

# Clinical Trials of a New Chlorin Photosensitizer for Photodynamic Therapy of Malignant Tumors

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## ABSTRACT

Photodynamic therapy (PDT) was performed with a new photosensitizer, a water soluble form of chlorins (“Radachlorin”, Russia) possessing an absorption peak around 662 nm. As light source there was used the diode laser (ML-662-SP, Russia) with 662 nm wavelength and 2.5 W optical power. The sensitizer had passed broad pre-clinical in vitro and in vivo studies, which showed safety and efficiency of it. PDT was applied to 51 patients with basal cell cancer of the skin (about 60% of all cases), breast cancer, lip cancer, melanoma, cancer of esophagus, stomach, and rectum, cancer and leucoplacia of vulva, malignant ganglioneuroma, sarcoma of soft tissue, cancer and reticular sarcoma of thyroid gland, cancer of ductus choledochus. Most of non-basalioma patients had either forth stage or recurrence of disease. The sensitizer was injected intravenously or applied externally (Radachlorin gel). There were used surface, endoscopic, and interstitial ways of irradiation. Full tumor regression with excellent cosmetic effect was reached in 100% cases of 1-3 stage basal cell cancer patients treated with intravenous Radachlorin injection. In most other (non-basalioma) cases significant regression of tumors and improvement of life quality of patients (recanalization and regain of conductivity) was obtained.

**Keywords:** photodynamic therapy, cancer, photosensitizer, chlorin, laser.

## 1. INTRODUCTION

Photodynamic therapy (PDT) of malignant tumors is a violently developing method based on selective accumulation of light-absorbing agents (photosensitizers) in tumor tissue, capable to stimulate photoreactions in biological tissues after irradiation by light of a certain wavelength. This paper is a first short report about results of efforts of many specialists in development and introduction to clinic practice of PDT with a new photosensitizer (Radachlorin) and new light source (diode laser ML-SP-662).

## 2. PHOTOSENSITIZER RADAHLORIN

### 2.1. Composition

Radachlorin is a registered trade mark of a drug substance. This drug substance represents an aqueous solution of three chlorins, including sodium chlorin e<sub>6</sub> (90-95%), sodium chlorin p<sub>6</sub> (5-7%), and a third chlorin (1-5%) which we would not like to disclose yet. These chlorin constituents (called “chlorin active substance”) form 98% of the drug substance in dry weight. The drug substance Radachlorin is stored in form of 7% aqueous solution, we have found that this form provides the most shelf life.

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Fig 1. Two components of Radachlorin: sodium chlorin e<sub>6</sub> (to left) and sodium chlorin p<sub>6</sub> (to right).

The Radachlorin drug substance and its technology have been patented by us<sup>1</sup>. The production process includes 4 steps: (1) acetone extraction of chlorophyll *a* from the dry mass of cyanobacteria *Spirulina Platensis*, magnesium removal with diluted hydrochloric acid and purification of the resulting phaeophytin *a*; (2) acid hydrolysis of the latter in the organic solvent-aqueous medium to phaeophorbide *a* and its purification; (3) saponification of phaeophorbide *a* to chlorin e<sub>6</sub>; (4) conversion of chlorin e<sub>6</sub> to Radachlorin.

The drug substance is used for preparation of two drug forms: Radachlorin solution for intravenous administration, 0.35%, 10 ml and Radachlorin gel for external application, 0.1%, 25 g. They have passed through the number of obligatory laboratory (non-clinical) studies in accordance with the official (GLP) requirements, and were applied for the registration in Russian Federation as pharmaceutical means in June, 2001. Currently, the drugs are approaching the phase IIa studies which are to be done in the strict compliance with GCP requirements in Russia and Western Europe.

## 2.2. History

Water-soluble derivatives of chlorophyll were first introduced as potential drugs by E. Snyder (USA) in 1942<sup>2</sup>. The other important step was done by E.A. Allen<sup>3</sup>. He revealed that chlorin mixtures, mainly containing chlorin p6, under peroral and intravenous administration possess low toxicity and hypotensive, antisclerotic, spasmolytic, anaesthetic, antirheumathoid action. The first PDT usage of chlorins relates to phaeophorbide *a* derivatives. Some of them were patented as prospective photosensitizers for PDT in Japan in 1984 by I. Sakata et.al.<sup>4</sup> In 1986 an American group reported about a photosensitizer meeting crucial PDT requirements: good tumor affinity and intensive absorption in the middle red part of the spectrum<sup>5</sup>. Their choice was mono-L-aspartyl-chlorin e<sub>6</sub> (MACE). At present it is at stage III of clinical studies in Japan. Afterwards the group has patented more functionally advanced chlorin and bacteriopheophorbide *a* derivatives as photosensitizers for PDT<sup>6</sup>. In 1990s a Byelorussian group at first headed by G. Gurinovich reported about their search on a water-soluble chlorin type photosensitizers derived from nettle<sup>7</sup>. As far as there was not reported about clear chemical composition of the drug substance, the investigators probably dealt with mixtures similar to chlorin *e* mixtures described in the above-mentioned works of E. Snyder<sup>2</sup> and E. Allen<sup>3</sup>.

Starting from the only chlorophyll *a* containing cyanobacteria of *Spirulina* species, the first chlorin e<sub>6</sub>-based water-soluble preparations for the medical purposes have been developed in 1994-2001 by one of the authors of the present paper (A. Reshetnickov) in Russia<sup>8</sup>. The investigations had a number of directions and were supported by various sponsors under the project names: "Photodithazine"<sup>9</sup>, "Photochlorin I", "N-methyl-D-glucamine chlorin e<sub>6</sub>". The Radachlorin, sponsored and patented by "RADA-PHARMA" Co. Ltd., Russia, is one of this set. To our opinion it is the most advanced water-soluble chlorin e<sub>6</sub>-based drug substance.

## 2.3. Radachlorin's properties

**Chemical stability** of Radachlorin and its drug forms have been estimated as 2 years storing at 0-8 °C in the dark. Therefore we have stated the shelf life as 1.5 years at these conditions (the stability experiments were performed by group of Dr. N. Dementyeva in Research Institute for Pharmacy, Moscow, Russia<sup>1</sup>).

**Photophysical characteristics** of Radachlorin are presented in table 1 and fig. 2 (the measurements were conducted by Dr. A. Gradyushko and Dr. V. Temeshov in N.N. Blokhin's Russian Cancer Research Center<sup>1</sup>).

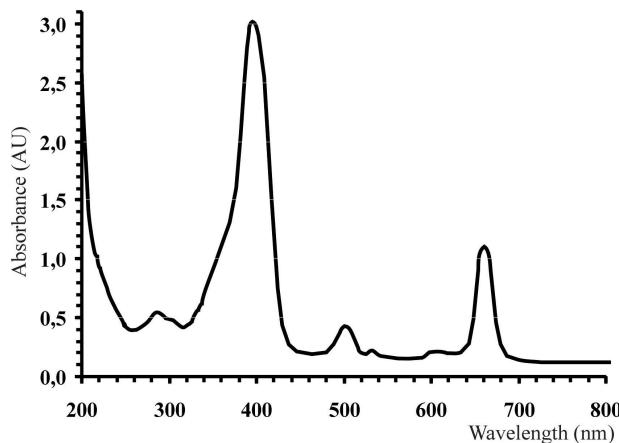


Fig.2. Absorption spectrum of Radachlorin (0.037% solution in ethyl alcohol with chlorin active substance concentration of 25.9 µg/ml).

Table 1. Optical properties of Radachlorin in 3 biological media in the red part of spectrum

Parameter	Medium		
	0.01 M borate buffer, pH 9.18	0.01 M borate buffer with 1% HSA added, pH 7.2	Ethanol
<b>Absorption maximum</b>	654,5 nm	662 nm	662 nm
<b>Fluorescence maximum</b>	661 nm	668 nm	668 nm
<b>Fluorescence quantum yield</b>	4%	4%	4%
<b>Interconversion quantum yield</b>	96%	96%	96%

Radachlorin possesses an intensive absorption band in the middle-red part of the spectrum where biological tissues are transparent to a larger extent than in case of the first generation photosensitizers (around 630 nm). The high value of interconversion coefficient, 96%, provides a high quantum yield of singlet oxygen (up to 75%) that makes Radachlorin highly cytotoxic under irradiation in the red absorption band. 4% of the fluorescence is very important, this value is quite enough for photodynamic diagnostics. It is very helpful for clinical use that the yields of interconversion and fluorescence are stable in different biological media.

**Toxicity** parameters are presented in table 2.

Table 2. Some parameters of dark and light Radachlorin toxicity.

Toxicity parameter. Biological object	value
Drug dose of S % acute survival in the dark. White breedless mice.* (Human therapeutic dose is around 1mg/kg)	S=90      119 mg/kg S=50      147 mg/kg
Cell survival in the dark at drug concentration 5 µM/l. Pheochromocytoma PC12 cell line**	96%
Drug concentration of 50% cell survival under 662 wavelength irradiation in light dose 50 J/cm <sup>2</sup> . Pheochromocytoma PC12 cell line**	1.8 µM/l

\* Experiments were conducted by Dr. N. Neugodova et.al. in Institute for State Control of Medicinal Drugs (Russia)<sup>1</sup>.

\*\* Experiments were conducted by Dr. O. Abakumova et.al. in V.N. Orekhovich Institite of Biomedical Chemistry of RAMS and Dr. A.Karmenyan (N.N.Blokhin Russian Cancer Research Center)<sup>1</sup>.

So, Radachlorin combines a high cytophototoxicity and low dark toxicity both in acute in vivo and in vitro cell experiments. Experiments on the chronic toxicity, allergenicity, immunogenicity, local irritating action, CNS action, cardiovascular action, biochemistry of blood and histology show the absence of serious Radachlorin side-effects on the organisms of mice, rats, rabbits, guinea-pigs. (Dr. G. Dolgova and Dr. A. Nikitin<sup>1</sup>, State research centre on antibiotics, Moscow, Russia). There are no pyrogenic and histamine-like actions at therapeutic doses (rabbits and cats in experiments of N. Neugodova et.al.<sup>1</sup>).

**Pharmacokinetic and contrast** properties of Radachlorin were studied in experiments on mice with inoculated lymphogenically embryocarcinoma T36 by the group of Dr. A. Ivanov in N.N. Blokhin Russian Cancer Research Center<sup>1</sup>. Fig. 3 shows the kinetics of accumulation of Radachlorin in different mouse's tissues and a tumor (diameter 10 mm, mass 1 g, within 14 days after inoculation) after intravenous injection of dose 20 mg/kg.

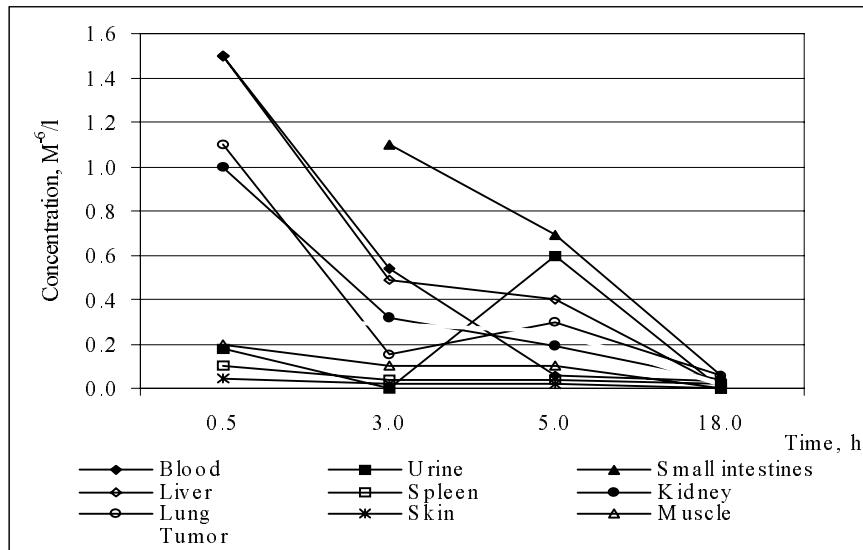


Fig. 3. Radachlorin pharmacokinetics in tumor-bearing mice with inoculated embryocarcinoma T36 after 20 mg/kg intravenous injection.

It is seen that the maximum of contrast is observed around 3 hour after injection: tumor-to-muscle ratio is about 3, and tumor-to-skin ratio is about 14. At intra peritoneum injection these numbers were higher: the maximum was observed in 18 hour, the ratios were 32 and 44 respectively. The full clearance period has been found to be 48 hour after any injection.

#### 2.4. PDT activity

Radachlorin showed an expressed specific PDT activity in the same mouse model. The experiment was performed (by A. Ivanov's group<sup>1</sup>) using male Balb/c mice with embryocarcinoma T36 inoculated in the muscle of hind leg. The PDT procedure was conducted in 2 weeks after tumor cell inoculation (tumor weight 0.9-1 g) at intra peritoneum (dose 40 mg/kg) or intra venous injections (20 mg/kg). The tumors were irradiated with 662 nm light in surface dose 300 J/cm<sup>2</sup> (diode laser ML-662-SP, Russia, see below) in 5-6 hour after the drug injection. The procedure resulted in complete tumor necroses in 2-4 weeks. The laser irradiation only did not produce any effect to the tumor. The result of Radachlorin action could be seen already in the first hours after irradiation. During the first two days the tumor grew darker from its bulk to periphery, and by the 3<sup>rd</sup> day tumor necrosis started, resulting in tumor contraction and upper crust formation, its peeling away with normal tissues beneath. Biopsy revealed no cancerous cells.

Basing on these and toxicity experiments we recommended to conduct the clinical trials with drug doses 0.8-1.2 mg/kg (intravenous injection) and light doses starting from 100 J/cm<sup>2</sup>.

### 3. LASER ML-662-SP

The Laser Diode Module ML-662-SP is a result of joint efforts of Milon Laser Ltd (Russia, Saint-Peterburg) and Sigm Plus Co. (Russia, Moscow). High brightness and high reliable laser diodes (660 nm, 500 mW, 200 mkm aperture) were used. Diodes have been made by Polaroid Laser Diode Department (Boston Laser Inc. now) and passed a special selection as to provide the parameters of table 3. Six LDs were coupled into high bright optical scheme in well known "MILON module". The ML-430 connectorized module is designed for high brightness, high reliability and ease of use. It contains: built-in thermoelectrical cooler, laser diode driver, standard optical SMA-905 connector. Parameters of the laser diode system is presented in table 3:

Table 3. Diode laser ML-662-SP.

Wavelength	$662 \pm 2\text{nm}$
Power in CW mode	0.1-2.5 W, continuously
Fiber core diameter	250mkm and more
Numerical aperture	0.22
Pulse duration in pulse mode	2 msec and more
Pause duration in pulse mode	2 msec and more
Mass	6 kg

### 4. CLINICAL TRIALS

Clinical trials of the method were performed in General Surgery Clinic of Chelyabinsk State Medical Academy (Chelyabinsk, Russia).

#### 4.1. Patients

The method was applied to 51 volunteer patients with different malignant tumors. Age: 10-93, mean age: 48, sex: 22 males and 29 females. All the patients were informed that the treatment method is an experimental one, each of them signed Informed Consent. Distribution of patients in type and location of tumors is presented in table 4.

8 patients with primary basal cell cancer had multiple form of the disease, one of them, female, age 48, had 112 tumors (fig. 4): 11 patients came in the clinic with IV stage in incurable state, one of them had 3 different tumors: basal cell cancer, cancer of lower lip, and lung cancer to which PDT was not applied. 20 patients had I or II stage, most of them were from basal cell group. A reason of PDT prescription for 8 patients was contra-indications to tradition methods. For 5 patients PDT was prescribed because they was earlier treated with traditional methods (surgery operation, chemo, radio and cryotherapy), and potential of these methods to act on the vestigial tumor (1 patient of 5) and on the recurrences (4 patients) was exhausted. PDT for these patients was performed in more than 1 month after radio or chemotherapy.

Table 4. Patient's tumors treated by PDT.

Hystological type and location of tumor	Number of patients /number of tumors	Peculiarity (patients/tumors)			
		Primary	Vestigial	Recurrence	Metastasis
Basal cell cancer of the skin	35/159	32/38		3/121	
Cancer of lower lip	1/1	1/1			
Melanoma of soft palate with limphogeneous metastasis	1/1	1/1			
Cancer and leucoplacia of vulva	2/2	1/1		1/1	
Breast cancer	2/2	1/1		1/1	
Cancer of esophagus, stomach, rectum	3/3	3/3			
Cancer of thyroid gland	3/3	1/1		1/1	1/1
Cancer of ductus choledochus	1/1	1/1			
Reticular sarcoma of thyroid gland	2/2	2/2			
Sarcoma of soft tissue	1/2		1/2		
Malignant ganglioneuroma	1/1	1/1			
Total number of cases*	52/177	44/50	1/2	6/124	1/1

\* The number of cases exceeds the number of patients because one patients had a few type of tumors.

## 4.2. Procedure

In most cases the photosensitizer Radachlorin was injected intravenously in dose 0.8-1.2 mg per a kg of patient's mass (usually 1 mg/kg). For 10 patients with basal cell cancer we used external local applications with Radachlorin gel.

Laser irradiation was carried out in 1-2 hours after the injection. We used four techniques of laser irradiation: 1) distant surface irradiation, 2) intracavity irradiation, 3) intratissue irradiation, 4) combination of surface and intratissue irradiation. Delivery of radiation was fulfilled by means of quartz fibers terminated in cylinder diffuser (in techniques 2-4) or microlens (1,4) or simple face plane (1,4). In the intracavity cases we used standard endoscopes, intratissue irradiation was carried out through the puncture needle under ultrasonic control. Surface light dose was 100-500 J/cm<sup>2</sup>.

Treatment was performed under local or general anesthesia. As a rule no anesthesia was required at surface irradiation of skin or lip. For a day after irradiation patients stayed in black-out wards without TV. Then patients were free in respect to light and TV.

## 4.3. Reactions

Effect of PDT was estimated on base of visual, endoscopic, roentgenological, ultrasonic, and cytomorphological investigations. The estimation was fulfilled during the procedure and just after it in 1-2 days, on 7-10 day, 15-20, 30 days, and then monthly. Observe period consisted from 3 to 16 months.

Injection of Radachlorin did not cause any side consequences. State of patients remained stable. Roof pressure, pulse did not change significantly. There were no complaints both during the injection and after it. We did not observe any complications, connected with heightened light sensibility of skin and mucous membrane. No sunburns, hyperemia, other signs of phototoxicity.

Typically a local photodynamic reaction began in a few minutes after the beginning of irradiation. Patients marked a local burning pain in the irradiation zone. To the end of irradiation the tumor and ambient skin became anaemic, and around a hyperemia "aureole" appeared. After the irradiation the surface of tumor got humid with dark color sections, which increased in size, merged with each other, and in 2-3 hours became violet or black. Obviously this is manifestation of haemorrhagic necrosis. In 20-60 minutes after the procedure an edema of surrounding tissues and intense hyperemia of skin formed. The most expressive edema formed on the face and hairy part of the head.

To the end of the first day local reaction was maximum. The edema reached maximum (fig.4), gradually decreasing to 3-4 day (fig.4). There developed dry necrosis in tumors with clear demarcation line between surrounding skin and necrotized tumor tissue (fig.6).



Fig.4. Primary multiple basal cell cancer of the skin on the face: before PDT (left), 12 hour after PDT (right), strong edema of face.

Tumors of oral cavity, larynx, hollow organs (esophagus, stomach) reacted to PDT in the same way: edema of surrounding tissues, change of tumor colour, appearance of fibrin patches. Patients marked pain and we sometimes gave

them analgesics. Temperature of some patients increased up to subfebrile numbers. Blood analysis, pulse, roof pressure remained stable.

To the end of the 1<sup>st</sup> week local changes were marked only in the tumor zone, they were black dense scabs. Surrounding tissues repaired to their original look completely. Tumors of esophagus, stomach, larynx, mucous membrane of oral cavity were covered with thick layer of fibrin.

In 2-4 weeks the necrosis was torn away and then there began gradual cicatrization and epithelization, which completed to 6th-8th weeks.

Reaction on PDT at external applications of Radachlorin gel (we used it only for treatment of 10 patients with basal cell cancer of the skin) was of the same type as in the injection cases, but less expressive. We had to increase light dose up to 500-800 J/cm<sup>2</sup>.

#### 4.4. Efficacy

We estimated effect of PDT in the following grades:

- Complete regression,
- Partial regression: (decrease of maximal size of tumor not less than 50%),
- No effect: (decrease of maximal size of tumor less than 50%).

The results are presented in table 5.

Table 5. Clinical results of PDT with Radachlorin

Hystological type and location of tumor	Total number of patients	Number of patients with the effect		
		Total	Partial	No
Basal cell cancer of the skin	35	28	7**	
Cancer of lower lip	1	1		
Melanoma of soft palate ***	1	1		
Cancer and leucoplacia of vulva	2	1	1	
Breast cancer	2		2	
Cancer of esophagus, stomach, rectum	3		3	
Cancer of thyroid gland	3	2	1	
Cancer of ductus choledochus	1		1	
Reticular sarcoma of thyroid gland	2		1	1
Sarcoma of soft tissue	1			1
Malignant ganglioneuroma	1			1
Total number of cases*	52	33	16	3

\* See the footnote to table 4

\*\* In all these cases the external applications of Radachlorin gel was used.

\*\*\* A combination of PDT procedure with laser ablation was used (see below).

Total regression of tumors of basal cell cancer of the skin was reached in 100% cases under intravenous injection of Radachlorin. A good cosmetic effect was reached in all these cases. At external application of Radachlorin the most cases have ended with partial regression.

At other locations of cancer the total regression was reached in 4 of 12 cases, and not a one case ended with "no effect". In this "partial" group of 8 patients there was reached: stabilization of local tumor growth – 8 patients, possibility to do a radical operation – 2, improvement of life quality – 8, regain of conductivity of hollow organs (recanalization) – 4, death because of dissemination and metastases growth in absence of local progression – 2.

Positive effect in small (4 patients) group of connective tissue tumors was reached in 1 case. It seems impossible to speak about the PDT effectiveness in treatment of this type of patients because of insignificant number of observations.

#### 4.5. Examples

As an illustration of PDT with Radachlorin we give two cases.

## **Basal cell cancer of the skin**

Patient B., age 65, male.



Fig. 5. Before PDT. Basal cell cancer of the skin of nasolabial area in the left (T3 N0 M0).  
There is a flat sore rising over the skin surface with erose edges and with scarce discharge.



Fig. 7. 20 days after PDT.  
Partial tearing away of necrosis in the tumor zone.  
Surrounding skin is of usual colour, no edema.



Fig. 9. 3 months after PDT.  
Total healing of the wound with epithelialization and formation of the flat hardly visible scar.



Fig. 6. Second day after PDT.  
In the tumor place there is a photochemical burn with the necrosis in the form of dry scab, and with the reaction of surrounding tissues, which are cyanotic and edemic.



Fig. 8. 6 weeks after PDT.  
Tearing away of necrosis in the tumor zone with edge epithelialization of the wound.



Fig. 10. 1 year after PDT.  
Hardly visible scar in the place of tumor.

## Melanoma

Patient S., age 50, male. During a year he had been noticing a gradually increasing formation on the mucous membrane of the soft palate. An oncologist's examination showed the possibility of soft palate melanoma. Patient S. refused from the offered operation with resection of upper jaw. He was hospitalized in Clinic of General Surgery at Chelyabinsk State Medical Academy.

At the clinical checkup the diagnosis was the soft palate melanoma (fig.13) with metastases in lymphatic glands of the neck in the right (T3 N2 M0).

On the 4<sup>th</sup> of January 2001 after trachea intubation there was injected intravenously 100 mg of Radachlorin. There was performed the operation of Crile's type with the ablation, as a single block, of cellular tissue of submaxillary and side neck triangles in the right, together with jugular vein, submaxillary salivary gland and lymphatic collector along general carotid. Urgent hystological investigation showed the melanoma metastases in the neck lymphatic glands. There was performed the PDT irradiation of the post-operative wound in the light surface dose of 200 J/cm<sup>2</sup>.

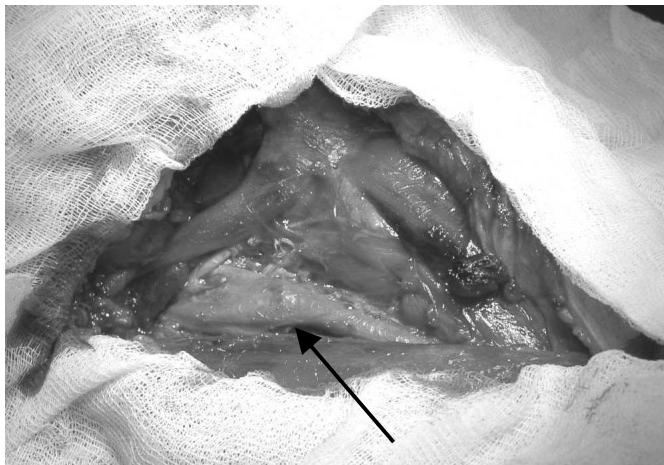


Fig. 11. Wound after Crile's operation on the neck in the right. Cellular tissue of submaxillary and side neck triangles is ablated. General carotid below its bifurcation is marked with an arrow.

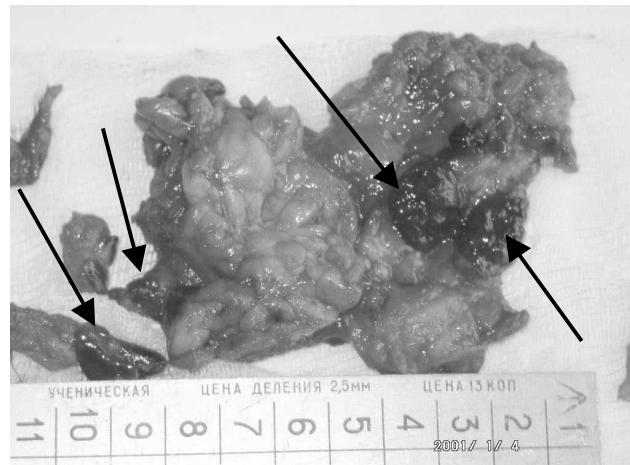


Fig. 12. The ablated neck cellular tissue with melanoma metastases (marked with arrows).

The neck wound was sewed layerwise with drainages. After this the PDT irradiation of soft palate melanoma in surface light dose of 500 J/cm<sup>2</sup> (fig. 14) was performed. Just after there was performed the laser ablation of melanoma with a diode laser of 980 nm wavelength, 30 W power.

### Photodynamic therapy and laser ablation of the soft palate melanoma



Fig. 13. Soft palate melanoma in patient S. before treatment.  
On the soft palate there is a round tumour-like formation of blue-black colour rising over the surrounding mucous membrane with satellite tumors along inner semi-circle of the melanoma.



Fig. 14. Melanoma just after PDT.  
Tumor surface and surrounding mucous membrane are pale.

After the PDT procedure the laser ablation of melanoma was performed with a diode laser of 980 nm wavelength, 30 W power.



Fig. 15. Wound just after the laser ablation.  
In the place of melanoma there is a crater-like defect of soft tissues covered with dark scab.

Post-operative neck wound healed up by primary tightening. Soft palate wound gradually cleaned up from the necroses and totally epithelialized by the 6<sup>th</sup> week.

### **Healing up of soft palate wound after PDT and laser ablation of melanoma**



Fig. 16. 2 weeks after the treatment.  
Tearing away of necroses. At the inner wound edge the area of necrotized tissue is covered with fibrin.



Fig. 17. 4 weeks after the treatment.  
Total cleaning up of the wound with the edge epithelization.  
No inflammatory reaction.



Fig. 18. 6 weeks after the treatment.  
In the place of melanoma there is a soft scar, partially of bright pink colour.



Fig. 19. 16 weeks after the treatment.  
Total epithelization of the scar in the place of melanoma.

The patient was subjected to two courses of polychemotherapy. No recurrence and melanoma metastases were revealed during a year observation.

### **5. CONCLUSION**

Photodynamic therapy with Radachlorin is an effective, safe and non-expensive (especially with diode lasers) method in treatment of malignant tumors.

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