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## **UKRAINIAN ANTI-CANCER INSTITUTE**

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# **NSC 631570 (UKRAIN)**

**EFFICACY,  
SAFETY,  
QUALITY**



Vienna, 05.08.2010

# NSC 631570 (UKRAIN): EFFICACY, SAFETY, QUALITY

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## **I. EFFICACY**

UKRAIN (NSC 631570) is chelidonii radix special liquid extract. This is a complex produced from two approved substances – greater celandine alkaloids and thiotepa (2, 9, 145). Its quality proof is specified in German Pharmacopoea and Pharmacopoea Austriaca.

Ukrain is the first and only drug effective against cancer and more than 300 times less toxic than its sources substances (12, 39, 41, 59, 111, 141, 179). It has been proven in numerous in vitro, in vivo and clinical studies that this medicine in smaller dosage (5 mg) exerts immune modulating properties (2, 5, 8, 10, 14) whereas in larger dosage its effect is malignocytolytic (3, 6, 11). The therapeutic index of Ukrain is 1250 (therapeutic index is relation between toxic dose and the therapeutic one and reflects the safety of a drug). This is rather unusual for an anticancer drug and explains the good tolerability of NSC 631570. Therapeutic index of the common cytostatic drugs is in the range 1.4-1.8 and their overdosage can cause fatal consequences.

### **Phase I Clinical Study**

The phase I clinical study was performed on 19 healthy volunteers on the out-patient basis. Beside general clinical condition, following parameters were evaluated: blood count, clinical chemistry, immune values, electrolytes, microelements, neopterin. NSC 631570 was administered intramuscularly or intravenously daily, on the alternate days or every third day at a daily dose of from 5 up to 50 mg for 7-40 days. In a special case, the medicine was administered during three years at a total dose of 3500 mg divided into several therapy courses. No significant changes in clinical status were revealed at the examination. In the case of intramuscular administration, volunteers reported local pain, sometimes sleepiness, increased thirst and polyuria. In some cases, a light non-significant increase of the body temperature and minor blood pressure decrease were observed. The authors concluded NSC 631570 at single doses of 5, 10, 20 and 50 mg were well tolerated, also at prolonged administration. (37)

### **The Dose Finding Study (Phase II)**

To find out the correct dosage for NSC 631570 a phase II clinical study was performed on 70 end stage cancer patients. The following parameters were estimated: physiologic values (heart beat rate, blood pressure, body temperature), blood count, clinical chemistry, electrolytes, immune values. The response on the therapy was evaluated by means of x-ray, ultrasonography and computed tomography (CT). NSC 631570 was administered intramuscularly or intravenously daily, on the alternate days, every third, every forth, or every fifth day. Single doses were 2.5, 5, 10, 15, 20, or 25 mg in ascending order (from 2.5 up to 25 mg), descending from 25 mg to 2.5 mg, or 5, 10, 15, 20 or 25 mg constantly. The duration of a therapy courses was 10-90 days. Breaks between courses varied from seven days up to three months. In all cases the therapy with NSC 631579 was well tolerated. In some patients the analgesics dosage could be reduced. The quality of

life improved in the most cases. Subjective as well as objective symptoms and signs were observed like headache, dizziness, thirst, sweating, polyuria, fever (with the body temperature increase of 1-2 °C), and pain at the tumor and/or metastases area. Increased temperature at the tumor area was observed also. Temporary tumor swelling, increased heart beat rate and minor blood pressure decrease were observed as well. The intensity of such concomitants correlated with the response to the therapy. After full remission these concomitants were not anymore observed (21, 45).

In healthy volunteers, such concomitant events are observed not so extensively or not observed at all. It can be suggested they are triggered by the tumor degradation products. The intensity of these concomitants can be decreased with the detoxication measures.

According to the recent findings, to achieve the best results high doses of NSC 631570 should be used in turn with small ones. Higher doses destroy tumors and smaller doses helps to eliminate the tumor degradation products. This is why alternate doses of NSC 631570 are used, e.g. 5-20 mg, or 5-30 mg, 5-40 mg daily or on alternate days. NSC 631570 can be diluted with 5% dextrose. In the case of administering 20 g NSC 631570, higher doses of vitamin C (2-4 g) should precede the injection. There are reports on tumor responses after 10 day in-patient therapy courses with daily dose of 20 mg NSC 631570 intravenously. Due to its antiangiogenic effect NSC 631570 can bring about the tumor encapsulation improving its respectability.

The clinical efficacy of NSC 631570 has been proven by many researchers and is subject of numerous publications. More than 40 original articles keep records on the treatment of more than 750 patients, 332 of these were treated with NSC 631570 in controlled clinical trials. All researchers note the efficacy and safety of NSC 631570.

### **Phase III Clinical Trials**

Ukrain can cause the full regression of the main tumour and also of metastases. In the treatment of advanced tumours Ukrain can improve the quality of life and prolong survival. Many clinical studies have proved this, such as those of the work groups led by Prof. Beger in Germany and of Prof. Zemskov in Ukraine with pancreatic cancer (182, 186, 187, 205, 247; 154, 185), as well as groups led by Prof. Susak and Prof. Bondar in Ukraine with colon cancer (67, 106, 108, 112). Neoadjuvant (before surgery) use of Ukrain can induce encapsulation of tumours as revealed the studies by the researchers of Grodno Medical University (Grodno, Belarus) in breast cancer (68-73, 114, 157-159).

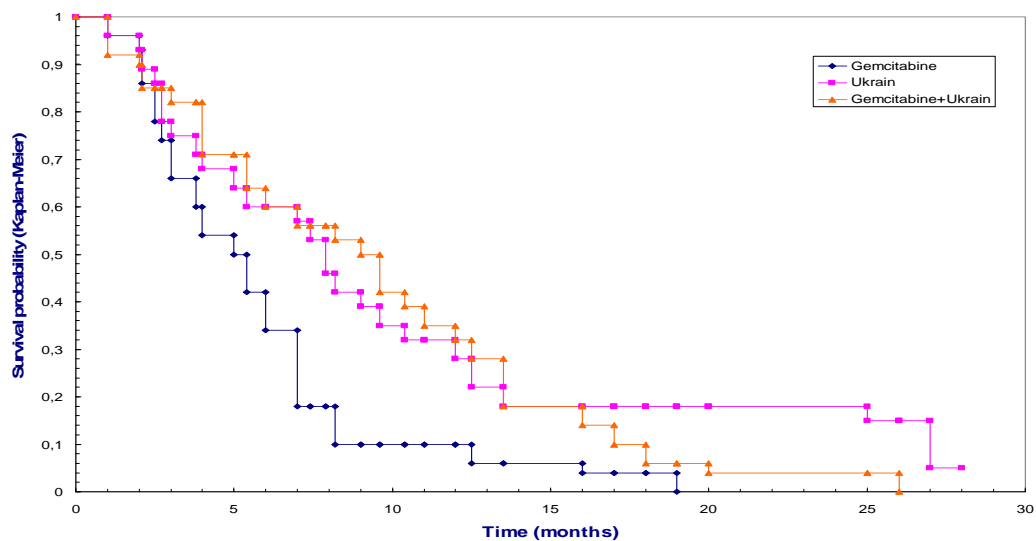
In an open study total 203 advanced cancer patients were treated with NSC 631570 and partially with local hyperthermia (37.4%) after all conventional treatment modalities had failed and the disease progressed or relapsed. Full remission was achieved in 41 cases (20.2%), partial remission – in 122 cases (60.1%). Seminoma and prostate cancer responded especially well with remission rate of more than 75% (144, 161).

## Pancreatic carcinoma

In a controlled randomised study by Prof. Beger et al. in the Ulm University Hospital, Germany, the therapy with NSC 631570 and gemcitabine doubled the survival rate in the 9 patients with inoperable advanced pancreatic cancer (182). The longest survival was 19 months in the group treated with gemcitabine alone, 26 months in the combined group, and in the NSC 631570 alone group two patients were alive after 28 months. NSC 631570 was well tolerated. The study authors consider further evaluation of NSC 631570 as justified whereas the quality of life of the patients improved (186).

### *Ukrain in pancreas cancer: palliative therapy*

Survival analysis in pancreatic cancer patients



*Gansauge, Beger et al Langenbeck's Archives of Surgery, 2002*

Patients were further observed after the conclusion of the study and it was noted that UKRAIN was well tolerated and could be administered without problem to all patients. UKRAIN brought about a significant increase in survival time in comparison to therapy with gemcitabine alone. Combination therapy with gemcitabine and UKRAIN showed no advantage over monotherapy with UKRAIN. The longest survival in the gemcitabine group was 19 months, 21 months in the gemcitabine+Ukrain group, and in the Ukrain group a patient was still alive after 28 months. The authors concluded: 'As a result of this study we highly recommend the treatment of patients suffering from advanced pancreatic cancer with Ukrain' (187).

2007 the results of another clinical study by the same research team were published. This time the efficacy of the adjuvant therapy with NSC 631570 has been demonstrated in the patients with advanced pancreatic cancer after surgery. The patients were treated with a combination of NSC 631570 and gemcitabine.

The median survival was 33.8 months and the 5-year survival rate was 23.3% which is clearly better than results reported in the earlier studies without NSC 631570, with the median survival of 20.1 months and the 5-year survival rate was 21% (<http://content.nejm.org/cgi/content/abstract/350/12/1200> ). Moreover, NSC 631570 at therapeutic dose range has only minimal adverse effects, improves the quality of life of patients and can be administered also on out-patient basis. All these features distinguishes this drug favourable compared to the standard cytostatic agents.

***Adjuvant therapy in pancreas cancer: comparison of three studies***

Author	Neoptolemos	Kurosaki	Gansauge
Year	2001	2004	2007
Number of patients	238	16	30
Therapy	5-FU/FS	Gemcitabine	NSC 631570 / Gemcitabine
Relapse free survival	k. A.	16,8 Mo.	26 Mo.
Median survival	19,7 Mo.	20,4 Mo.	37,6 Mo.

Again, this publication supports the efficacy (and safety) of the use of Ukrain as it demonstrates a considerable prolongation of survival compared to what is known from other clinical studies (247).

Other researcher confirmed the efficacy of NSC 631570 in pancreatic carcinoma (205, 208, 209), while the partial remission rate was as high as 85.7% in one study (207). The longest survival in palliative therapy was more than six years (185, 186).

Histological changes caused by the treatment with NSC 631570 in the pancreatic tumor and in the surrounding tissue were profoundly studied. NSC 631570 has been revealed to bring about the fibrotic and sclerotic transformation of the tumour. Perivascular sclerosis has also occurred (206).

**Colorectal cancer**

In a controlled randomized clinical study by the National Medical University (Kyiv, Ukraine) colon cancer patients were treated with NSC 631570 or with 5-fluorouracil and x-ray therapy. The survival rate after 21 months was 78.6% in the NSC 631570 group and 33.3% in the group treated with 5-FU and radiotherapy (67).

Within a randomized study in the Doneck Regional Cancer Center (Ukraine) rectal cancer patients received either high-dose radiotherapy and 5-FU before surgery, or the therapy with NSC 631570: one course before surgery (10 mg every second day up to 60 mg) and another course afterwards (up to 40 mg). During following 14 months, relapses occurred in six patients (25%) from the combined group and in 2 patients (8.3%) in the NSC 631570 group. Two year relapse rate was 33.3% (8 patients) in the combined group and 16.7% (4

patients) in the NSC 631570 group (112). Now, 11 years after this publication 18 from 24 patients (75%) in the NSC 631570 group are still alive.

### Prostate cancer

The efficacy of NSC 631570 in prostate cancer has been confirmed in a controlled clinical study. In the study patients, all standard treatment modalities had been exhausted. The cancer relapsed and/or progressed and no therapy protocol was available. The patients were treated with NSC 631570 and partially with local hyperthermia. Following results were achieved: full remission in 54 patients (73%), partial remission in 16 patients (22%). Only in 4 patients (5%) the therapy did not affect the course of the disease (201).

Total number of patients	Full remission	Partial remission	Disease progression
74	54	16	4
100%	73%	22%	5%

The good efficacy of NSC 631570 in prostate cancer has been confirmed in another study (155).

### Breast cancer

In a controlled clinical study conducted at the University Grodno (Grodno, Belarus), after the therapy with NSC 631570 the hardening of the tumor, a slight increase in the tumor size (5-10%) and proliferation of connective tissues were observed. The T4/T8 lymphocytes ratio increased by 30%. The tumours appeared harder and slightly enlarged after NSC 631570 therapy, and were easier to detect by ultrasound or radiological examination. Metastatic lymph nodes were also hardened and sclerotic (fibrous). Tumours and metastatic lymph nodes were clearly demarcated from healthy tissue and therefore easier to remove. Complications such as prolonged lymphorrhoea (leakage of lymph onto the skin surface), skin necrosis (death of skin tissue), suppuration of the wound, and pneumonia, all occurred in patients from the two NSC 631570 groups at only half the rate that they appeared in patients from the control group. Based on the results of this study the scientists from Grodno recommended the use of NSC 631570, at the higher dosage, in all breast cancer operations (54, 68-70, 114). Other parameters were also evaluated, e.g. hormones (T3, T4, cortisol, progesterone, estradiol, prolactin; 71), immune values (lymphocytes, immune globulins, complement, phagocytic activity; 72), morphologic and cytochemical changes (73, 110), amino acids and their derivatives in plasma (74, 109) and in the tumor tissue (75).

In a series of articles the researchers have studied the effect of NSC 631570 on various parameters in breast cancer patients (157-160). Best results were achieved with higher dosage of NSC 631570. Almost every patient noted the improvement of the general well-being, sleep and appetite. During the surgery, the tumors as well as involved lymph nodes were presented sclerotic and well demarcated from the surrounding tissue. This alleviated the surgical removal of the tumor considerably (158). In the tumor tissue, increased concentration of the amino acid proline was revealed indicating augmented production of connective tissue demarcates the tumor from surrounding tissue (159). NSC 631570 improved also the amino acid balance of patients (160).

### **Bladder cancer**

In a study NSC 631570 caused full remission in three patients for six months (113, 137).

Biochemical evaluation revealed NSC 631570 had favourably affected the amino acid metabolism (156).

### **Malignant melanoma**

The first publication on the using NSC 631570 in malignant melanoma describes the full remission in a patient with metastases to the lung (91).

A long lasting remission (more than 10 years without recurrence) has been observed in a patient with malignant nodular melanoma after the treatment with NSC 631570. At the beginning of the NSC 631570 therapy liver metastases were present and melanin was excreted with urine (92).

The effects of NSC 631570 alone and in combination with the pathogen associated molecules (PAM) on the cell cycle and apoptotic induction were compared in two melanoma cell lines MM-4 and MM-4M2 with different metastatic properties (cell division rate, hematogenous metastazing, sensitivity to the TNF-induced apoptosis). Apoptosis induction and cell viability were analyzed using trypan blue exclusion test, morphological criteria, DNA gel electrophoresis, and flow cytometry. Cell cycle distribution of tumor cells was estimated by flow cytometry. The therapy with NSC 631570 induced apoptosis in both melanoma cell lines in a dose-dependant matter. The cell line with higher metastatic potential was more sensitive to NSC 631570. In the cell line with low metastatic potential, combined use of NSC 631570 and PAM induced apoptosis more effectively (261).

### **Brain tumors**

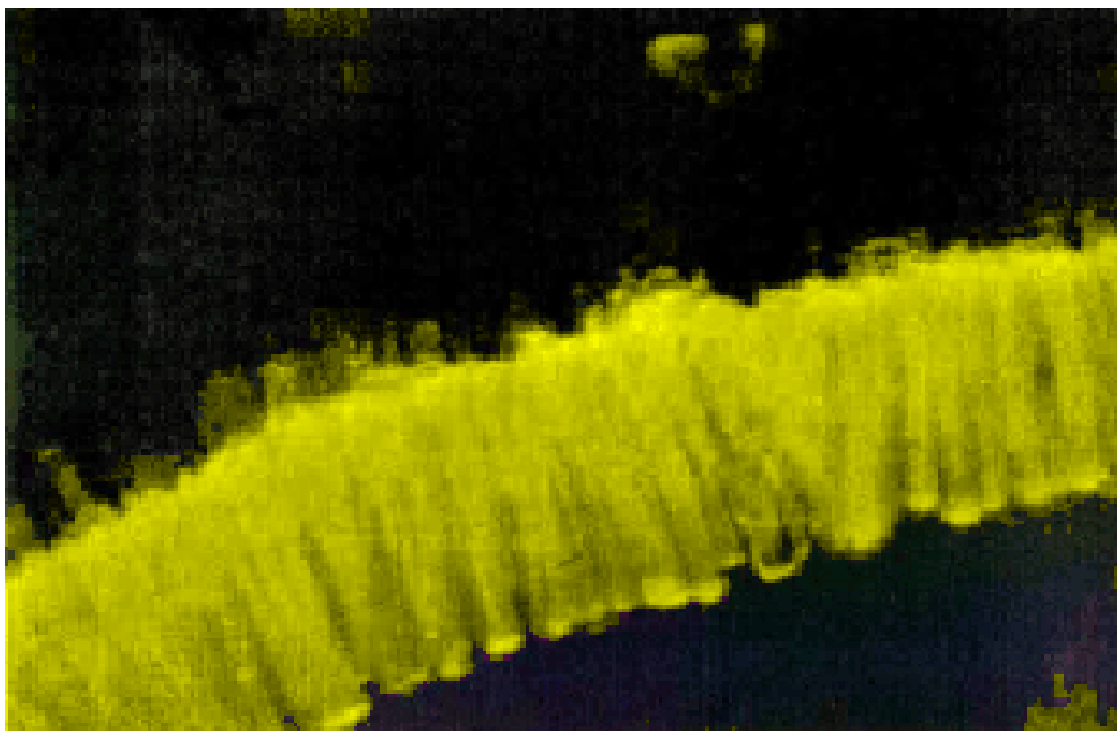
NSC 631570 has been successfully used in the treatment of brain tumors (101, 102).

In a review on the clinical studies with NSC 631570 performed so far the researchers from the Universities of Exeter & Plymouth suggested this agent to have potential as an anticancer drug (238).

## **Malignant gynaecologic tumors**

Earlier, there was a report on the successful using NSC 631570 in the treatment of ovarian cancer (97). Also in the tests of National Cancer Institute (Bethesda, Maryland, USA) NSC 631570 was toxic against all ovarian cancer cell lines tested (190). Other authors reported on good results in the therapy of cervical cancer (27, 96).

### ***Ukrain (NSC 631570): autofluorescence under UV-light***



*In ultraviolet light Ukrain fluoresces in the yellowish green range of spectrum. Excitation frequencies are within a range of 220 to 490 nm. The spectral width of the fluorescence extends from 410 to 665 nm (4).*



*Thin-layer chromatography plate with drops of Ukrain under UV light. On the left, Ukrain in a concentration of 10 mg/ml in distilled water. Then serial dilutions by a factor of 10 each time (1 mg/ml; 0.1 mg/ml,...).*



*Patient N.E., aged 82. 1½ year history of multiple and exulcerating basaliomas at the cheek-nose area.*



*The same patient under UV-light at 254 nm three minutes after the first intramuscular injection of 5 ml Ukrain. Strong fluorescence of the tumours and surrounding tissue is visible. Ukrain administered after one week produced only slight fluorescence. A considerable regression of the tumours was also observed.*

## **The selective accumulation of NSC 631570 in the tumor tissue proven due to autofluorescence**

The selective effect of NSC 631570 on cancer cells has been confirmed due to its autofluorescence under UV light (4). First time this feature of NSC 631570 was presented 1983 at 13<sup>th</sup> International Congress of Chemotherapy in Vienna. It has been confirmed in this work NSC 631570 to selectively accumulate in cancer cells. The accumulation of NSC 631570 in cancer cells correlates with the efficacy of the drug. With the elimination of the preparation from the body the intensity of the fluorescence decreases also (1).

At this congress, the first reports on the successful using NSC 631570 in the treatment of the end-stage cancer patients, where all standard therapy modalities had failed. Though poor prognosis, NSC 631570 brought about full remission in a part of the patients and some of those are still alive.

## **CASE REPORTS**

Besides clinical studies, NSC 631570 has been also used by many physicians in the treatment of various tumors, for example cervical cancer (96), ovarian cancer (97), testicular cancer (98), esophageal carcinoma (99), urethral carcinoma (100), and neuroblastoma (116). Such reports complete the pattern of the clinical using NSC 631570.

### **Breast cancer**

Physicians report on the successful treatment of a stage IV breast cancer patient. After the NSC 631570 therapy the tumor size decreased and the surgical removal was alleviated (162).

There are also more reports on the successful treatment of breast cancer (94, 95), including a relapsing breast carcinoma with lung metastases (94).

### **Sarcomas**

Beside common solid cancers, infrequent sarcomas present a real problem in oncology because of therapy resistance (93). All the more interesting is the case of retroperitoneal sarcoma successfully treated with NSC 631570. At the time of the publication, e.g. four years after the start of the NSC 631570 therapy, the patient was in full remission (163).

The Ewing's sarcoma is a tumor from connective tissue cells of bone marrow. It is a rare disease with the incidence of 3 new cases per million. Ewing sarcoma can occur on every site of skeleton. In most cases, legs, pelvic bone, scapula and ribs are concerned. Usually, children between 10-15 years suffer from it, but also children under 10 years can be affected.

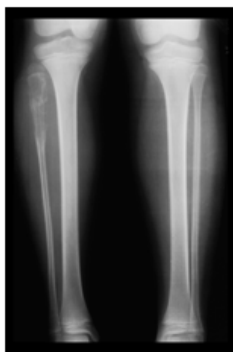
A 9 year-old girl from Poland was diagnosed with Ewing's sarcoma. The girl received chemotherapy and radiotherapy but the tumour growth could not be stopped. The girl was declared to have exhausted all possible forms of treatment (i.e. had been given up by mainstream medicine) and was sent home with infaust prospects. The parents took their daughter to Vienna to St. Anna Children Hospital because they hoped to receive the state-of-the-art treatment. The doctors carried out new examinations and had to declare that they too could not help her. The complete repertoire of mainstream medicine was used up but the tumour growth continued. At this point her parents heard about Ukrain by chance and contacted Dr. Wassil Nowicky. On 21 January 1984 treatment with Ukrain was begun.

After six months of treatment with Ukrain the girl was examined again at St. Anna Children's Hospital and to the great astonishment of the doctors not only had the tumour growth been stopped but even better, the tumour had become smaller. However, there was still no interest in Ukrain.

Treatment with Ukrain was continued and every six months the progression of the disease was checked at St. Anna Children's Hospital at the instigation of Dr. Nowicky. In this way Dr. Nowicky wanted to arouse the interest of doctors in Ukrain (unfortunately to no avail).

Gradually the tumour disappeared completely and at the X-ray examination on October 31, 1990 it was seen that even the bone which had been eaten away had regenerated (28).

***Ewing's sarcoma, first diagnosed 22.11.1983, histologically verified, tumour resistant both to chemotherapy and radiotherapy. UKRAIN therapy started on 21 January 1984.***



22.11.1983



18.1.1984



14.12.1984

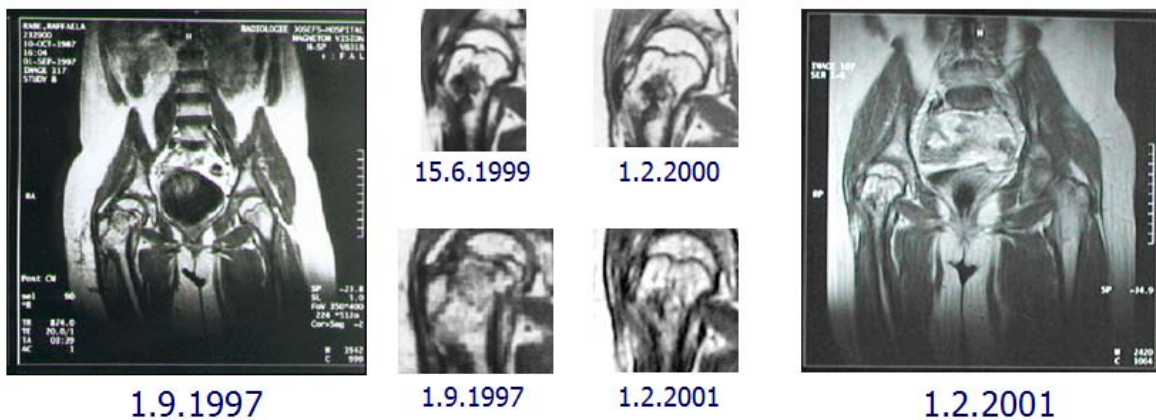


31.10.1990

*A 9 year-old girl had felt marked pain below the right knee joint in November 1983 following a slight injury. X-ray revealed Ewing's sarcoma in the proximal portion of the right fibula. Hospital treatment included chemotherapy and cobalt therapy. X-rays confirmed that the patient's tumour had not responded to radiation or chemotherapy and the tumour mass increased rapidly. One month after the end of chemotherapy, UKRAIN treatment was started at a dose of 5 mg i.m. for a total of 10 injections, combined with regional deep hyperthermia. The first series of UKRAIN therapy included three identical courses with a two-week pause between them. Six series of UKRAIN treatment were administered over the course of one year. Repeated x-rays showed reduction of the tumour mass.*

In a 10 year girl the Ewing's was verified histologically. She was treated in the high risk group of the EICESS 92 study. The MRI of the pelvis revealed the disease progression; the tumor was resistant to chemotherapy and to radiotherapy as well. The treatment with NSC 631570 was started: 15 mg mixed with 250 ml dextrose 5% and 5 g vitamin C followed by local hyperthermia on alternate days, 10 sessions totally. The control MRI revealed the stop of the progression. The followed therapy cycles brought about the regression of the tumor. At the MRI after four years no signs of a relapse or metastases could be revealed (115). The patient is in remission till now.

***Ewing's sarcoma, first diagnosed 18.3.1996; the tumour was resistant to both chemotherapy and radiotherapy. UKRAIN therapy started on 13 October 1997 (115).***



*The patient, a 10 year-old girl, was treated in the high-risk arm of the EICESS 92 study. MRI examination of the pelvic region on 1.9.1997 showed progression in the cystic-edematous process. She was then treated with combined Ukrain and local hyperthermia therapy. The therapy series consisted of 15 mg Ukrain in an infusion with 250 ml glucose and 5 g vitamin C, followed by local hyperthermia treatment. Treatment was administered every second day up to a total of 10 therapy sessions. MRI examination on 8.1.1998 showed no progression of the tumour. Subsequent therapy cycles caused regression of the tumour (see MRI on 15.6.1999 and 1.2.2000). MRI on 1.2.2001: Cystic residual defect in right femur, as observed in previous examinations. No sign of a relapse or of metastases.*

In vitro studies (studies on cell cultures) by scientists at the University of Tübingen have demonstrated the effectiveness of Ukrain with Ewing's sarcoma. These studies and clinical successes, such as with Dr. Aschhoff, were repeatedly submitted to the Ministry of Health. At Dr. Aschhoff's clinic 'Cancer patients were treated whose disease had already been treated with all mainstream medicine therapy protocols and as a result of relapse and/or progress of the disease no further mode of therapy was available for them and who had thus exhausted all modes of therapy' (161). In these patients, Dr. Aschhoff achieved high remission rate using NSC 631570, for example, 50% for Ewing's sarcoma.

### **Renal cell carcinoma**

NSC 631570 has been used for the treatment of renal cell cancer after the therapy with vinblastin had failed and the tumor had spread metastases. Full remission was achieved with Ukrain therapy and lasted 32 months at the time of publication (164).

### **Testicular cancer**

NSC 631570 has been used in the combined treatment of a nonseminoma patient and improved his immune parameters (98).

### **Esophagus carcinoma**

The therapy of an esophagus carcinoma patient with NSC 631570 induced a prolonged remission (99).

### **Bladder cancer (urothelial cell carcinoma)**

A case of a successful treatment of urothelial cell carcinoma was described. The authors reported on a sustained remission and improved quality of life of the patient (100).

### **Neuroblastoma**

After chemotherapie had failed, the patient was treated with NSC 631570. The tumor markers decreased, the metastases responded well on the therapy and the quality of life of the patient improved (116).

### **Hereditary diseases**

#### ***Tuberous sclerosis***

As an example of the using NSC 631570 in hereditary diseases, a case of a girl with tuberous sclerosis can be considered. After successful treatment the patient is now an adult woman and has given birth to a healthy son (210).

#### ***Generalised lymphangiomatosis***

Another publication reports on the successful treatment of a severe hereditary disease generalised lymphangiomatosis (211). The three year-old Stefan Dan with the diagnosis of generalised lymphangiomatosis was sent home by doctors in 1995 as having exhausted all forms of therapy. To “comfort” his parents they were told that Stefan would never be

able to speak and walk in his life. After two years of UKRAIN therapy with a general practitioner the child was most certainly able to speak and also to walk. However, treatment with UKRAIN was stopped. The tumour then began to grow and brought about a compression of the spinal cord. Stefan – in the meantime eight years-old – underwent surgery. Despite the surgical intervention the condition of the young patient deteriorated continually, resulting in him having to be connected to a lung ventilator. Paraplegia appeared. In this condition the child was discharged to be cared for at home. He had to be given morphine four times a day to relieve his pain and his breathing had to be supported with a home respirator. The doctors then after all recommended his parents to turn to UKRAIN therapy again. Although the child's condition could be improved with UKRAIN therapy, Stefan can speak, read, is very intelligent, but will no longer be able to walk.

### ***Xeroderma pigmentosum***

Xeroderma pigmentosum (XP) is a genetic disorder of DNA repair in which the ability to repair damage caused by ultraviolet (UV) light is deficient. Multiple basal cell carcinomas (basaliomas) and other skin malignancies frequently occur at a young age in those with XP. In fact, metastatic malignant melanoma and squamous cell carcinoma are the two most common causes of death in XP victims.

This is a very rare disease. The incidence differs regionally and is between 1:40000 (Japan) and 1:250000 (USA). About 250 XP patients live in the USA, about 50 in Germany, mostly children. The life expectancy is low; usually they die in the first decade. If left unchecked, damage caused by UV light can cause mutations in individual cells DNA. XP patients are at a high risk (more than 2000 times over the general population) for developing skin cancers, such as basal cell carcinoma, for this reason.

A report on the successful using NSC 631570 in a XP patient suggests this drug can be very useful also in this hereditary disease (212).

### ***Ukrain (NSC-631570) bei Xeroderma pigmentosum***

*Patient S.S., an eight year old boy, was presented with an ulcerating lesion of the nose. As he was 10 month old, xeroderma pigmentosum was diagnosed. Until the age of three years the number of skin lesions increased considerably. In May 2002 skin cancer (squamous cell carcinoma) at the nose was diagnosed, T4NXM0, histologically verified. From May till June 2002 three cycles of chemotherapy were administered (cyclophosphamide, vincristine, vinblastine). The therapy failed and the tumors grew up. Clinical investigation in April 2004 revealed deforming malignant melanoma of the nose with invasion into the cartilage of nasal septum, measuring 3x3 cm. On 20 May 2004 the therapy with Ukrain was started, 5 mg intravenously twice a week, up to a total dose of 85 mg. One month after the last administration of Ukrain a complete regression of the tumor was revealed. The skin defect was partially replaced with connective tissue. Xeroderma skin lesions improved throughout the body.*



*Photo 1. Patient S.S. before the therapy with Ukrain. Deforming invasive malignant melanoma of the nose. April 2004.*



*Photo 2. Autofluorescence of NSC-631570 at the melanoma area under UV-light during the first intravenous injection. May 2004.*



*Photo 3. Patient S.S. in December 2004. Complete regression of the tumor, with connective tissue substitution.*

## **ANTIVIRAL PROPERTIES**

The researchers from St. Petersburg, Russia have used the antiviral properties of NSC 631570 in their works with viral hepatitis C (HVC). They reveal NSC 631570, at optimal dosage, has brought about the elimination of the virus from the blood in 40 (80%) of 56 patients (165).

In their further study these researchers compared the effects of NSC 631570 at various dosages with the recombinant human interferon-alpha-2b (IFN) in 75 HVC patients. The best results were achieved when NSC 631570 was administered at a single dose of 1 mg (203).

Pilot studies evaluating the effect of NSC 631570 on HIV virus and related diseases were also performed (12). The authors noted, for example, improved immune values after the therapy (103).

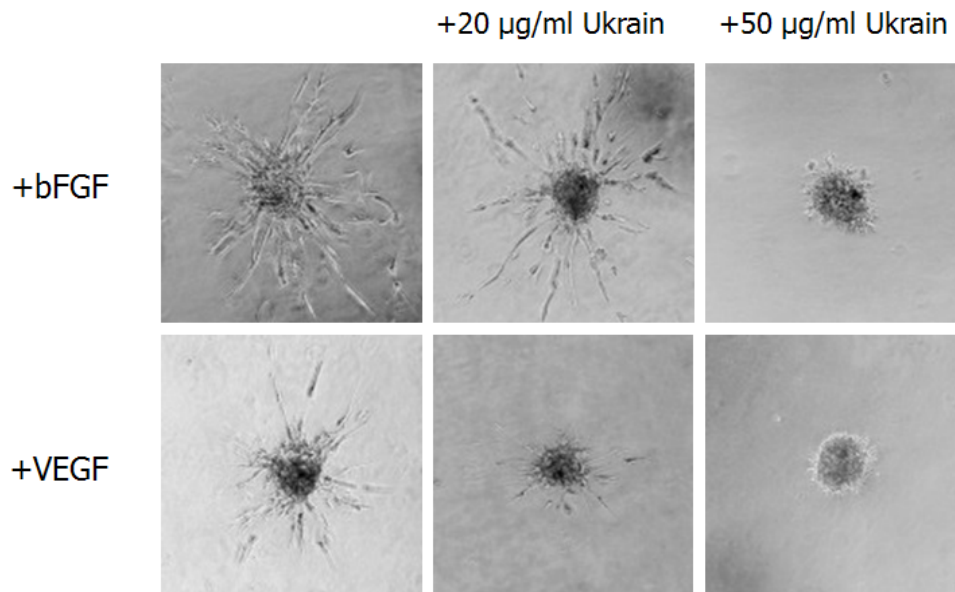
The antiviral properties of NSC 631570 were confirmed in the *in vivo* experiments (42, 51, 52, 88, 90).

## **THE INHIBITION OF THE TUMORAL ANGIOGENESIS**

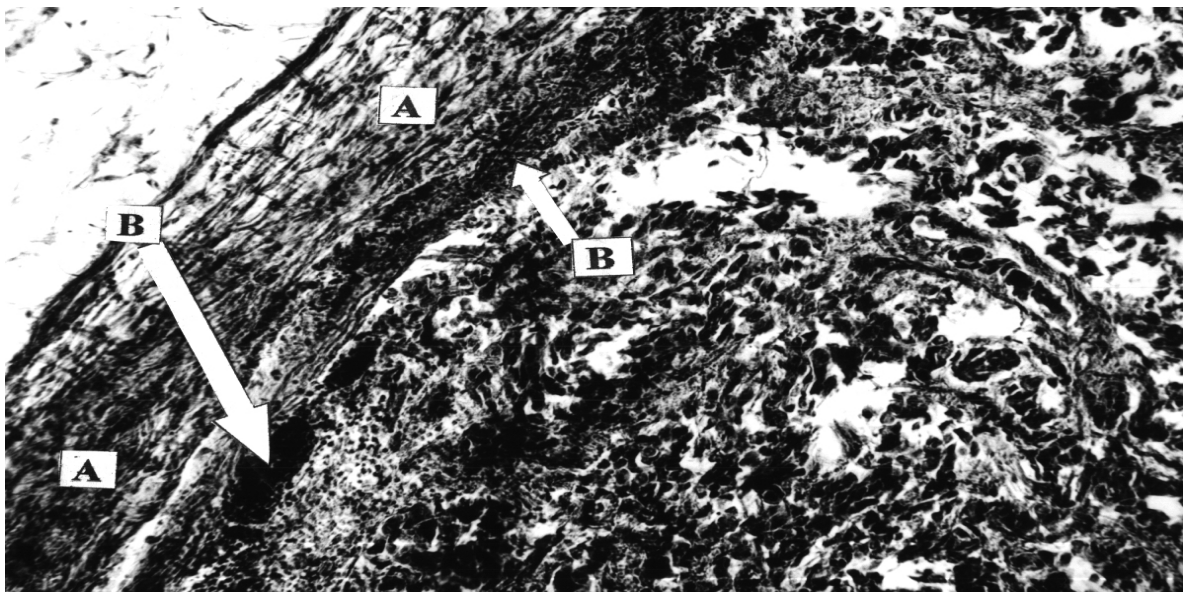
NSC 631570 inhibits the formation of the new blood vessels supplying a tumor. Due to these antiangiogenic properties NSC 631570 administered before surgery brings about better demarcation of the tumor from surrounding tissue and the tumor encapsulation. This alleviates the surgical removal of tumors what has been confirmed in breast cancer studies (68-73, 114). It is recommended to reduce the tumor burden 7-10 days after the start of the therapy with NSC 631570.

In tests *in vitro*, NSC 631570 inhibited dose-dependent the proliferation of human endothelial cells without exerting cytotoxic effect. The angiogenesis inhibition was observed on the capillary formation model. The tumor angiogenesis is the formation of new blood vessels supplying a growing tumor with nutrients. Angiogenesis is of critical importance for the tumor growth (136).

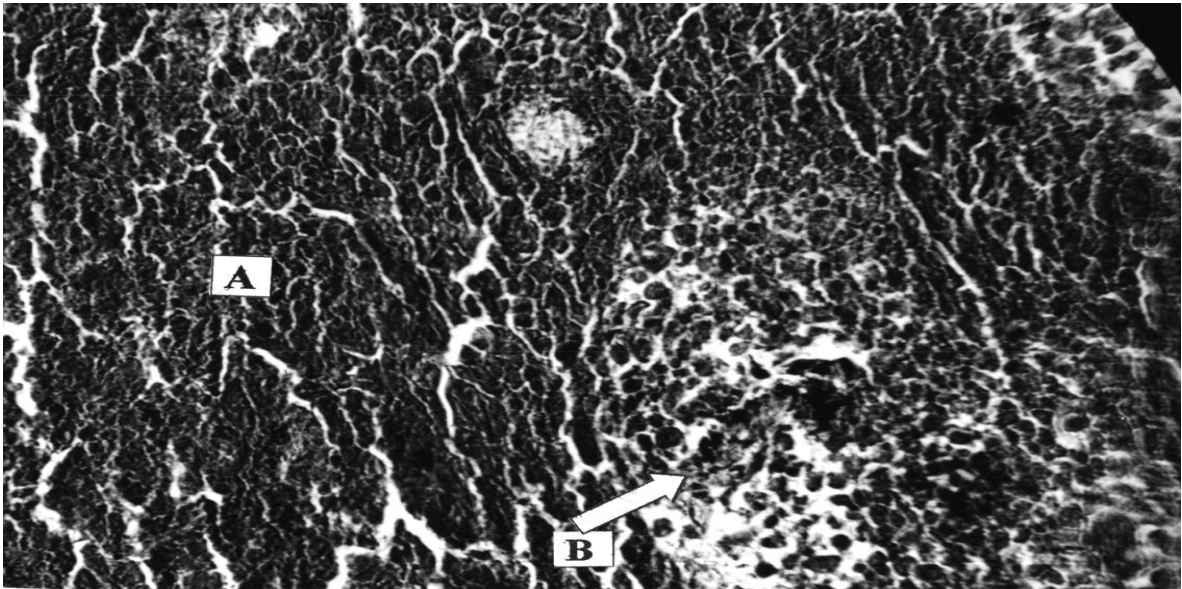
***Inhibition of the capillary sprouting by NSC 631570 in the endothelial spheroids model***



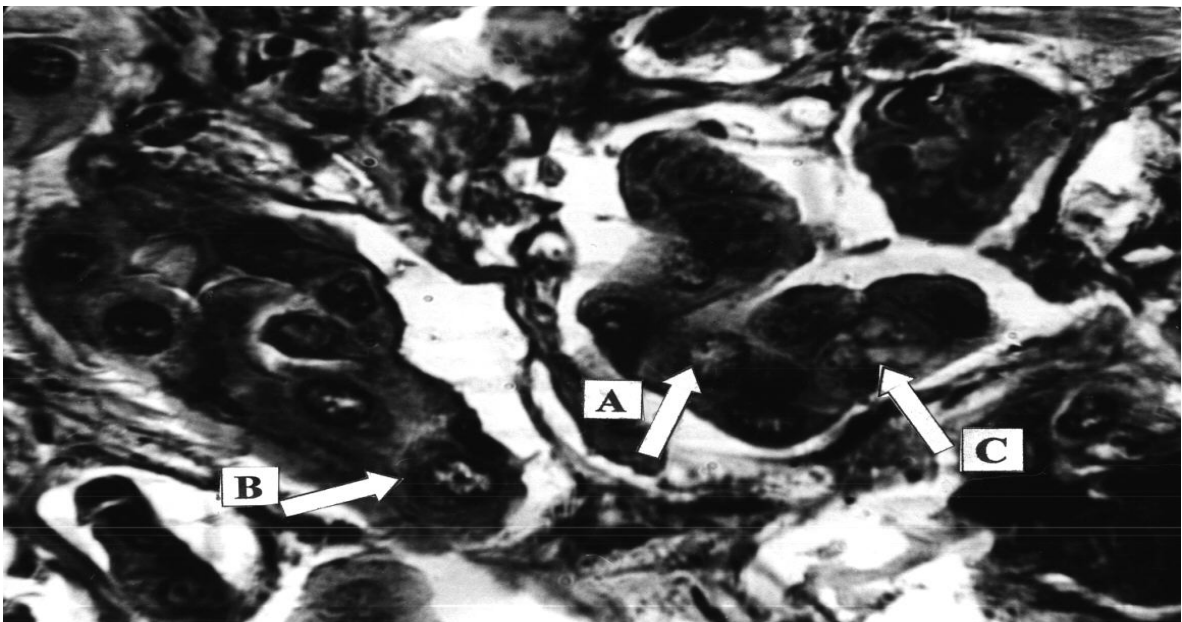
*Pancreatic adenocarcinoma, preoperative treatment with Ukrain, total 100 mg, 10 days prior to surgery (pancreatico-duodenectomy). Formation of a capsule (A) around the tumour. Tumour cells do not infiltrate the capsule. Massive round cell infiltration (B) of the tumour–capsule border area. H-e, x100.*



*Pancreatic adenocarcinoma, preoperative treatment with Ukrain, total 100 mg, 10 days prior to surgery. Necrosis of tumour tissue (A), inhibition of new vessels formation (defect in capillary wall, defect in endothelial covering, B). H-e, x200.*

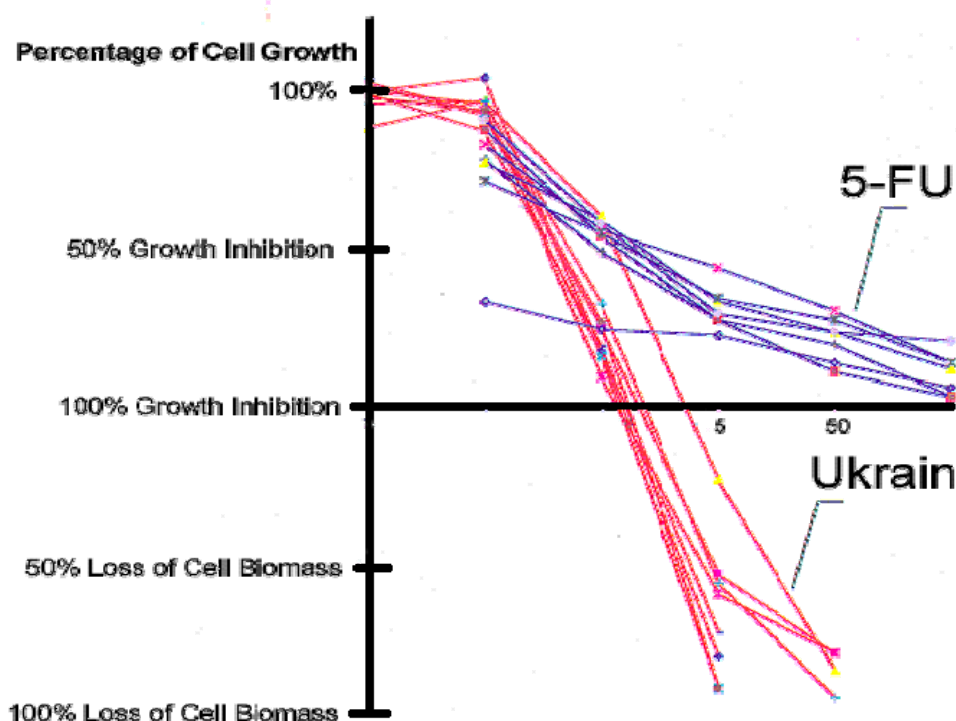


*Pancreatic adenocarcinoma, preoperative treatment with Ukrain, total 100 mg, 10 days prior to surgery. Nuclear changes: Chromatin dispersion (A) and fragmentation (B), hydropic cytoplasm degeneration (C). "Iron" hematoxylin – van Gieson, x500.*



## THE *IN VITRO* STUDIES

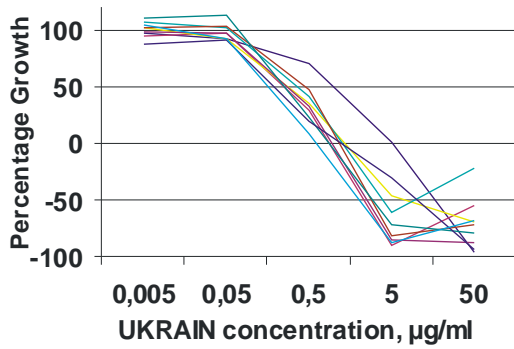
Clinical efficacy of NSC 631570 is not coincidental or even 'spontaneous remission' but rather a consequence of its mechanisms of action confirmed in various *in vitro* and *in vivo* studies. NSC 631570 has been tested on more than 100 cancer cell lines so far. Among others, NSC 631570 was tested at the National Cancer Institute (Bethesda, Maryland, USA) on 60 cell lines representing eight important human malignant tumors: brain tumors, ovarian, small cell and non-small lung cancer, colon cancer, kidney cancer, leukaemia and malignant melanoma. NSC 631570 exerted toxic effects against all these cell lines (40, 190). Compared to 5-fluorouracil (5-FU) and gemcitabine, two standard cytotoxic agents in the treatment of digestive tract tumors, NSC 631570 achieved better results and not only inhibited the cell growth but reduced the cell mass, also.



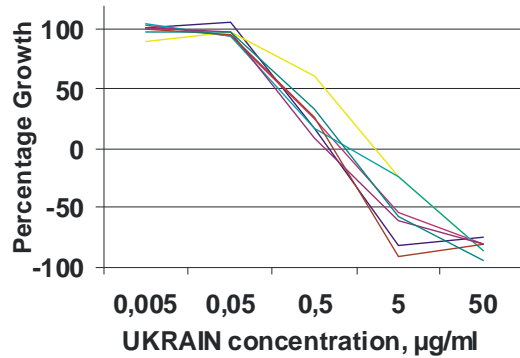
The cytotoxic effect of NSC 631570 on the cancer cell lines M-HeLa and Hep-2/0-6-5 was more pronounced than the effect of a synthetic agent Oliphen (61). NSC 631570 inhibited the growth of four Ewing's sarcoma cell lines dose- and time-dependent. In this experiment the effect of NSC 631570 was more pronounced than this of thiotepa (243, discussed in 244). In the tests on Ehrlich's carcinoma cells and lympholeucemia P-388, the researchers revealed that the sensitivity of the cancer cells to NSC 631570 depends highly on the cell cycle phase, the first sensitivity peak being at the end of G1 phase and the second - in G2 phase (148). The effects of glucose, succinat, pH value and increased temperature on the efficacy of NSC 631570 against cancer cells were investigated *in vitro*. Glucose reduced the cytotoxic effect of NSC 631570, but succinat intensified it. The most pronounced effect was at pH 7.3-8.0. The temperature up to 41.5 °C did not impact the effect of NSC 631570 (117).

**Results of the Ukrain (NSC 631570) study at the National Cancer Institute, Bethesda, Maryland, USA. The effects of NSC 631570 on the 60 various human cancer cell lines**

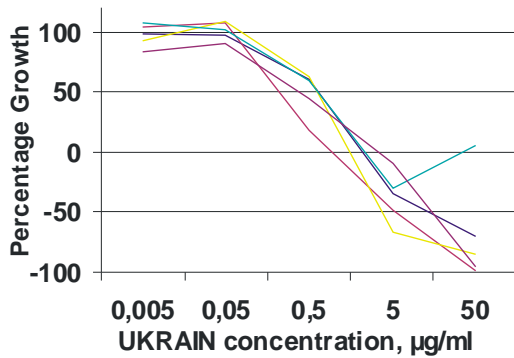
**Malignant Melanoma**



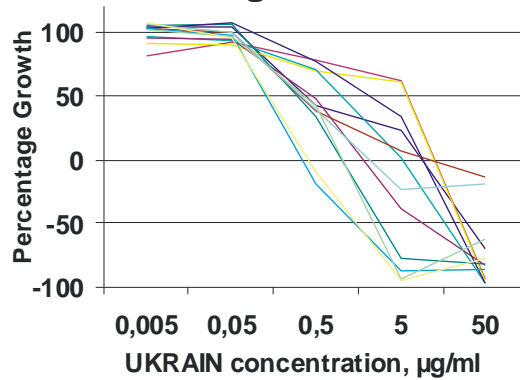
**Colon Cancer**



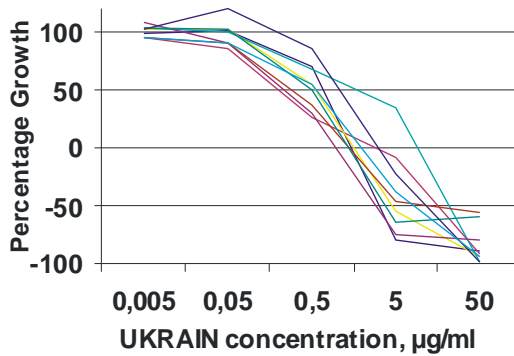
**Ovarian Cancer**



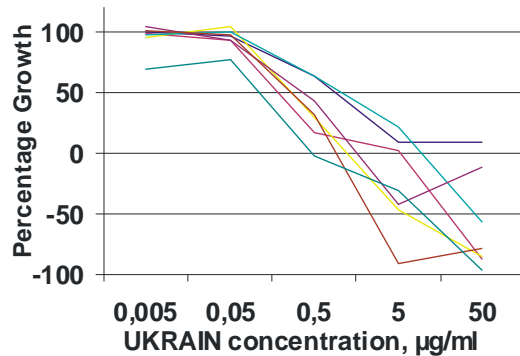
**Lung Cancer**



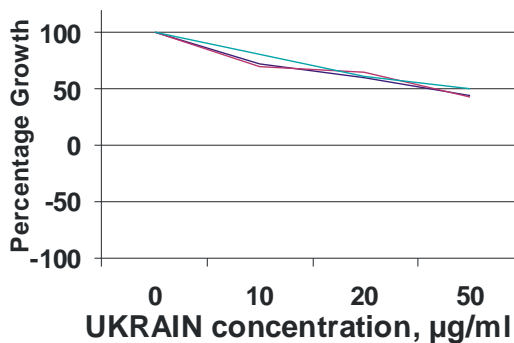
**Renal Cancer**



**Brain Cancer**



**Normal Cells**



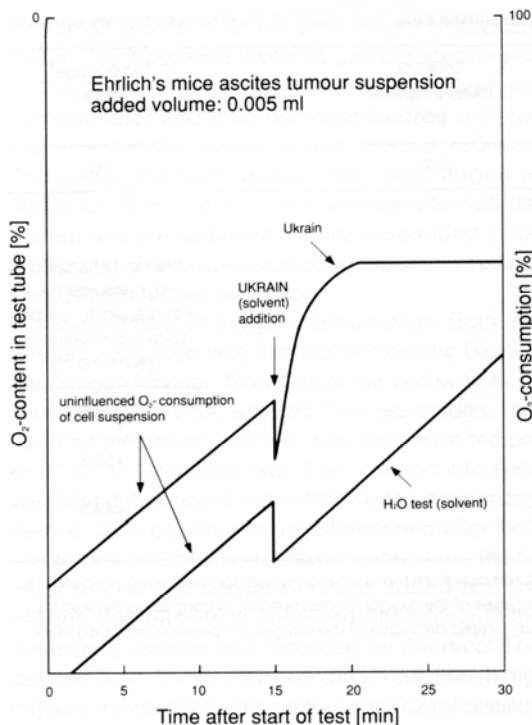
## The Selective Effect of NSC 631570

In comparative studies NSC 631570 was tested on 18 malignant and 12 non-malignant cell lines at identical conditions (36, 38, 63, 143, 147, 149, 181, 184, 190, 245, 255). These experiments eliminated all doubts on the selective effect of NSC 631570. Numerous studies have confirmed NSC 631570 to be the first and only anticancer agent being toxic against cancer but not normal cells. This explains also its good tolerability in clinical use.

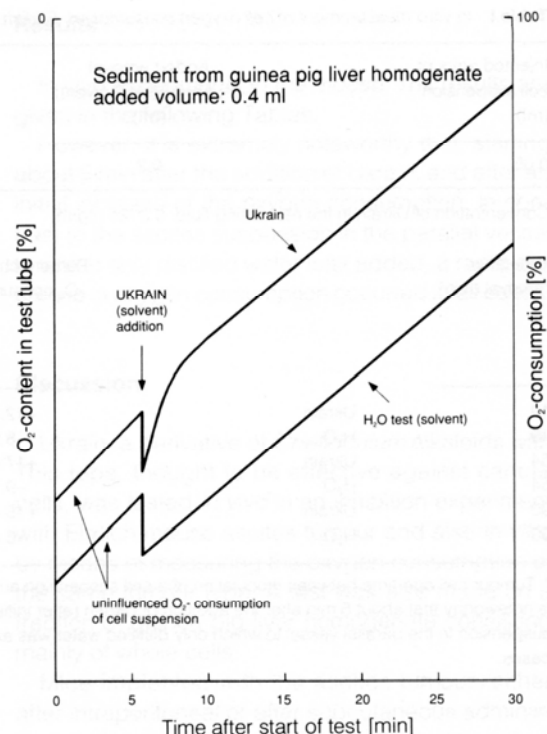
First indications on the selective effect of NSC 631570 on the cancer cells provided an early study in 1976 of the Bundesstaatlichen Anstalt für Experimentell-Pharmakologische und Balneologische Untersuchungen (Vienna, Austria). This study revealed different oxygen consumption by normal liver cells and Ehrlich's tumor ascitic cells after the incubation with NSC 631570 was revealed (38).

About at the same time, the researchers from the Vienna University of Agriculture compared the inhibiting effect of NSC 631570 on the proliferation of malignant and normal cells. For 50% growth inhibition of normal endothelial cells, the NSC 631570 concentration had to be tenfold higher than for the same growth inhibition of human osteosarcoma cell line (36).

### The different influences of Ukrain on oxygen consumption in healthy cells and cancer cells

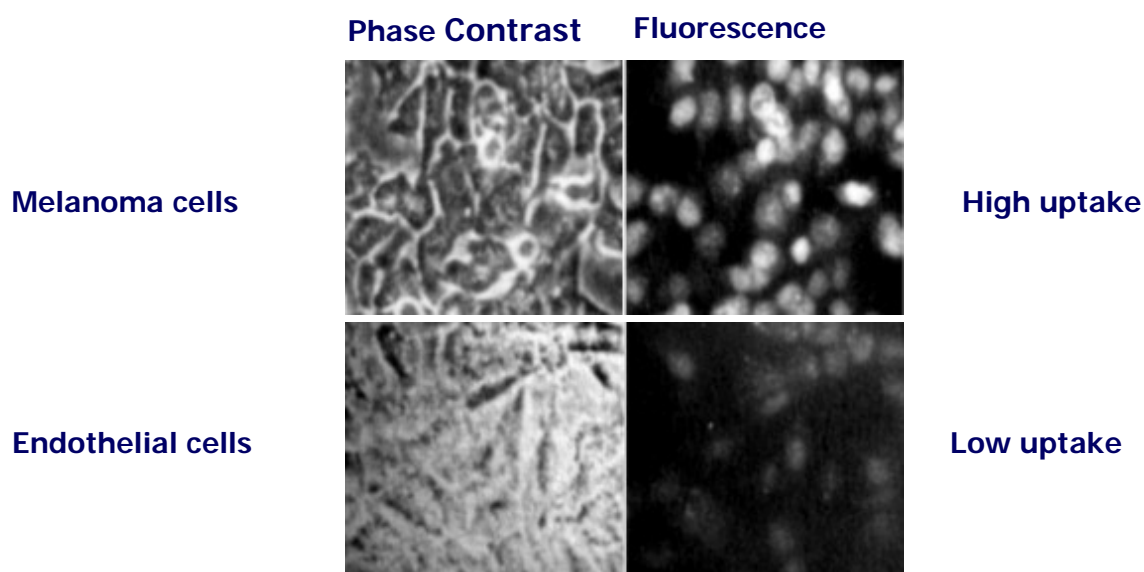


*The effect of Ukrain on the oxygen consumption in malignant cells (murine Ehrlich's ascites tumour suspension)*



*The effect of Ukrain on the oxygen consumption in normal cells (sediment from guinea pig liver homogenate)*

***The uptake of Ukrain in melanoma cells compared to normal cells (in vitro)***



*Hohenwarter O. et al. Selective inhibition of in vitro cell growth by the anti-tumour drug Ukrain, Drugs under Experimental Research, 1992 (36).*

This selective effect of NSC 631570 on cancer cells has been confirmed in numerous studies at the renowned universities and research facilities.

In the study from European Organisation for Research and Treatment of Cancer (EORTC), human tumor xenografts (HTX) were implanted into nude mice. These tumor cells were later incubated with NSC 631570 at various concentrations. Following HTX were used: colon cancer CXF 1103/11, stomach cancer GXF 217/17, lung cancer LXFL 529/14, breast cancer MAXF 401/13, melanoma MEXF 276/10, and ovarian cancer OVXF 899/9. NSC 631570 was active against OVXF 899/9 at 10 µg/ml and in all colonies tested at 100 µg/ml with the T/C-ratio ('test to control') 1/135 in OVXF, 8/109 in CXF, 10/98 in GXF, 15/187 in LXFL, 34/133 in MAXF, and 10/122 in MEXF (64, 189).

In 1998, at the 89th Annual Meeting of the American Association for Cancer Research in New Orleans, USA, researchers from the University of Pretoria, South Africa presented their work on the selective effect of NSC 631570 on various cancer cell lines. The authors concluded 'that Ukrain is selectively toxic to malignant cells by causing a metaphase block which is characterised by abnormal chromosomal distribution, and results in the formation of micronuclei and in apoptosis' (139). In 2000 the same group discovered NSC 631570 to inhibit the tubulin polymerisation. In this paper they denied the selective mode of action of NSC 631570 on cancer cells (140).

Researchers from Eberhard-Karls-University Tubingen, Germany, investigated the effects of NSC 631570 on cell survival, alteration of the cell cycle and induction of apoptosis without and in combination with ionising radiation (IR) at a dose of 1-10 Gy. The tests

were performed on the exponentially growing human tumor cells MDA-MB-231 (breast), PA-TU-8902 (pancreas), CCL-221 (colon cancer), U-138MG (glioblastoma), and human skin and lung fibroblasts HSF1, HSF2 and CCD32-LU. Without IR, NSC 631570 exerted a time- and dose-dependant cytotoxic effect, more pronounced against the cancer cells. Flow cytometry revealed NSC 631570 to modulate radiation toxicity against human cancer cell lines and to protect normal cells from radiation. The combination of NSC 631570 plus IR gave enhanced toxicity in CCL-221 and U-138MG cells with their accumulation in the G2/M phase of the cell cycle, but not in MDA-MB-231 and PA-TU-8902 cells. A radio protective effect was found in normal human skin and lung fibroblasts. The authors suggest a reasonable use of NSC 631570 in combined radiochemotherapy (184).

### **Induction of the Apoptosis in Cancer Cells**

In the study on the erythroleucemia cells K-562, it was revealed NSC 631570 to bring about the bimodal death of cancer cells. At lower concentrations of NSC 631570, cancer cells die in as a consequence of apoptosis. At higher concentrations, the formation of microtubules is inhibited and polyploidy occurs (56, 62).

The researchers from the Rochester University, USA revealed NSC 631570 causes the accumulation of prostate cancer cells as well as epidermoid carcinoma cells in the G2/M phase, however, not of normal cells (149, 150).

In the tests on human cervix carcinoma cells HeLa, squamous carcinoma cells WHCO5, normal kidney cell line Graham 293, and transformed kidney cell line Vero from African green monkey the researchers of the University of Pretoria, South Africa revealed NSC 631570 'is selectively toxic to malignant cells by causing a metaphase block which is characterised by abnormal chromosomal distribution, and results in the formation of micronuclei and in apoptosis' (139).

The scientists of the Eberhard Karl University (Tübingen, Germany) investigated the effect of NSC 631570 on the cell survival, the cell cycle modification and the apoptosis induction alone and combined with radiation (IR). They discovered NSC 631570 combined with IR increased the toxicity against the cell lines CCL-221 and U-138MG. The normal human skin and lung fibroblasts were protected from the damaging effects of IR (184).

In the tests at the Nacional de Cancerologia, Mexico City, Mexico NSC 631570 revealed that Ukrain induced apoptosis in a panel of cancer cell lines (cervical cancer HeLa, HeKB, HeKS32, HeBcl3, HeNFR and HeIKK, human colon cancer SW480, human renal carcinoma HEK293, human osteosarcoma MG-63) by activating the intrinsic cell death pathway. Interestingly, non-transformed fibroblasts (hTERT) cell line was insensitive to the drug (255).

## ***Ukrain's (NSC 631570) deadly action against cancer cells - Induction of apoptosis***

Apoptosis



Estimating the cell proliferation according to the BrdU uptake in the cell lines AsPC1, BxPC3, MiaPaCa2, Jurkat, and THP-1 and the cell cycle phases by means of Giemsa staining, the authors established NSC 631570 at a dose of 10  $\mu\text{g}/\text{ml}$  brought about a considerable accumulation of cancer cells in the G2/M phase after 24 h incubation. The apoptosis rate in the peripheral mononuclears was similar at the same incubation conditions. Moreover, the mitogene stimulated lymphocytes showed increased blastogen reaction (181).

The apoptosis induction by NSC 631570 has been also confirmed in the *in vitro* tests on the Chinese hamster ovarian cells. In this experiment the effects of NSC 631570 and etoposide were synergistic (167).

### **Inhibition of the Tubulin Polymerisation**

In the experiments with cow brain tubulin, the scientists from the Pretoria University (South Africa) discovered NSC 631570 to inhibit the tubulin polymerisation (146).

Later on, the researchers from the University of Ulm revealed in the tests with pancreas cancer lines AsPC1, BxPC3, Capan1, MiaPaCa2, and Panc1 NSC 631570 brings about a dose dependant cell cycle arrest in the G2/M phase. Moreover, NSC 631570 stabilizes the tubulin monomers in cancer cells and consequently inhibits the formation of microtubules (143).

## **Activation of Mitochondrial Caspases**

Caspases, or cysteine-aspartic proteases are a family of enzymes, which play essential roles in apoptosis (programmed cell death) hence in cancer therapy.

In the tests on the Jurkat lymphoma model, NSC 631570 has been proven to be a strong apoptosis inductor. Profound research showed NSC 631570 brought about the depolarisation of mitochondrial membranes and consequently the activation of caspases (246).

There are two types of apoptotic caspases: initiator (apical) caspases and effector (executioner) caspases. Initiator caspases CASP 8 and CASP10 induce the death factor dependant or extrinsic apoptosis pathway, whereas effector caspase CASP9 mediates the intrinsic or mitochondrial apoptosis. To establish the exact mechanism of apoptosis induced by NSC 631570, caspases were assigned with Western blot in the cancer cell line HeLa after incubation with NSC 631570. The results showed NSC 631570 to trigger the intrinsic apoptosis pathway (255).

## **The Effect on Cyclins and Cyclin-Dependant Kinases**

Cyclins are a family of proteins which control the progression of cells through the cell cycle by activating cyclin-dependent kinase (Cdk) enzymes. A cyclin forms a complex with Cdk. Complex formation results in activation of the Cdk active site. When concentrations in the cell are low, cyclins dissociate from Cdk, thus inhibiting enzymatic activity; this probably occurs due to a protein chain of the Cdk blocking the active site upon cyclin dissociation. There are several different cyclins which are active in different parts of the cell cycle and which cause the Cdk to phosphorylate different substrates. Increasing concentration of cyclin A triggers the cell into the G2 phase, whereas cyclin B is essential for the mitosis initiation.

The researchers from the Rochester University explored the effect of NSC 631570 on cyclins and Cdk in the epidermoid cell lines ME180 and A431 as well as in the prostate carcinoma line LNCaP. They found changes in the concentrations of mitotic cyclin A and B1 as well as Cdk1 and Cdk2. Increased expression of Cdk inhibitor p27 has been also observed in both cancer cell lines. This can promote accumulation of the cells in the G2/M phase (147, 149).

## **The Effect on the Expression of hENT1**

The interactions between NSC 631570 and the molecular determinant expressions involved in the metabolism of gemcitabine, such as hENT1 and dCK were evaluated in *in vitro* studies on pancreatic ductal adenocarcinoma cells (PDAC). Two ATCC cell lines (PL45 and Mia PaCa-2) and 2 Primary Cell Cultures obtained from PDAC patients underwent surgical resections (PPTCC78 and PPTCC109) were used. Cells were treated with Ukrain at IC<sub>50</sub> concentration levels for 48h. All the amplifications were carried out with

normalization of gene expression against the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping control gene, and the quantitation of gene expression was performed. NSC 631570 positively modulated the expression of hENT1 mRNA in all PDAC cell cultures treated with IC50 ( $p < 0.001$ ). The analysis revealed a mean increase of 2.8 fold ( $p = 0.001$ ) with respect to untreated control cells. In PL45 and Mia PaCa-2 cells NSC 631570 positively affects mRNA expression of dCK gene as well.

Based on the previous clinical data the NSC 631570-gemcitabine combination appears a promising regimen and the results of this study provided the experimental basis for the further clinical testing of the NSC 631570-gemcitabine schedule in PDAC patients (Funel et al, 'Molecular mechanisms underlying the synergistic interaction of the novel anticancer drug Ukrain with gemcitabine in preclinical models of pancreatic cancer', 44<sup>th</sup> Annual Pancreas Club Meeting, New Orleans, USA 2010).

### **The Effect on the DNA and Protein Synthesis**

The effect of NSC 631570 on the DNA and protein synthesis in cancer cells was studied *in vitro* on the cell lines HeLa, Yoshida, mice lymphoma, mice myeloma, human WiDr tumor, EB, EsB, YAC-1, and P815. NSC 631570 (1-100  $\mu\text{g/ml}$ ) has been proven to inhibit in dose-dependant way the DNA, RNA and protein synthesis in these cell lines. At the same test conditions, the inhibition in normal cells (human tonsil cells and guinea pig liver cells) was much lesser distinct (58, 63).

Among many possible mechanisms of action of NSC 631570, its effect on the endonucleases of the rat liver and on topoisomerases was studied (topoisomerases play an important part in the DNA metabolism). In these experiments NSC 631570 inhibited the metal-dependant endonucleases and the topoisomerase I as well (166).

### **The Effect on the Proteins Involved into the Remodelling of Extracellular Matrix**

An Italian research team from the University of Milan used RT-PCR, Western blot analysis and SDS-zymography to evaluate the effect of NSC 631570 on the expression of genes and proteins involved into the remodelling of the extracellular matrix. This mechanism plays an important role in the tumor invasion of human glioblastoma cells. There was a significant dose-dependant decrease of the proliferation of glioblastoma cells and a trend to the down-regulation of secreted protein acidic cysteine rich (SPARC). This study provided theoretical reasons for the using NSC 631570 in glioblastoma. The authors concluded: NSC 631570 'may be a useful therapeutic tool for brain tumors' (245). This has been proved in clinical use (see "Brain tumors").

In their further work with glioblastoma cells the Italian researchers revealed NSC 631570 to increase the expression of the glial fibrillary acidic protein (GFAP). The connexin 43 expression was not modulated by NSC 631570. These results 'support the possible potential of NSC 631570 for the therapy of brain tumors' (250).

This Italian team investigated also whether NSC 631570 is able to modulate the *in vitro* expression of some proteins involved in tumor progression on three clear cell type renal carcinoma cell lines (ccRCC). CAKI-1, CAKI-2 and ACHN clear cell carcinoma (ccRCC) cells were treated with three doses of NSC 631570 (5, 10, and 20  $\mu$ M) or left untreated, and cultured for 48 h in duplicate. SPARC protein levels were assayed in Western blot, MMP-2 and MMP-9 protein levels and activity were determined by SDS-zymography in cell culture supernatants, and the distribution of the different cell cycle phases was calculated using FACS analysis. The results suggest that NSC 631570 modulates two major aspects involved in tumorigenesis of RCC cancer cells, that are extracellular matrix remodelling and cell proliferation. The tendency to MMP-2 and MMP-9 down-regulation in UK-treated cells suggests that UK may decrease RCC cell invasion. Moreover, SPARC protein down-regulation in supernatants point to an inhibition elicited by UK also on extracellular matrix remodelling in the tumor microenvironment, possibly rendering the tumor microenvironment less permissive for tumor invasion. At the same time, SPARC intracellular protein levels up-regulation in ccRCC cells suggest that NSC 631570 may be involved in the inhibition of cell proliferation by cell cycle inhibition, as shown by FACS analysis. (Pettinari et al, 'Matrix metalloproteinases activity and SPARC expression are targeted by Ukrain administration in renal cell carcinoma', presentation at the symposium 'Targeting Cancer Invasion and Metastasis', Miami, USA, 2010).

The effect of NSC 631570 on the modulation of some of the key markers of tumor progression in pancreatic cell carcinoma was investigated on three cell lines HPAF-II, PL45, and HPAC. The cell lines were treated with NSC 631570 (5, 10 and 20  $\mu$ M) for 48 h, or left untreated. Secreted protein acidic and rich in cysteine (SPARC) mRNA levels were assessed by real-time PCR. Matrix metalloproteinases (MMP)-2 and -9 activities was analyzed by SDS zymography; SPARC protein levels in cell lysates and supernatants were determined by Western blot. Cell cycle was determined by flow cytometric analysis, and invasion by matrigel invasion assay. NSC 631570 down-regulated MMP-2 and MMP-9, suggesting that UK may decrease pancreatic cancer cell invasion, as confirmed by the matrigel invasion assay. SPARC protein downregulation in supernatants points to an inhibition NSC 631570 of extracellular matrix remodelling in the tumor microenvironment. At the same time, SPARC mRNA and cellular protein level up-regulation suggests that NSC 631570 can affect cell proliferation by cell cycle inhibition, showing a cell cycle G2/M arrest in UK-treated cells (Funel et al, 2010, submitted).

### **The Effect on the Heat Shock Proteins**

In the tests with human lymphoma cells the effect of NSC 631570 on the lines U-937 and U-937/hsp70 was determined. These cell lines differ in the expression of the heat shock protein 70 (Hsp70). The line U-937/hsp70 turned out to be more resistant against the effect of NSC 631570. The authors attribute this to the protective effect of Hsp70 (200).

## **The Effect on the Electrokinetic Potential**

A study examined how NSC 631570 affects the electrokinetic (EKP) potential of malignant and benign cells. The EKP of the Ehrlich's carcinoma cells dropped after incubation with NSC 631570. The EKP decrease of normal cells was less pronounced (221).

## **THE *IN VIVO* STUDIES**

The anticancer effect of the intravenous administration of NSC 631570 (4 µg/day) was demonstrated in the tests on BALB/c mice with the implanted rapidly proliferating mammary adenocarcinoma D1 DMBA-3. Three routes of administration were employed for NSC 631570, e.g. intravenous, intraperitoneal and subcutaneous. Intravenous administration was proven to be the most effective and inhibited the tumor growth significantly. The authors evaluated also the role of immune factors in the effect of NSC 631570 and revealed this agent can restore the cytotoxic effect of macrophages (26, 43).

Later this mode of action was confirmed in a study on CBA mice. Once again the role of macrophages in the antitumor effect of NSC 631570 was stressed by the authors of the study (120).

In an experimental study NSC 631570 inhibited the growth of the primary melanoma B-16 and its metastases in mice (107).

In the tests with mice C57BL6 NSC 631570 inhibited the growth and metastazing of the primary implanted Lewis' carcinoma compared to the control group (219). This effect of NSC 631570 was confirmed in another study. Here too, the intravenous administration was the most effective (254).

In a study on 18 mice CBA, the mixture of 0.1 mg NSC 631570 and 10000 carcinoma cells Krebs-2 was injected intramuscularly. In the control group only tumor cells were injected. After 11 days a significant inhibition of the tumor growth was established in the group treated with NSC 631570. Similar effect was achieved also in mice with implanted hepatoma cells (118).

In mice with implanted hepatoma HA-1, NSC 631570 increased the concentration of procathepsin B in the ascitic fluid whereas the concentration of cathepsin B decreased. Moreover, the concentration of the macrophageal p-hexosaminidase increased. This indicates an inflow of macrophages into the peritoneum (119).

The anticancer effect of NSC 631570 was also confirmed on Wistar rats with implanted sarcoma-45 (122).

Two different forms of Ehrlich's carcinoma cells were transplanted intraperitoneally and subcutaneously to mice. NSC 631570 was administered intraperitoneally for six days. The effect of the drug on the tumors was estimated by the indexes of the tumor growth inhibition (TGI), total number of tumor cells in ascitic fluid, number of viable tumor cells and average life span of experimental animals. Cell cycle distribution of cancer cells was

determined by flow cytometry. The number of circulating phagocytes was estimated by flow cytometry using FITC-labelled *S. aureus*. Intraperitoneal administration of NSC 631570 in mice with ascitic form of Ehrlich's carcinoma resulted in moderate tumor growth inhibition, but was accompanied by acute local inflammation and caused reduction of life span of experimental animals. In the mice bearing the solid variant of Ehrlich's carcinoma the treatment with NSC 631570 led to significant TGI and slight increase of life span. The treatment caused restitution of the number of circulating phagocytes in peripheral blood in the both groups of animals. The authors concluded the antitumor effect of NSC 631570 was mediated by its direct proapoptotic action as well as by the interactions with the murine immune competent cells (262).

### **The Effect on Cystein Proteases**

In mice with implanted LS-lymphosarcoma and HA-1 hepatoma, the concentrations of cystatin C, cathepsin B and cathepsin L were estimated after the treatment with cyclophosphamide and NSC 631570. An increased concentration of cystatin C was found only in hepatomas treated with NSC 631570. The concentrations of cathepsin B and cathepsin L were increased in LS lymphosarcoma. These results indicate apoptosis as the mechanism of action of NSC 631570 in these tumor models (169).

On the same tumor models the effect of NSC 631570 on cathepsin D was studied. Cathepsin D is an important lysosomal protease, modulating apoptosis induced by interferon-gamma and TNF-alpha. NSC 631570 combined with cyclophosphamide increased cathepsin D activity in mice with LS-lymphosarcoma. NSC 631570 alone did not exert this effect (170).

Cystatin C is a well known extracellular protease inhibitor and has been used as a possible marker of the tumor growth and efficacy of the anticancer therapy (204). The using NSC 631570 in mice brought about a 4fold increase of cystatin C in the tumor tissue (171).

In the tests on male mice CBA/C57Bl/6J the serum concentration of cystatin C after the implantation of Lewis' adenocarcinoma was only half as high as in the control group. The combined treatment with NSC 631570 and cyclophosphamide normalised the cystatin values (218).

On the experimental mice tumors LS-lymphosarcoma, hepatoma HA-1 and Lewis' adenocarcinoma, the effect of NSC 631570 on the cystatin A (stefin A) was studied. The treatment with NSC 631570 caused the increase of stefin A in the liver of animals with Lewis' adenocarcinoma, but not in the tumor itself. In other tumor models the concentration of cystatin A both in the liver and tumor tissue did not change. In LS-lymphosarcoma and hepatoma HA-1 the serum concentration of cystatin A increased in the course of the treatment with NSC 631570 (220).

The treatment of the hepatoma HA-1 implanted mice with NSC 631570 caused the deceleration of the tumor growth and increased the survival of the animals. The macrophages count increased whereas the number of tumor cells in the ascetic fluid diminished. NSC 631570 did not affect the activity of cathepsin B and cathepsin L (199).

## MODULATION OF THE IMMUNE SYSTEM

Unusual for an anticancer agent NSC 631570 possesses some distinct immune properties (24, 44). In several immune target-effector systems NSC 631570 significantly amplified the malignotoxic activity of macrophages (231), lymphocytes and NK cells (47), and stimulates dendritic cells maturation *in vitro* (258). While the parameters like B-lymphocytes count, immune globulin concentrations, complement and acute phase proteins did not changed significantly, it can be postulated NSC 631570 modulates the cellular part of the immune system whereas the humoral part remains unaffected.

### Clinical Immunology

The incubation of peripheral lymphocytes of healthy blood donors with NSC 631570 resulted in the increase of lymphocytes with the T-helper phenotype, decrease of the lymphocytes with T-suppressor genotype as well as increase of T-helper/T-suppressor ratio (7, 18).

NSC 631570 was administered to nine advanced stage cancer patients (4 with liver cancer, 4 with head and neck carcinomas, and one breast cancer). In three cases the tumors responded partially on the therapy, in one case a minimal response was noted, in 3 cases the disease was stabil, and in 2 cases the tumors did not respond on the treatment. After the therapy, the number of T-helper cells (DC4) as well as the CD4/CD8 ratio increased (13).

In eight oncological patients immune parameters were compared before and after the treatment with NSC 631570. It was revealed NSC 631570 affected basically the thymus dependant cells (T-cells). The number of rosette-forming T-lymphocytes was significantly higher after the treatment. No significant changes were observed in the humoral immune parameters (22).

In nine male lung cancer patients lymphocytes subpopulations were determined before and after the therapy with NSC 631570. The therapy resulted in increased total T-cells and reduced T-suppressor fraction. The helper-suppressor ratio normalized. There was no sign of the activation of NK cells, T-helpers as well as B-cells. The restoration of the cellular immunity correlated with the better clinical course of the disease (25).

The effect of NSC 631570 on the functional activity of monocytes from 20 patients with lung cancer or peritonitis was studied using nitro blue tetrazolium chloride test (NBT-test). The authors reported on the positive effect of NSC 631570 on the functional activity of the macrophages as well as antioxidant systems of monocytes and erythrocytes (46).

23 patients with various tumors were treated with NSC 631570 and the immune e parameters were evaluated before and after the therapy. The authors observed the increase of lymphocytes and the decrease of the blood sedimentation rate. Following immune changes were also noted: increase of T-lymphocytes, T-helpers, NK-cytotoxicity, phagocytic activity, normalisation of the T-helper/T-suppressor ratio, and occurrence of large granular lymphocytes (48, 106).

NSC 631570 was effective in the therapy of recurring lung diseases in children from the Chernobyl area (202).

### **Immunological properties of Ukrain (NSC 631570)**

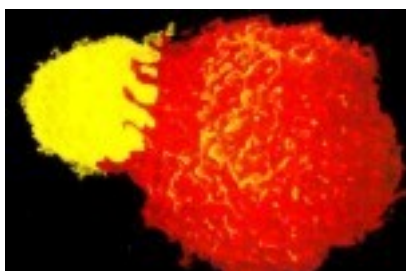
**NSC 631570 modulates the natural defense system against developing cancer**



***Under attack – Ukrain-activated lymphocytes start to recognize the tumor cells.***



***Victory – Ukrain-activated lymphocytes begin to destroy a tumor cell.***



***Kiss of death – Ukrain activates lymphocytes to kill a cancer cell.***

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### **The Studies *in vitro* and *in vivo***

In the tests on alloimmunised mice NSC 631570 augmented the lytic activity of splenic lymphocytes by up to 48fold. The lytic activity of the IL-2 treated spleen cells and peritoneal exudate lymphocytes were also increased significantly by the addition of NSC 631570 to the assay medium (17, 66).

Human lymphocytes as well as guinea pig lymphocytes were activated more pronounced when incubated with Ukrain than with PHA. In rats NSC 631570 caused a clear increase of macrophages with NK-activity. A similar modulating effect on the macrophages was observed also clinically (49).

It was revealed in the tests on CBA mice and Wistar rats that NSC 631570 stimulates macrophages. As marker of this activity the enzyme chitotriosidase - a part of the native immunity was used (168).

In a study on intact and thymus-ectomised mice NSC 631570 augmented the endocrine function of thymus. After NSC 631570 administration, increased production of substances with thymosin-like activity was detected. Repeated administration of NSC 631570 caused a 2fold rise of T-cells in blood, a 4.5fold rise of large granulocytes and increase of the NK-activity of splenocytes. The production of interferon and antibodies after the antigen administration was increased as well (180).

The effect of well-known immune modulators interferon-gamma, NSC 631570 and pokeweed mitogen on the selective uptake of technetium-99m(<sup>99m</sup>Tc)-labelled tumor necrosis factor (TNF) was studied in the intramuscular implanted murine embryonic carcinoma. The highest absolute tumor uptake of <sup>99m</sup>Tc- TNF was achieved when NSC 631570 was used, followed by IFN-γ and pokeweed mitogen (104).

In the experiments on BALB/c and F1 (BALB/c x C57BL/6J) mice it was revealed that NSC 631570 inhibits the allergic sensitization of animals against ovalbumin, expressed in the weakened IgE-reaction and decreased histamine release. The incubation of ovalbumin with NSC 631570 induced decreased antigenicity of this protein (84).

The immune modulating effect of NSC 631570 was studied in several studies in mice. Repeated subcutaneous injections of NSC 631570 to mice infected with the twofold LD50 of *E. coli*, *S. aureus*, or influenza virus increased the survival rate of the animals significantly (60, 87, 89).

When human lymphocytes were incubated with phytohemagglutinin (PHA) and NSC 631570, increased absorption of <sup>3</sup>H-thymidin in the cells was observed. The authors point out the strong synergetic effect of NSC 631570 and phytohemagglutinin (76).

By means of the cell proliferation assay the mitogenic effects of PHA and NSC 631570 on human peripheral blood mononuclear cells (PBMC) were studied. It was revealed even a short pretreatment of the PBMC with NSC 631570 has a strong synergetic effect on the PHA-mitogenesis. Consequently, the cell stimulation parameters were much higher after combined stimulation than after using PHA alone (65).

## **RADIOPROTECTIVE EFFECT**

When NSC 631570 has been used in clinic, it was observed that the patients treated with this drug tolerate the radiotherapy much better. This gave reason to study radioprotective properties of NSC 631570.

In the tests on mice was proven the radioprotective effect of NSC 631570 is much more pronounced than that of its source materials (132).

Further studies showed NSC 631570 to modulate the cell components of the hemopoietic system (stem cells, proliferating, maturing, and competent cells) so that the total radioresistance increases (77, 133, 249).

The radioprotective effect of NSC 631570 has been confirmed by the infection models in mice where its effect was superior to the effect of the known radioprotector cysteamine (134).

Compared to other drugs NSC 631570 exerted a strong radioprotective effect similar to lymphokinin (135).

These radioprotective properties of NSC 631570 were confirmed in further studies in rats (172).

NSC 631570 exerted a protective effect on the hormonal system of irradiated rats (173).

The protective effect of NSC 631570 against radiation was also studied and confirmed on *in vitro* models.

The effect of NSC 631570 as protector against radiation was studied on human skin fibroblasts HSF1 and HSF2 as well as lung fibroblasts CCD32-LU. As evaluation parameters were chosen cytotoxicity, apoptosis induction, cell cycle course, and the expression of TP53 and p21. Additionally, following malignant cell lines were used: MDA-MB-231 (human breast tumor), PA-TU-8902 (pancreas cancer), CCL-221 (colorectal cancer), and U-138MG (glioblastoma). The cytotoxicity of NSC 631570 was time- and dose dependent. The combination of NSC 631570 plus ionizing radiation (IR) enhanced toxicity in CCL-221 and U-138MG cells, but not in MDA-MB-231 and PA-TU-8902 cells. Most strikingly, a radioprotective effect was found in normal human skin and lung fibroblasts. Flow cytometry analyses supported differential and cell line-specific cytotoxicity of NSC 631570. CCL-221 and U-138MG cells accumulated in G2 after 24h treatment with NSC 631570, whereas no alterations were detected in the other tumor cells and normal fibroblasts tested. Differential effects of NSC 631570 in modulating radiation toxicity of human cancer cell lines and its protective effect in normal human fibroblasts suggest that this agent may be beneficial for clinical radiochemotherapy (184).

In their next study on the role of the proteins fibronectin and laminin in the radiation protection mechanisms of the cells, the researchers from the University of Tübingen used NSC 631570 as a reference substance (198).

In the experiments on male Wistar rats irradiated with microwaves 53.57 MHz during 14 days, NSC 631570 was revealed to normalise the activity of aminotransferases ALT and AST as well as the serum concentration of the alpha-fetoprotein compared to the irradiated control group (233).

Microwaves are electromagnetic waves with wavelengths ranging from as long as one meter to as short as one millimetre, equivalent to frequencies between 300 MHz and 300 GHz. As the devices operating in this wavelength range have been more and more used, including therapeutic applications, the importance of the studies of their health effects increases constantly. The effect of NSC 631570 (7 mg/kg intraperitoneal for 10 days) on the serum

chemistry parameters of male Wistar rats during concomitant microwave irradiation (53.57 GHz, 10 mW/cm<sup>2</sup>, 20 min daily for 10 days) was studied. At the end of the study no significant changes were observed in the combined treated group compared to the control group. The authors concluded NSC 631570 can be used combined with microwave therapies (242).

## **TOXICOLOGIC STUDIES**

On an induced hepatitis model the researchers studied whether NSC 631570 can protect liver cells from the toxic effect of acetaminophen overdosage. Indeed, NSC 631570 exerted a protective effect with the stimulation of liver macrophages (217).

In the tests on rats, the effects of chelidonine (50 or 100 mg/kg intraperitoneal) and NSC 631570 (7 or 14 mg/kg intraperitoneal) on some blood chemistry parameters after intoxication with copper chloride or lead chloride were studied. Both agents normalised the serum values of beta-2-microglobulin, creatinine, and urea. The effect of chelidonine was more pronounced (229, 234, 236). The treatment with NSC 631570 normalised also increased values of ALT and alpha-fetoprotein (193, 241).

In male Wistar rats with experimental acute ethylene glycol intoxication (3 or 3.5 g/kg intraperitoneal), the effect of NSC 631570 (7, 14 or 28 mg/kg intraperitoneal) on blood chemistry parameters was studied. Compared to the control group, the 10 day administration of NSC 631570 caused a significant decrease of serum urea concentration and increase of serum beta-2-microglobulin concentration (235, 257). In the study with lower ethylene glycol dose (2.5 g/kg), an increased serum concentration of beta-2-microglobulin was observed (240).

In male Wistar rats with experimental acute alcohol intoxication (methanol 4.5 g/kg intraperitoneal, ethanol 3 g/kg intraperitoneal, or ethylene glycol 3.5 g/kg intraperitoneal) the effect of NSC 631570 (28 mg/kg intraperitoneal as single dose or 10 day administration) on the aminotransferase activity (ALT and AST) and the serum alpha-fetoprotein concentration was studied. Compared to the control group, the single administration of NSC 631570 caused the drop of the increased AFP concentration (except the ethylene glycol group). The serum AFP concentration increased after the 10 day administration of NSC 631570. There were no changes in other parameters compared to the control (237, 248, 256, 257).

To study the effect of NSC 631570 on some chemistry parameters in the acute methanol intoxication, Wistar rats were treated with NSC 631570 for 10 days. The therapy alleviated the unfavourable consequences of the methanol intoxication, expressed by the normalization of the increased serum concentrations of beta-2-microglobulin and urea (192, 196, 227, 228).

## **NORMALISATION OF THE METABOLISM**

### **Bone Metabolism and Osteoporosis**

A series of animal tests was aimed to study the effect of NSC 631570 on bone density and mineral metabolism.

In a 6 month study on female ovariectomized rats, NSC 631570 has been revealed to inhibit the development of some indications of early osteoporosis (78). The blood parameters and transaminase activity in the NSC 631570 group did not differ from ones in the control group (79). Prolactin and progesterone concentrations were elevated, those of corticosterone and aldosterone diminished compared to the control ovariectomized group (57, 80).

The treatment of mature female rats with NSC 631570 at high dosage had no negative effect on the bone mineral density. A slight decrease of bone mineral content was observed (128).

After intermittent 3 month administration of NSC 631570 at high dosage to ovariectomized female rats the bone mineral density decreased (129).

The effect of NSC 631570 on the bone metabolism in rats was reviewed in an article. The author concluded this drug to affect especially the estrogen dependant mechanisms and has protective effect against osteoporosis (174).

In a set of animal tests, rat femur was used as a model for the study of the long-term effect of NSC 631570 on various bone parameters. NSC 631570 was revealed not to diminish the bone strength and density also at higher dosage (175, 176).

It was also studied how various doses of NSC 631570 affect the intensity of electron spin resonance (ESR) signal in intact and ovariectomized rats. The intensity of the signal correlates directly with the amount of free radicals in the tissue. The signal intensity was the lowest in the ovariectomized group with highest NSC 631570 dose and the highest in the intact group at the lowest dose of NSC 631570 (177).

In intact female rats the effect of NSC 631570 on the hormones involved into calcium metabolism was studied. In the highest dosage group the serum concentrations of corticosterone and progesterone decreased significantly and non-significantly, respectively. In the lowest dosage group the serum concentration of parathormone increased significantly. The serum values of calcitonin were not affected in either group (223).

The effect of NSC 631570 on the same hormones was also studied in female ovariectomized rats. In the middle dose group the corticosterone serum concentration dropped. There were no other changes in hormone values in all dose groups (224).

In the tests in male Wistar rats irradiated with 53.57 MHz microwaves for 14 days, NSC 631570 exerted practically no effect on the water content as well as organic and inorganic phases of pelvis bone compared to the irradiated control group (232).

The effects of NSC 631570 at different doses and/or strontium on the rat tooth intertubular dentine were analysed in cuts perpendicular to the dentinal tubes. The tooth surfaces and cross-section morphology and roughness were investigated with atomic force microscopy. The cross-section surface of intertubular dentine was analysed by roughness and fractal parameters. Histograms were prepared for the typical samples from all groups teeth analysed. Dentine cross section surfaces showed significant differences between the nano structures of normal rat teeth and those from animal treated with NSC 631570 and strontium (260).

## **THE EFFECT ON VARIOUS ENZYMES**

In the tests on liver cells NSC 631570 was revealed to inhibit the alcohol dehydrogenase activity (178, 253).

The effect of NSC 631570 on the activity of trypsin-like enzymes was also studied (183, 222).

The effect of NSC 631570 on the concentration of the vasoactive intestinal peptide (VIP) was studied on the diabetes model in mice as well as on intact animals. NSC 631570 exerted no effect on the VIP concentration in intact mice. In diabetic mice the 10 day administration of NSC 631570 caused a significant increase of the VIP concentration in the lowest dose group (194, 225).

In the experiments on the rat liver the researchers revealed that NSC 631570, beside chelidonine, caused the strongest inhibition of the enzyme monoamine oxidase (MAO), indicating its antidepressant properties (197, 252).

In a review the researchers summarized the articles on the effect of NSC 631570 on the amino acid metabolism. They noted NSC 631570 exerts different effects on the amino acid pools in the body and in the malignant tissue (213).

In a set of experiments in rats the effects of NSC 631570 on various liver enzymes were studied. In a test, the effects of the NSC 631570 seven day administration on the lipid peroxidation and antioxidative function of the rat liver were explored. At daily dose of 2 mg/kg NSC 631570 the serum concentration of reduced glutathione decreased at 25% and the antioxidative function of the rat liver decreased at 49%. The glutathione reductase activity in the postmitochondrial fraction of the rat liver increased at 43% compared to the control group. There was no activation of the lipid peroxidation after the administration of 2 mg/kg NSC 631570, and the catalase as well as peroxidase activity did not differ from those in the control group. The authors suggested an assumption NSC 631570 to cause an oxidative stress in the tumor resulting in apoptosis (214).

The effects of NSC 631570 on the liver enzymes involved into the drug metabolism were studied. After the six day administration of NSC 631570 at a daily dose of 2 mg/kg, the activity of aminopyrine-N-demethylase increased by 35% and the one of glutathione-S-transferase by 55%. The concentrations of microsomal cytochromes P450 and b5 as well as the rate of ethylmorphine-N-demethylation were not affected (215).

## **INTERACTION WITH OTHER DRUGS**

### **Non-Opioid Analgesics**

In mice and rats the interaction of NSC 631570 and aminophenazone was studied. It was found that NSC 631570 affects the analgesic effect of aminophenazone in various ways. In the writhing syndrome test in mice as well as in tail-flick test in rats the antinoceptive effect of the analgesic was potentiated, whereas in the hot-plate test the analgesic effect of aminophenazone was reduced (20).

The analgesic effect of NSC 631570 was augmented by the nitric oxide synthase inhibitors in mice. These results suggest the endogenous nitric oxide can modify the analgesic effect of NSC 631570 (130).

### **Opioid Analgesics**

The tests on mice and rats revealed NSC 631570 to modify the antinoceptive effect of opiates. In mice, for example, NSC 631570 potentiated the analgesic action of morphine in the hot plate test and in the tail flick test, however, reduced this effect in the writhing syndrome test (35).

In the studies in mice was revealed the 10 day intraperitoneal administration of NSC 631570 at high dose has an analgesic effect in mice. Combined administration of morphine and NSC 631570 reduced their antinoceptive effects reciprocally. The authors propose to avoid combined clinical use of these drugs (85, 86).

The analgesic action of NSC 631570 in mice was completely abolished by naltrexon, a pure opioid antagonist acting on all opioid receptors as a competitive antagonist (131).

### **Streptozotocin**

The streptozotocin diabetes model was used in rats to explore how changes the liver and kidney function in these animals under the impact of NSC 631570. The 10 day administration of NSC 631570 to streptozotocin rats did not change the serum creatinine concentration, but increased the urea value. The authors concluded NSC 631570 should not be combined with streptozotocin (195, 226, 239).

### **Porphyrin Derivates**

In the tests on murine sarcoma, mammary carcinoma, human colon carcinoma and melanoma cell lines NSC 631570 and porphyrin amino acid derivates to have synergetic action against cancer cell lines (53, 83).

## **Anticonvulsants**

In Albino mice the interaction of NSC 631570 and various anticonvulsants such as diazepam, carbamazepine, diphenylhydantoin, phenobarbital, and valproate was studied. NSC 631570 potentiated the protective effect of valproate, whereas the effects of other anticonvulsants were not affected (34).

## **INTERACTION WITH OTHER THERAPY MODALITIES**

### **Local hyperthermia**

Hyperthermia is a type of treatment in which body tissue is exposed to high temperatures (up to 45°C), to damage and kill cancer cells, or to make cancer cells more sensitive to the effects of radiation and certain anticancer drugs. Local hyperthermia treatment (heat applied to a very small area, such as a tumor) is a well-established cancer treatment method with a simple basic principle: If a rise in temperature to 45°C can be obtained for one hour within a cancer tumor, the cancer cells will be destroyed. Primary malignant tumors have a bad blood circulation, which make them more sensitive to changes in temperature. The therapy with NSC 631570 has been successfully combined with local hyperthermia since many years (115, 144, 208).

In his speech at the 5th Vienna Dialog on the Holistic Medicine, Dr. B. Aschhoff reported on his experience in the NSC 631570 therapy and local hyperthermia. Summing up, following theses were noted: significant improvement of the quality of life; few adverse effects; NSC 631570 is safe and can be used in children also; wide indication range of the therapy (142).

### **Endovascular Laser Therapy / Photodynamic Therapy**

The endovascular laser therapy as a part of photodynamic therapy (PDT) is a new method for systemic laser treatment and energy transfer to the human body. For therapeutic application red, infrared, green, and blue lasers are used. The photon stream applied intravenously leads to an improved microcirculation, activation of the immune system and mitochondria. Recently, NSC 631570 has been used as a sensitizer (Weber M, 'The intravenous laser blood irradiation, a new therapeutic approach in immunology and cancer therapy', presentation at the 2nd International Conference on Drug Discovery and Therapy, SL-341, Dubai, UAE, 2010).

NSC 631570 was administered to the patients with rheumatic diseases or high susceptibility to infections. In the lymphocytes from both patients group and from a control group, expression of IgG, proliferation marker Ki-67 and other marker molecules were estimated. There were no significant changes in the control group whereas in the NSC 631570 group the expression of IgG and Ki-67 increased significantly. Further increase of marker expression was achieved using additional endovascular low-level laser therapy. Circulating tumor cells from cancer patients were incubated with NSC 631570.

Patients were treated with NSC 631570 alone or with NSC 631570 and endovascular laser. The tumor mass reduction was achieved in both groups but in the combined group this effect was more pronounced (Andrae F, 'Ukrain based laser therapy in oncology', presentation at the 2nd International Conference on Drug Discovery and Therapy, SL-274, Dubai, UAE, 2010).

### **Ozone**

Empiric experience revealed NSC 631570 should not be combined with the ozone therapy. Generally, NSC 631570 should preferably be used as monotherapy. Other therapy modalities can be used in the breaks between the treatment cycles.

### **Others**

In a pilot study it was elaborated how a method for determination of an optimal dosage for the therapy with NSC 631570 could be elaborated (the same for interferon-alpha, too). The method is based on the estimation of SS/SH groups ratio in serum (138).

## II. SAFETY

At therapeutic dose the product has no appreciable adverse effects and does not damage healthy cells but only attacks cancer cells. Due to its very high therapeutic index of 1250 – in contrast to common cytostatics with a low TI of 1.4-1.8 – there is no danger of an overdose with Ukrain therapy (therapeutic index is the ratio between the toxic dose and the therapeutic dose of a drug, used as a measure of the relative safety of the drug for a particular treatment. From 'The American Heritage® Dictionary of the English Language, 4th Edition'). Ukrain also does not cause necroses when administered intramuscular, which is a proof for its safety (37).

The Austrian Research Center Seibersdorf is the leading Austrian research institution in life sciences. Now it is a part of the Austrian Institute of Technology (AIT), the largest non-university research institution in Austria. At the Seibersdorf branch of AIT the state-of-art toxicological studies are performed according to the GLP guidelines (Good Laboratory Practice). Following GLP conformed studies with NSC 631570 were performed at the ARCS.

### **Acute Intravenous Toxicity with Rats**

The study was performed in conformance with the EC-Guideline 92/69 and the OECD-Guideline 401. The test substance was administered undiluted as a slow intravenous injection to male and female Him:OFA rats at doses of 33 mg/kg, 57 mg/kg, or 100 mg/kg (only females) body weight (b.w.). The administration induced immediate effects, which, if not lethal, soon lead to an almost complete recovery. Most probable cause of death was a shift in the blood pH or in the blood ion homeostasis. Males were more susceptible than females. Based on the results obtained, the LD50 (intravenous) of the active substance was calculated as 43 mg/kg b.w. for males and 76 mg/kg b.w. for females (151, OEFZS-A-4483, October 1998).

### **Acute Intravenous Toxicity with Mice**

The study was performed according to the Directive 92/69/EEC and the OECD Guideline 401, 1987. NSC 631570 was administered as a slow intravenous injection to three groups of five male and of five female Him:OF1, SPF mice at doses of 33 mg/kg b.w., 74 mg/kg b.w., and 165 mg/kg b.w. All animals in the high dose group and two males and 3 females in the mid dose group died. All other animals survived until 14 days after administration. All survived animals were normal at the end of the study. All animals were normal at necropsy. There was no marked sex difference in the response to the test substance. Based on the results obtained, the LD50 (intravenous) was calculated as 80 mg/kg b.w. for males and 68 mg/kg b.w. for females (OEFZS-L-0400, May 2000).

### **Acute Intramuscular Toxicity with Rats**

The aim of the study was to reveal acute toxic effects of NSC 631570 after a single intramuscular administration to rats. The study was performed according to the EC-Directive 87/176/EWG and the OECD-Guideline 401, as far as these were useful for intramuscular administration. The test substance was administered undiluted as an intramuscular injection to five male and five female Him:OFA rats at the highest technically feasible dose for the intended route, i.e. 150 mg active ingredient per kg b.w. All animals survived until the scheduled termination of the study. Body weight gain was not impaired. Sedation and less pronounced disturbed locomotion was noted on the day of the administration. Local changes at the injection site (crusts) were present in about half of the animals. There was no sex difference in the response to the test substance. The authors concluded intramuscular injection of NSC 631570 in the highest feasible dose induced some transient signs of minor clinical importance but was otherwise well tolerated and did not become life threatening. Based on the results obtained in this study the LD50 (intramuscular) of NSC 631570 was calculated to be higher than 150 mg/kg b.w. (151, OEFZS-L-0194, October 1999).

### **Acute Oral Toxicity Study with Rats**

This study aimed to investigate acute toxic effects of NSC 631570 after a single oral administration to rats and was performed according to the EC-Directive 92/69 and the OECD Guideline 401. NSC 631570 concentrated was administered undiluted once by stomach intubation to three groups of five female Him:OFA Sprague Dawley rats and to one group of five male Sprague Dawley rats at the doses of 450 mg/kg b.w., 810 mg/kg b.w., and 1500 mg/kg b.w. (females) or 810 mg/kg b.w. (males). All females in the highest dose group died spontaneously on the day of administration. All females in both other dosage groups as well as all males survived until the scheduled termination. All animals of the mid and high dosed groups were affected with most of the signs observed on the day of administration of the test substance. The effects lasted until maximal three days after administration. All surviving animals were normal at the end of the study. There was no relevant sex difference in the response to the test substance.

Hence the test substance induced immediately effects which, if not lethal, led to an almost complete recovery soon. The LD50 (oral, females) was calculated as 1110 mg/kg b.w. (151, OEFZS-L-0195, October 1999).

### **Local Tolerability Study**

NSC 631570 was administered as a slow intravenous, intraarterial, paravenous or intramuscular injection to two male and two female rabbits per route to study its local tolerability. Normal saline was administered correspondingly to the other ear or muscle of each animal as a control. NSC 631570 was well tolerated at intravenous and intraarterial administration. The paravenous injection caused mild local irritation, and the intramuscular administration caused a mild to moderate local inflammation. There was no sex difference in the response to the test substance (OEFZS-A-4204, October 1997).

### **Micronucleus Test with Mice**

The study was performed to detect the possible production of micronuclei induced by NSC 631570 as a result of chromosomal damage or of damage to the mitotic apparatus in an *in vivo* test system. NSC 631570 was diluted with normal saline and administered once at doses of 1.25, 2.50, or 5.00 ml/kg b.w. intravenously to three groups of five male and five female Crl:NMRI BR mice each. Additionally, one high dose group of one male and one female spare animal was included to replace possible unscheduled deaths in the high dose group. Two negative control groups (normal saline) and one positive control group (thiotepa) were also included into the study. Preparation of bone marrow cells and investigations were performed in conformance with the OECD Guideline 474.

All animals survived until scheduled sacrifices. Thiotepa (positive control group) caused cytotoxicity and produced micronuclei in polychromatic erythrocytes. NSC 631570 did not cause cytotoxicity. All data were in the range of historical negative control data. There were no significant differences in micronucleated normochromatic erythrocytes (MNE) between the test substance group animals of both sexes and the corresponding negative controls, neither 24 nor 38 hours after administration. Also no statistically significant differences in the amounts of micronucleated polychromatic erythrocytes (MPE) were noted in any sex at any dose used. The rates of MPE were at both sampling times within the limits of historical negative control groups. No sex difference in the response to the test substance was noted. The authors concluded NSC 631570 not to cause cytotoxicity to the bone marrow at doses up to 5 mg/kg b.w. and not to produce micronuclei in the polychromatic erythrocytes in mice of both sexes at the doses used (OEFZS-L-0225, November 1999).

Similar results were obtained in the second micronucleus study with NSC 631570 concentrated (OEFZS-L-0224, November 1999).

### ***Salmonella typhimurium* Reverse Mutation Test**

NSC 631570 was tested for mutagenic activity in the *S. typhimurium* reverse mutation test (Ames test) according to the OECD Guideline 471 and the EEC Guideline 92/69, part B14. The test substance was tested at the range of concentrations according to the direct plate incorporation method without and with external metabolism system S9-mix. The bacterial strains *S. typhimurium* TA97a, TA98, TA100, and TA1535 were used as test system. Negative and positive controls were included and an independent repetition of the experiment was performed. The test substance was not toxic to the *S. typhimurium* strains used. According to the results obtained in this study, NSC 631570 is non-mutagenic in the Ames test with the strains TA97a, TA98, TA100, TA102, and TA1535 (OEFZS-L-0003, January 1999).

### **Liver Toxicity**

As the source materials for the production of NSC 631570 - celandine alkaloids and thiotepa are toxic for liver cells, a study was initiated to evaluate the possible toxic

potential of NSC 631570 to the liver. The study was performed in rats and proved NSC 631570 is not toxic to the rat liver (Müller 2004, original report).

### **Other Toxicity Studies**

Many other toxicity studies were performed in Eastern Europe (15, 16, 105, 123, 127). The first toxicity studies were performed on Albino Swiss mice and Wistar rats. NSC 631570 was administered intraperitoneally at the single daily dose of 0.025, 0.05, and 0.1 of LD50 (e.g. 4.75, 9.5, 19 mg/kg in mice and 7, 14 and 28 mg/kg in rats) during three months. The treatment had no effect on the organ weight with except of spleen in rats where the organ weight was up to 3fold higher than in the control group (29). The estimation of catecholamines in brain revealed the three month administration of NSC 631570 at middle and higher dose diminished the dopamine concentration in mice and rats. The concentrations of noradrenalin, 5-hydroxytryptamine and 5-hydroxyindolacetat were not changed (29). The single administration of NSC 631570 had no effect on the serum prolactin concentration in rats. On the contrary, after the three month administration of NSC 631570 the serum prolactin concentration increased in all dose groups compared to the control (32).

Regarding blood count, the administration of NSC 631570 had similar effects in the middle and higher dose groups: increase of white blood cells (WBC) and decrease of platelets. The changes in the differential count were also observed, e.g. lymphocytes increase and neutrophil bands decrease. No significant changes in red blood cells (RBC) were observed compared to the control (33).

Also there were no changes in the transaminase activity in rats. In mice, increase of ALT and AST activity occurred in the middle and higher dose groups. A slight decrease of the total protein was observed in male mice in the groups 9.5 and 19.5 mg/kg, in female mice only in the group 19 mg/kg. The single administration of NSC 631570 did not affect the evaluated parameters (31).

In the experiments with hamster cells it was revealed NSC 631570 is not mutagen as well as not genotoxic and does not cause morphologic cell transformations (19).

Tests in mice and guinea pigs have revealed NSC 631570 not to cause anaphylactic sensitization (23).

In the tests in rats, NSC 631570 was confirmed not to exert embryotoxic or teratogenic effect. In hamsters, the embryotoxic effect was observed at the very high dosage of 28 mg/kg body weight (30).

In a series of experiments the effect of the six week administration of NSC 631570 in rabbits was studied (subacute toxicity).

The daily treatment at three various dosage caused no effect on the organ morphology and body mass of the animals. In blood count, the numbers of red blood cells and white blood cells did not change. In the high dosage group, the percentages of lymphocytes, monocytes, and

eosinophils increased. The blood chemistry parameters were not affected apart from increase of urea and uric acid (124).

Among sex hormones, estradiol increased in some groups as did testosterone in the lowest dose male group. In females, progesterone increased only in the highest dose group (125).

NSC 631570 caused the increase of thyroid hormones in males. In females, triiodothyronine increased and thyroxin concentration did not change (126).

In a study in male Wistar rats the researchers revealed **that** the six day administration of NSC 631570 in both doses of 1 mg/kg and 2 mg/kg caused no significant changes in the following parameters: albumin, mucopolysaccharides, aminotransferase activity, and thymol test. Similarly unaffected compared to the control group was the liver tissue morphology. Significantly lower was the blood concentration of the middle molecules (216).

In the Wistar rats with implanted Guerin carcinoma, NSC 631570 was revealed to reduce the number of thiol groups in the tumor homogenates (121).

## **PHARMACOKINETICS**

Pilot studies of pharmacokinetics gave first suggestions on the methods which should be used in the later studies (105, 127).

To estimate the plasma concentration and pharmacokinetic parameters of NSC 631570 at intravenous administration, first a method for its determination in plasma was developed (152). This enabled consequently the study of pharmacokinetics of NSC 631570 in rats. It was revealed the tumor tissue and the liver had the strongest affinity to NSC 631570. In brain and muscles the NSC 631570 accumulation was the least (153).

## **BIOPHYSICAL PROPERTIES**

The examinations with physical methods (spectrometry, polarized light microscopy) revealed NSC 631570 to be resistant against the UV light of middle intensity. The absorption peaks 210 and 230 nm can be used for the detection of NSC 631570 in plasma (50, 81).

In the tests with the rat liver homogenates the oxygen consumption was detected by means of polarography. The addition of NSC 631570 caused complex redox reactions in the substrate. This was dependant on the NADP induced oxidation (82).

### III. QUALITY

The proof of quality has been described in the Pharmacopoea Austriaca VIII, European Pharmacopoeia and in the Deutsches Arzneibuch (German Pharmacopoeia), 6th edition.

#### Greater celandine - Extract from the European Pharmacopoeia 6.0

6.0/1861

#### GREATER CELANDINE

##### *Chelidonii herba*

##### DEFINITION

Dried, whole or cut aerial parts of *Chelidonium majus* L. collected during flowering.

*Content:* minimum 0.6 per cent of total alkaloids, expressed as **chelidone** (C<sub>20</sub>H<sub>19</sub>NO<sub>5</sub>; M<sub>r</sub> 353.4) (dried drug).

##### CHARACTERS

Macroscopic and microscopic characters described under identification tests A and B.

##### IDENTIFICATION

- A. The stems are rounded, ribbed, yellowish to greenish-brown, somewhat pubescent, about 3 mm to 7 mm in diameter, hollow and mostly collapsed. The leaves are thin, irregularly pinnate, the leaflets ovate to oblong with coarsely dentate margins, the terminal leaflet often three-lobed; the adaxial surface is bluish-green and glabrous, the abaxial surface paler and pubescent, especially on the veins. The flowers have 2 deeply concavo-convex sepals, readily removed, and 4 yellow, broadly ovate, spreading petals about 8 mm to 10 mm long; the stamens are numerous, yellow, and a short style arises from a superior ovary; long, capsular, immature fruits are rarely present.
- B. Reduce to a powder (355). The powder is dark greyish-green to brownish-green. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: numerous fragments of leaves in surface view, the epidermal cells with sinuous walls; anomocytic stomata (2.8.3) occur on the abaxial surface only; covering trichomes long, uniseriate, with thin walls and usually fragmented; vascular tissue from the leaves and stems with groups of fibres, pitted and spirally thickened vessels and associated latex tubes with yellowish-brown contents; occasional fragments of the corolla with thin-walled, partly papillose cells containing numerous pale yellow droplets of oil; spherical pollen grains about 30 µm to 40 µm in diameter with 3 pores and a finely pitted exine.
- C. Thin-layer chromatography (2.2.27).

*Test solution.* To 0.4 g of the powdered drug (710) add 50 ml of dilute acetic acid R. Boil the mixture under a reflux condenser in a water-bath for 30 min. Cool and filter. To the filtrate add concentrated ammonia R until a strong alkaline reaction is produced. Shake with 30 ml of methylene chloride R. Dry the organic layer over anhydrous sodium sulphate R, filter and evaporate *in vacuo* to dryness. Dissolve the residue in 1.0 ml of methanol R.

*Reference solution.* Dissolve 2 mg of papaverine hydrochloride R and 2 mg of methyl red R in 10 ml of alcohol R.

*Plate:* TLC silica gel plate R.

*Mobile phase:* anhydrous formic acid R, water R, propanol R (1:9:90 V/V/V).

*Application:* 10 µl as bands.

*Development:* over a path of 10 cm.

*Drying:* in air.

*Detection:* spray with potassium iodobismuthate solution R and dry the plate in air; spray with sodium nitrite solution R and allow the plate to dry in air; examine in daylight.

*Results:* see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other weaker zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Methyl red: a red zone	A brown zone
Papaverine: a greyish-brown zone	A brown zone
	A greyish-brown zone
	A brown zone
	A brown zone
<b>Reference solution</b>	<b>Test solution</b>

#### TESTS

Foreign matter (2.8.2): maximum 10 per cent.

Loss on drying (2.2.32): maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) by drying in an oven at 100-105 °C for 2 h.

Total ash (2.4.16): maximum 13.0 per cent.

#### ASSAY

*Test solution.* To 0.750 g of the powdered drug (710), add 200 ml of *dilute acetic acid R* and heat on a water-bath for 30 min, shaking frequently. Cool and dilute to 250.0 ml with *dilute acetic acid R*. Filter. Discard the first 20 ml of the filtrate. To 30.0 ml of the filtrate add 6.0 ml of *concentrated ammonia R* and 100.0 ml of *methylene chloride R*. Shake for 30 min. Separate the organic layer, place 50.0 ml in a 100 ml round-bottomed flask and evaporate to dryness *in vacuo* at a temperature not exceeding 40 °C. Dissolve the residue in about 2-3 ml of *alcohol R*, warming slightly. Transfer the solution to a 25 ml volumetric flask by rinsing the round-bottomed flask with *dilute sulphuric acid R* and dilute to 25.0 ml with the same solvent. To 5.0 ml of the solution, add 5.0 ml of a 10 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R* in a 25 ml volumetric flask, stopper the flask and mix carefully. Dilute to 25.0 ml with *sulphuric acid R* and stopper the flask.

*Compensation solution.* At the same time and in the same manner, place in a 25 ml volumetric flask 5.0 ml of *dilute sulphuric acid R* and 5.0 ml of a 10 g/l solution of *chromotropic acid, sodium salt R* in *sulphuric acid R*, stopper the flask and mix carefully. Dilute to 25.0 ml with *sulphuric acid R* and stopper the flask.

Place both solutions on a water-bath for 10 min. Cool to about 20 °C and dilute if necessary to 25.0 ml with *sulphuric acid R*. Measure the absorbance (2.2.25) of the test solution at 570 nm.

Calculate the percentage content of total alkaloids, expressed as **chelidone**, from the expression:

$$\frac{A \times 2.23}{m}$$

i.e. taking the specific absorbance of **chelidone** to be 933.

$A$  = absorbance at 570 nm,

$m$  = mass of the substance to be examined, in grams.

## Thiotepa – Extract from the US Pharmacopoeia, 24th edition

### „Thiotepa

C<sub>6</sub>H<sub>12</sub>N<sub>3</sub>PS 189.22  
Aziridine, 1,1',1''-phosphinothioylidynetris-,  
Tris(1-aziridiny)phosphine sulfide [52-24-4]

» Thiotepa contains not less than 97.0 percent and not more than 102.0 percent of C<sub>6</sub>H<sub>12</sub>N<sub>3</sub>PS, calculated on the anhydrous basis.

*Caution — Great care should be taken to prevent inhaling particles of Thiotepa or exposing the skin to it.*

**Packaging and storage** — Preserve in tight, light-resistant containers, and store in a refrigerator.

**USP Reference Standards** (11) — USP Thiotepa RS.

**Identification, Infrared Absorption** (197S) — *Solution*: 3 in 400. *Medium*: carbon disulfide.

**Melting range** (741): between 52° and 37°

**Water, Method I** (92I): not more than 2.0%.

**Assay** —

*Mobile phase* — Prepare a suitable filtered and degassed mixture of water and acetonitrile (9:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation* — Dissolve an accurately weighed quantity of USP Thiotepa RS in *Mobile phase* to obtain a solution having a known concentration of about 1.5 mg per mL.

*Assay preparation* — Transfer about 75 mg of Thiotepa, accurately weighed, to a 50-mL volumetric flask, dissolve in *Mobile phase*, dilute with *Mobile phase* to volume, and mix.

*Resolution solution* — Transfer about 10 mg of USP Thiotepa RS to a 4-mL vial, add 2 mL of methanol, and mix. Add 50 µL of 0.1% phosphoric acid solution. Place a cap on the vial, and heat at 65° for 50 seconds. Cool the solution, add 1 mL of methanol, and mix.

*Chromatographic system* (see *Chromatography* (621)) — The liquid chromatograph is equipped with a 215-nm detector and a 4-mm x 15-cm column that contains packing L1. The flow rate is about 0.8 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 1.25 for methoxythiotepa and 1.0 for thiotepa, and the resolution, *R*, between the methoxythiotepa peak and the thiotepa peak is not less than 3.0. Chromatograph the *Standard preparation*, and record the responses as directed under *Procedure*: the tailing factor for the thiotepa peak is not more than 1.8, the column efficiency is not less than 2600 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure* — Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>6</sub>H<sub>12</sub>N<sub>3</sub>PS in the portion of Thiotepa taken by the formula:

$$50C(r_u/r_s),$$

in which  $C$  is the concentration, in mg per mL, of USP Thiotepa RS in the *Standard preparation*, and  $r_u$  and  $r_s$  are the thiotepa peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.”

During the production of Ukrain thiotepa is washed out so that there is no thiotepa in the end product. This has been proved using the most sensitive method of gas chromatography.

### **Stability**

The stability of the Ukrain solution for injection was investigated in a real time stability study with 3 batches stored for 60 months at 25°C and 60% relative humidity as well as for 6 months at 40°C and 75% relative humidity (accelerated condition stability study). The study was performed according to the OECD GLP Guidelines. At the end of the study all parameters were within reference range having proved the stability of the end product (Stability Study of Ukrain Ampoules Stored under Controlled Conditions. Final Study Report. March 2003. SGS Lab Simon S.A. Healthcare and Bioscience Services 796.529).

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