6 patients, increased body temperature up to 38°C in 6, thirst in 8, fatigue in 5, sleepiness in 3 and mild nausea in 2 cases (personal communication). All adverse events were lasting for approximately 24 hours after injection.

Four of these 10 patients with breast carcinomas (stage T2-3N0-1M0) mentioned above (24) were treated also postoperatively with 2 cycles of 50mg Ukrain (treatment-free interval of 1 month between the cycles). No reoccurrence of the tumor was observed in the group receiving Ukrain pre- and postoperatively (0/4) up to 1 year after operation (43). **24:** 180-183 **43:** 295-297

Ten patients with histologically verified cervical carcinoma (squamous or

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adenocarcinoma, average age of 39.3 years) were treated with Ukrain (10mg i.m., every 2nd day for 20 days); 2-3 weeks after the end of Ukrain treatment hysterectomy + lymphadenectomy was performed (26). At this time, response was also assessed. Partial remissions were achieved in 3/9 (33%) of cases and stable disease in 6/9 (66%) of cases. One patient was not assessed as she withdrew her consent after two injections and received radiotherapy thereafter. Other changes reported were an increase in the lymphocyte, T-helper and NK cell counts.

26: 187-190

No serious adverse events occurred. One patient had mild nausea and another complained of moderate pain at the site of injections.

In an open clinical trial, 9 patients with lung carcinoma have been treated with Ukrain (10 injections of 10 mg i.v. every 3rd day) (27). All cases were histologically verified and previously untreated; 7 had small cell- lung cancer (6 patients with limited disease, 1 with extensive disease), and 2 non-small cell lung cancers (stage IIIb and IV). Tumor regression was observed in 4/9 (44%) cases, one with a CR; 4 of the 9 patients died during therapy (2 NSCLC, 2 SCLC). Survival rate after response to therapy was 8 - 18.5 months (average 14 months), for therapy-resistant cases 1.2 months. In addition, an increase in the T-lymphocyte count, improvement in the T4/T8 ratio in favour of the T-helper cells, and an increase in NK cells were observed. No notable change in immunoglobulins or acute phase proteins occurred. No adverse events were reported.

27: 191-195

In a pilot study, 8 patients with various carcinoma (3 breast cancer, Stage II, 1 uterine-cervical cancer, Stage I, 1 lung cancer, Stage II, 1 uterine-ovarian cancer, Stage III, 1 ovarian cancer, Stage III, 1 malignant melanoma, all tumors histologically verified) have been treated with Ukrain (5 mg, i.v. or i.m. every 2nd day in 3 - 6 courses of 12 - 15 ampoules; total dose 60-75mg/course) (44). C-reactive protein increased in 7/8 patients but no significant changes to immunoglobulins or acute phase proteins have been observed. General condition improved in all cases and those patients of stage I and II who have not been operated showed regression or stabilisation of the tumor. No patient of stages I and II (5 cases) had tumor recurrence after 18 months of observation. In one patient who was known to be allergic against a number of antigens, complete disappearance of allergic reactions after 5 cycles has been observed as an unexpected finding (no further confirmation available).

44: 298-305

In most patients a transient increase of the body temperature by 0.5 to 1.5°C occurred after injections. During injections, patients reported a feeling of warmth. Other observations were: transient pain in the area of the tumor, short lasting nausea or vertigo, and enlargement of regional lymph nodes (that revealed reactive lymphangitis but without neoplastic changes when examined histopathologically).

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3.3. Case reports (selected)

Mixed tumors :
A total of 203 advanced-stage cancer patients suffering from various types of cancer and who had exhausted all conventional forms of therapy have been treated with Ukrain in a private clinic that published a summary of the experiences; 76 (37.4%) have been treated simultaneously with deep hyperthermia. Patients also received selen, cimetidine, thyme extract and vitamin A. A total of 41 (20.2%) achieved total remission, 122 (60.1%) partial remission. Seminoma and prostate cancer responded particularly well with remission rates >75% (45). Among these patients there were 14 children, between 22 months and 16 years old, treated with 0.3mg/kg b.w. 3 times per week.

45: 306-309

Malignant melanoma, stage III:

A long lasting benefit (12 years of no recurrence) was observed in a patient with a malignant, nodular melanoma, echo-reflexing zones in the ultrasound-scan of the liver and positive melanin excretion indicating diffuse metastasation at the time of diagnosis and who was subsequently treated with Ukrain (10mg i.v., twice weekly) for 21 months (46).

46: 310-312

Invasive ductal breast cancer:

A patient with invasive ductal breast cancer, signet cells, G2, pT1 and 3/16 lymph nodes positive during surgery. The patient refused chemo- or radiotherapy. She was treated with mistletoe extracts starting 8 months later when a new lymph node, infiltrated with carcinoma, was excised. Six years after the first surgical intervention, lung metastases were suspected that increased over the following 16 months. Treatment with Ukrain started 7 years after the first intervention (10mg, 3 times per week for 3 weeks). A total of 7 cycles have been administered over a period of 14 months, with treatment-free intervals of 4-6 weeks. The patient could be observed up to 10 months after the last cycle and showed a full clinical remission (47).

47: 313-315

Breast cancer:

A 69-year old female patient with histologically verified breast cancer and numerous metastatic defects of the skull as demonstrated by X-ray examination was treated with Ukrain (5mg i.m./day for 20 days followed by a 10-day treatment-free interval, total of 12 cycles). After 1 year, the whole body and skeletal scintigraphy demonstrated the absence of metastases. Injection of Ukrain was continued but with 2-months treatment-free intervals between cycles (48).

48: 316-320

Breast cancer:

A 50-year old female patient with T4N2M1 (stage IV) carcinoma received, after previous unsuccessful treatment with radiation, doxorubicin and Zoladex, 5mg Ukrain+ 3g ascorbic acid i.v. every 2nd day for 20 days. In addition, Ukrain was also administered locally (10mg with 1% lidocaine every 2nd day). After this 1st course, operation became possible. A demarcation between tumor and healthy tissue was observed as well as of lymph nodes. After a 2nd and 3rd course (5mg i.v. every 2nd day) X-ray and ultrasound examination showed clinical remission (49).
During the first 5 injections, a short lasting pain in the tumor area, itching and paresthesia (for 0.5 to 3 minutes) was noticed. Two hours after, body temperature increased to 38.4°C but returned to normal 14 hours later.

49: 321-322

Oesophageal cancer:

A patient with poorly differentiated squamous cell oesophageal carcinoma, inoperable according to clinical and X-ray contrast examinations, was treated first

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with 40 courses of radiation (maximal dose), followed by three courses of chemotherapy (cisplatin, methotrexate, bleomycin) without response. Nine months after the diagnose, treatment with Ukrain was started (20mg every 2nd day for 2 weeks, followed by 10mg every 2nd day up to 230mg). The patient was free of tumor reoccurrence up to the last observation 42 months later (50). **50:** 323-325

Astrocytoma of the optic nerve:

In a 33 months- old female patient an astrocytoma of the optic nerve was diagnosed and extirpated sub-totally. The tumor progressed over the next 52 months. The patient received neither chemotherapy nor radiation. Treatment with Ukrain was started 52 months after the first operation (2mg to 15mg i.v., up to a total dose of 723mg Ukrain over 13 months). Disease progression was slowed down and an almost stable condition was achieved (51). **51:** 326-328

Ewing's sarcoma:

In a 9-years old girl an Ewing's sarcoma was diagnosed and treated unsuccessfully with radiation. Despite subsequent chemotherapy (actinomycin D, vincristin, adriamycin, one 5-day cycle and two 3-days cycles) tumor progressed rapidly. Three months later treatment with Ukrain was started (3 cycles of 5mg i.m./day, 10 injections, followed by a 2-weeks treatment-free interval). A slow improvement (as demonstrated by a slow reduction of the tumor in repeated X-ray examinations and the clinical condition) was observed after each cycle. After the 5th cycle no pathological findings could be observed. The patient received a total of 6 cycles of Ukrain within 12 months and is considered as complete response (48). **48:** 316-320

Ewing's sarcoma:

In a 10-years old girl an Ewing's sarcoma was diagnosed and treated unsuccessfully with radiation. Despite subsequent radio- and chemotherapy (2 blocks EVAIA, 12 blocks VAIA) over 1.5 years, tumor progressed. Treatment with Ukrain was started (cycles of 15mg i.v./every 2nd day, 10 injections, combined with local hyperthermia, three courses within 8 months). No further tumor growth was observed already after 6 weeks. Examinations showed complete remission after 8 months (52). **52:** 329-331

Neuroblastoma:

In a 22-months old male patient a stage IV neuroblastoma was diagnosed. He received polychemotherapy for 6 months, laparotomy with tumor removal including nephrectomy and lymph node removal but was still positive for a remaining abdominal tumor, with a retro peritoneal tumor and metastases in the lung, brain, pelvis, kidney and distal femur when treatment with Ukrain was started (5mg, every 2nd day for 3 weeks, with a treatment-free interval of 3 weeks between each course of Ukrain). During the next 12 months of treatment (still ongoing at the time of the publication) the tumor masses and metastases either disappeared or have been considerably reduced. Stage IV neuroblastoma have a particular poor prognosis with a 6-years survival rate after conventional treatment of 9% (53). **53:** 331-333

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3.4. Global analysis of efficacy, indications

Ukrain has been investigated as neoadjuvant and adjuvant therapy. In most trials, Ukrain was given in a dose of 10mg i.v. every 2nd day for 20 days, in a total dose of 100mg/cycle, with a treatment-free interval of 2 (-4) weeks between cycles. More cycles or longer treatment duration respectively may improve the clinical result (32).

32: 224-227

Even in cases where all previous treatments had failed, without any further hope, Ukrain has been reported repeatedly to achieve a complete or partial clinical response (45). No controlled clinical trials are possible in these patient groups.

45: 306-309

Treatment of over 500 patients with Ukrain is documented in various publications (≈200 in controlled, ≈150 in non-controlled clinical trials, and over 210 in individual case reports or summaries of experiences); this includes 102 patients with colo-rectal carcinoma, 51 with pancreas cancer, 28 with bladder cancer and more than 97 patients with breast cancer.

40: 275-280
22: 167-170
42: 289-394
23: 171-179
26: 187-190
27: 191-195
28: 196-203
29: 204-206

Ukrain has a positive effect on a number of laboratory parameters known to be prognostic for survival and that may serve as surrogates: Tumor markers such as Ca-125 (ovarian cancer), AFP, MCA and CEA are markedly decreased, CEA by approximately 35 to 75% (40, 22, 42). Other changes of laboratory parameters that have been repeatedly observed after treatment with Ukrain are the T4/T8 ratio increasing by 30% (23, 26, 27, 28, 29), the NK-cell activity increasing by 50% (28, 26) or increased lymphocyte counts (by 5-15%, in particular T-cells, when compared to control groups) (23, 25, 26, 28)

23: 171-179
25: 184-186
28: 196-203
44: 298-305

After treatment with Ukrain, unspecific inflammatory parameters such as ESR and CRP are transiently increased (by ~10%). After surgery however, they are normalised more rapidly and were much lower than in controls. The effect is slightly more pronounced with a higher dose /day (23, 25, 28, 44).

Ukrain clearly improved patient's condition as described by the Karnofsky index. The index improved by approximately 10 points in patients treated with Ukrain but decreased by about 5 points in control groups (23, 42, 31). It should however be mentioned that the evaluation of the Karnofsky index may not have been performed under blinded conditions.

23: 171-179
31: 212-223
42: 299-304

Post-operative complications such as prolonged lymphorrhea, skin necrosis, suppuration of the wound and pneumonia have been less frequent in Ukrain-treated patients than in controls (23, 42). This could be seen as further hint of immunostimulating effects of Ukrain.

23: 171-179
42: 299-304

All controlled clinical trials consistently demonstrated higher response rates with Ukrain than with control treatments. "Hard endpoints" such as survival and/or the rate of tumor free-patients is about 2-3 times higher in groups treated with Ukrain than in controls (42, 28). Clinical response seems to be more pronounced with a longer treatment or a higher number of cycles respectively (e.g., as demonstrated for bladder cancer, 32) thus with the total dose and/or treatment duration. A clear dose-effect study in order to define the best dose but also frequency schedule is however missing.

42: 299-304
28: 196-203
32: 224-227

Preoperative treatment with Ukrain improved the operability.

Ukrain can thus be considered as therapy in patients with pancreas cancer and possibly with colorectal cancer. It can also be recommended for cancer patients where all other treatments have failed. The observation that Ukrain improves operability, paired with an antineoplastic effect, makes Ukrain an interesting candidate for neoadjuvant therapy in patients with cancers such as e.g., colorectal

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carcinoma, breast cancer, bladder cancer and cervix cancer.

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Tumor	Dosage	Response (CR+PR)	CEA (ng/ml)	Survival	Reference	
Colorectal ca.	2x100mgU each over 20d	Meta: 6/15	-	18/48 (5y)	Susak, Zemskov 1996	**28:** 196-203-
	600mg 5-FU/qm/d tot. 5.5 to 6.0g	Meta: 0/15	-	7/48 (5y)		
Rectal ca.	2x100mg U each over 20d	5/24 (22%)	-75%	22/24 (14m)	Bondar 1998	**42**: 289-294
	600 mg 5-FU/qm/d up to 5g	4/24 (18%)	-6%	18/24 (14m)		
Pancreas ca.	100mg U over 20d (10mg every 2nd d) + vitamin C	-	-	8/21 (2y)	Zemskov et al., 2000, 2002	**31**: 212-223 **59**: 355-360
	5.4g vitamin C	-	-	0/21 (2y)		
Pancreas ca.	20mg U i.v./ w., for 7 w, 1 w of rest	2/20	-	29% (1y)	Gansauge et al., 2002	**41**: 281-288
	G i.v., 1000mg/ qm/ w for 7 w, 1 w of rest	1/28	-	13% (1y)		
	U+G i.v. for 7 w, 1 w of rest	6/28	-	32% (1y)		

CR-complete response, PR-partial response, d - day, w-week , m - month, y - year;
U - Ukrain, 5-FU – 5-fluorouracil; G – gemcitabine;

3.5. Global analysis of safety, tolerance

No harmful effects or serious adverse reactions have been observed with Ukrain until now. In up to about 40% of the patients a transient increase of the body temperature by 0.5 to 1.5°C, rarely up to 2.0°C, occurs after injections. During injections, patients may observe a feeling of warmth, with a transient pain in the area of the tumor and sites of metastases. Thirst occurs with almost the same frequency, seems to occure also independently of fever, and has been observed also in animals. Other observations include short lasting nausea or vertigo, enlargement of regional lymph nodes (that revealed reactive lymphangitis but without neoplastic changes when examined histopathologically), increased pulse rate, a marginal reduction in blood pressure, tumor bleeding and an increased urine excretion (44). Slight, transient pain after i.m. injection was also reported. (40). Two patients with rectal carcinoma who have been treated with Ukrain before being operated, developed a transient bladder atony.

40: 275-280
44: 298-305

The maximum dose bringing harmful effects in man is not known. Ukrain has been well tolerated up to injections of 50mg (i.v. or i.m.) and up to total doses of 3,500mg administered over 3 years.

No cardiovascular-, gastrointestinal-, metabolic/endocrine- or haematological toxicity or any interactions with organs, systems and diseases has been reported. Allergic, cutaneous reactions are also absent. A specific patient monitoring during treatment with Ukrain is not necessary according present data.

No data are available concerning the application of Ukrain during pregnancy, lactation or in infants. Various case reports of children in the age of 22 months to 6-16 years treated with Ukrain do not show any specific risks (see case reports).

No contraindications for Ukrain are known.

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4. Post-marketing experience

According to the manufacturer, over 2,000 patients with various malignant tumors of stage I-III have been treated with Ukrain, before or after surgery, with tumor regression in 40-75% (32). During the 7, 3, and 2 years respectively where Ukrain is on the market in Belarus, Ukraine and Georgia no particular adverse drug reactions have been reported.

32: 224-227

5. Other information

In animal experiments, Ukrain had a protective effect against bacterial infections, with best results at a dose of 0.4mg/kg, injected s.c. every 2nd day for 20 days before the infection with lethal doses of E. coli (tested dose range: 0.04, 0.4, 4.0mg Ukrain/kg). Short term injections of Ukrain before and after infection produced best results at a dose of 0.04mg Ukrain/kg. Repeated injections of Ukrain before infection were also effective against S. aureus (54). This dose would be within therapeutic ranges, given that a dose of 10mg corresponds to 0.15mg/kg for a subject of 67kg. A protective effect of Ukrain has also been demonstrated against experimental virus infections (55).

54: 334-338
55: 339-343

These experiments should be seen in the light of observations from clinical trials where a 2-3 times lower rate of post-operative complications (42, 23) or a reduced rate of infections has been reported (50).

42: 289-294
23: 171-179
50: 323-325

Patients with malignancies are known to have disturbances in the amino acid pool metabolism with hyperaminoacidemia except taurine, proline and glutamine that are decreased by approximately 25% in comparison to healthy control subjects. In breast cancer patients it was shown that Ukrain (10 injections of 5mg Ukrain i.v. every 2nd day) significantly increased the total pool of free thiol-containing amino acids (mostly taurine), but also of proline and glutamine in blood plasma, and decreased the level of alanine in comparison to an untreated patients-control group. This was interpreted by the authors as sign of tumor cytolysis and formation of connective tissue (56).

56: 344-346

Ukrain increased also the ratio of free ethanolamine to phosphoethanolamine in cancerous tissue. The depression of this ratio has been reported to be an indicator for a decreased malignancy (56, 57).

57: 347-349

Significant changes of amino acid concentrations after treatment with Ukrain (10mg i.v. every 2nd day for 20 days) have also been reported in tumor tissues of patients with bladder cancer when compared with normal, healthy tissue (58).

58: 350-354

6. Conclusion

Despite weaknesses of many of the clinical trials with Ukrain (most have the character of pilot trials, blinding and randomisation may not have been optimal, assessment of response according to standard criteria is missing or not described in details) a number of endpoints can be identified that are relatively free of bias (e.g., overall survival, changes of tumor marker levels, objective response as demonstrated by X-ray examinations in particular of those patients not responding any more to chemotherapy). In addition, controlled cinical trials also consistently demonstrate a treatment benefit with Ukrain. These endpoints suggest a therapeutic

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effect of Ukrain in cancer patients.

Furthermore, it can be concluded from the clinical results that neoadjuvant (pre-) treatment of cancer patients with Ukrain, before surgery, but also adjuvant treatment with Ukrain does not present particular risks. On the contrary, it may improve the operability as well as the general condition of patients.

The optimal treatment schedule with Ukrain is still not known. Systematic dose optimisation studies are missing. However, from indirect comparisons it may be concluded that efficacy with doses of 35mg/week may be better than with lower doses and therapeutic benefits increase with the number of cycles. Most patients have been treated with 5-10mg i.v. injected every 2nd day over 20 days with treatment-free intervals between cycles in the order of 2-6 weeks.

In summarising, data of systematic, controlled clinical trials with Ukrain, in particular the number of studies, but also the number of patients may be considered as scarce. On the other hand, results of those few controlled clinical trials with Ukrain were consistently better than in control groups. Furthermore, some of the case reports describe treatment effects in patitents resistant to any other therapy. This is unexpected and justifies further investigations.

Ukrain seems to be better tolerated than other tumor therapies. It may also help surgeons to localize the primary tumor as well as metastatic lymph nodes.

Although further data from carefully planned, prospective controlled clinical trials are highly warranted, Ukrain can be taken into consideration for the treatment of patients in particular with pancreas cancer, but also as a “last line” treatment after previous failure of chemotherapy.

6.1 GCP Compliance statement

Most of the protocols and original reports have not been available to the expert (except for one trial on pancreas carcinoma and pharmacokinetics). Most of the studies seem to be investigator-initiated and publications result from the work of only a few clinical institutions. It cannot be excluded that data may have been taken of the same patient groups, thus raising the problems of repeated analyses. Whenever this was observed it is mentioned in the report.

Concerning the conduct of the clinical investigations according to good clinical practice it should be stressed that data on Ukrain have been collected over a period of more than 20 years, starting before July 1991, thus long before the date when the EC-GCP Note for Guidance came into force. Studies have been performed in the former Soviet Union where GCP was also not legally binding. They have, however, been conducted in compliance with the Declaration of Helsinki; studies received also the permission by regulatory authorities according to national laws. Furthermore, it should be pointed out, that, according to the experience of the expert, it is common praxis in Eastern European countries that the regulatory body granting authorisation of a clinical trial, acts as “central ethics committee” and approves also a study with regard to ethical, but also scientific aspects. Some of the publications include also a statement that the protocol has been approved by the responsible (local) ethics committee (e.g., 22, 31).

22: 167-170
31: 212-223

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8. Information on the Clinical Expert

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Education:	School and bacchalaureat in Insbruck (summa cum laude) University education in Vienna
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Training:	Internal Medicine (Sophien-Hospital Vienna, Elisabeth-Hospital Vienna) Clinical Pharmacology (University of Vienna) Radiology and Radioprotection (Centre for Nuclear Research, Seibersdorf, Austria)
Occupation:	General Manager of CIS GmbH since 1990 Head of Medical Dep., Bender & Co (affiliated to Boehringer Ingelheim Ltd.) (1987-1991) Head of Medical Dep., Biochemie GmbH. (affiliated to Sandoz) (1983-1987) Medical Departement, Biochemie GmbH. (affiliated to Sandoz) (1978-1983) Basic Research on Experimental Chemotherapy, Sandoz Research Institute, Vienna, (1974-1978) The expert is involved since 1987 in the clinical development of drugs used for treatment of cancer such as biologic response modifiers, monoclonal antibodies, anti-sense proteins and cytostatics.
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