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ABSTRACTS

EPPM-1

I-2

Vascular-targeted photodynamic therapy (VTP) of cancer: Acute blockade of tumor blood supply

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In vascular-targeted photodynamic therapy (VTP) of solid tumors, the Pd-bacteriochlorophyll-based photosensitizer (Tookad) is intravenously injected/infused and systemically circulating, while the tumor vasculature is locally illuminated. As a result, cytotoxic reactive oxygen species (ROS), mainly super oxide and hydroxyl radicals, are photo-generated in the blood vessels with consequent oxygen consumption confined to the treated tumor target. Local hypoxia and vasodilatation swiftly follow with development of intravascular damage and thrombus formation, resulting in blood vessel occlusion and stasis in the target tumor within minutes. Downstream tumor tissue becomes ischemic (hours), with a consequent development of necrosis that culminates with tumor eradication (2—4 weeks) as observed in preclinical studies. The hemodynamic response to VTP was explored online by fluorescent intravital microscopy and photosensitized (ps)MRI in tumor mouse and rat models. Monitoring blood supply and drainage at the tumor boundary, upon illumination, demonstrated a rapid slow-down in arterial blood flow, with vascular collapse around 1 min; venal flow however, declined hesitantly with delay. Blood stasis in the tumor was found to remain reversible following partial treatment (5 min), but became committed to irreversible stasis towards the end of the complete (10 min) VTP-treatment protocol, as verified 24 h later. Localized illumination and differential vascular response to ROS of abnormal tumor, as compared to healthy vasculature, provides VTP with significant tissue/organ selectivity. Tookad is the first of this Pd-bacteriochlorophyll derived sensitizer family developed in our laboratories. Using this approach, Tookad-VTP is presently in phase II/III clinical trials of patients in several countries with recurrent or primary-localized prostate cancer, in collaboration with Steba Biotech. Information that points at efficacy and selectivity of Tookad-VTP will be presented, along with highlighting data from the clinical trials.

Acknowledgment

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O-1

Correlation between depth of necrosis and fluorescence during PDT

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Photodynamic therapy (PDT) uses a photosensitizer (PS), light, and oxygen to create tissue damage. The activation of PS by light leads to degradation of drug (phototransformation) and photodynamic effect. These two processes may well be connected and can be visualized by decreasing of fluorescence intensity of PS. This work evaluated the degradation of photogem *in vivo* and in solutions through the study of necrosis depth in normal rat liver when irradiated at different light doses. It was observed that the degradation rate of Photogem has a strong correlation with depth of necrosis. Such fact creates an alternative to observe the effect of photodynamic activity measuring the fluorescence of the photosensitizer.

I-3

Selective targeting of photodynamic sensitisers to tumour tissue

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A wide range of PDT sensitisers are now available and these vary in wavelength of activation, physicochemical properties and photodynamic activity *in vivo*. One parameter critical to the clinical outcome of PDT is the localised concentration of photosensitiser in the target tissue at the time of treatment, and the relative ratio of this tumoural concentration to that in surrounding peritumoural tissue. Most PDT sensitisers currently used in the clinic have relatively poor tumour: peritumoural tissue ratios, with values for intravenously administered drug very rarely achieving >100: (1) significant improvements in this important parameter, and hence clinical outcomes, are unlikely to be achieved by simple manip-

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ulation of photosensitiser structure. It may be possible, however, by the use of delivery vehicles, such as liposomes and/or covalent attachment of the photosensitiser to biologically active molecules with affinity for cell surface structures that are over-expressed on tumour tissue.

In this paper the requirements for targeting will briefly be discussed and *in vitro*, mechanistic, and *in vivo* data relating to bioconjugates of PDT sensitisers with monoclonal antibodies will be presented.

O-2

Phthalocyanine—nanoparticle conjugates for photodynamic therapy

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Phthalocyanine—nanoparticle conjugates have been designed and synthesised for the delivery of hydrophobic photosensitisers for photodynamic therapy (PDT) of cancer. The phthalocyanine photosensitiser stabilised gold nanoparticles have an average diameter of 2—4 nm. The synthetic strategy interdigitates a phase transfer reagent between phthalocyanine molecules on the particle surface that solubilises the hydrophobic photosensitiser in polar solvents. The phthalocyanine is present in the monomeric form on the nanoparticle surface, absorbs radiation maximally at 695 nm and catalytically produces the cytotoxic species singlet oxygen with high efficiency. These properties suggest that the phthalocyanine—nanoparticle conjugates are ideally suited for PDT. An intracellular assessment of the nanoparticle conjugates has been successfully achieved using a cervical cancer cell line (HeLa). We have recently obtained *in vivo* pharmacokinetic data for the nanoparticle conjugates which had been intravenously injected (1.5 μmol/kg body weight) into C57BL/6 mice bearing a subcutaneously transplanted amelanotic melanoma. These data show that the particles are localised within the tumour, with *ca*. 2.5—3-fold increase in the efficiency and selectivity of tumour targeting as compared with the corresponding non-conjugated phthalocyanine. Preliminary *in vivo* photodynamic therapy data show that the nanoparticle conjugates induce a significant tumour response especially when irradiation is performed at 3 h after injection.

O-3

Photocytotoxicity of mTHPC (temoporfin) loaded polymeric micelles mediated by lipase-catalyzed degradation

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Meta-tetra(hydroxyphenyl)chlorine (mTHPC, temoporfin) is a second-generation photosensitizer, which is currently clinically used as a formulation in ethanol and propylene glycol (Foscan®). It is registered for the palliative treatment of advanced head and neck squamous cell carcinoma. In this report, we describe a novel mTHPC formulation in biodegradable polymeric micelles composed of the penta(ε-caprolactone) ester of monomethoxy poly(ethylene glycol) (750 Da) containing a terminal benzoyl group at the hydrophobic block. Physically stable mTHPC loaded micelles were prepared by hydration of a mixed film of the photosensitizer and the polymer, with an extremely high drug loading capacity of up to 30% (w/w), resulting in a solubilisate of 3 mg/mL. mTHPC-loaded micelles at a relatively high polymer concentration (0.5 mg/mL polymer) above the critical aggregation concentration (CAC) did not display photocytotoxicity up to an mTHPC concentration of 2 μM, nor any mTHPC-uptake by UM-SCC-14C cells at 10 μM mTHPC, in contrast to free mTHPC and liposomal mTHPC (Fospeg®). Interestingly, after dilution of the loaded micelles below the critical aggregation concentration (CAC), or after incubation with lipase causing micelle degradation, photocytotoxicity and cellular uptake of mTHPC were restored. In conclusion, the high loading capacity of the micelles, the high stability of mTHPC-loaded micelles above the CAC, and the lipase-induced release of the photosensitizer makes these micelles very promising carriers for photodynamic therapy *in vivo*. Biodistribution and therapeutic efficacy studies are currently performed in animal tumor models.

O-4

Photochemical internalization (PCI) as adjuvant treatment after inadequate resection of an invasive sarcoma model

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Local recurrence after surgery of soft tissue sarcomas is dependent on surgical margins. Wide margins require large resections which may lead to impaired function or loss of limb. In some cases it may be technical impossible or even ethical unacceptable to achieve ideal oncological margins. Standard adjuvant treatment in such cases is ionising radiation, which may cause serious side effects.

PCI is a unique procedure for site-specific delivery of several types of membrane impermeable molecules from endocytic vesicles to the cytosol of the target cells. The technology is based on the photochemical-induced cytosolic release of endocytosed macromolecules from endosomes and lysosomes. PCI has in this study been evaluated as an adjuvant to the surgical resection of sarcoma.

Human fibrosarcom (HT1080) was transplanted to athymic mice. The photosensitizer aluminium phthalocyanine disulfonat (AlPcS2a), and bleomycin (BLM) were systemically administrated 48 h and 30 min, respectively, prior to surgery and light exposure. After resection with intralesional margin the tumor bed was illuminated at 670 nm (15 J/cm^2).

PCI was found to induce longer delay in tumor growth than PDT. In combination with surgery little was achieved with respect to tumor growth delay by adding PDT while PCI-induced synergistic effect. The results indicate that PCI targets the viable peripheral zone of the tumor where PDT is apparently less effective. PCI with BLM seems promising as an adjuvant treatment after inadequate resection of sarcomas.

O-5

Synthetic strategies for porphyrin-based photosensitizers

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Photodynamic therapy is an excellent modality of treatment for cancer which uses the combination of a photosensitizer and light to generate active species able to destroy tumour cells in the organism. While a large number of porphyrin structures have been investigated as potential photosensitizers, only a few have received legal approval for clinical use. Ongoing research indicates that further improvements of these systems are needed with regard to their photophysical and absorption properties, localization, clearance, and amphiphilic character. In chemical terms this requires the development of simple syntheses for unsymmetrically substituted porphyrins. Current synthetic tools include condensation

methods, organolithium reactions, halogenations and Pd-catalyzed coupling reactions for the generation of various types of ABCD porphyrins. Based on the development of regioselective and high yield functionalization reactions more complex dimeric and oligomeric systems, and systems with extended π-conjugation suitable for further modifications and binding with bioconjugates are now in sight. An ongoing methods development program and critical analysis of available the synthetic repertoire indicates that almost all members of the A_x-, AB-, ABC-, A_2BC-, A_2B_2-, and ABCD-families of porphyrins are now accessible.

I-4

Photodynamic diagnosis and therapy in dermatology

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Exogenously applied or endogenously formed photosensitizer might preferentially locate in cancerous tissues. UV irradiation of the photosensitizer-enriched cancerous tissue leads to the emission of fluorescence. This diagnostic procedure has been known as photodynamic diagnosis (PDD). In the presence of oxygen, light activation of photosensitizer induces cell death via the formation of cytotoxic singlet oxygen and other reactive oxygen species. This therapeutic procedure has been known as photodynamic therapy (PDT). The feasibility of PDD and PDT for skin diseases is appreciated due to the easy accessibility of the skin to the topical application of photosensitizer and light. The benefit of PDD includes the delineation of peripheral borders of precancerous and cancerous lesions. The dermatological indications of PDT include non-melanoma skin cancers (BCC, SCC), actinic keratoses, and Bowen's disease.

Since the discovery of endogenous protoporphyrin IX (PpIX) fluorescence and photosensitization induced by exogenous administration of aminolevulinic acid (ALA), ALA (or its derivatives, e.g. methyl-ALA)-based PDD and PDT has become established modalities in dermatology. The advantage of ALA-PDT is its selectivity, good tolerance and generally good cosmetic effect. Local application of ALA can also eliminate the long-term cutaneous photosensitivity caused by the systemic administration of photosensitizer. ALA-based PDD and PDT might be further improved by enhancing PpIX production and accumulation.

Our recent study demonstrates that ALA-PDD could also be employed for identifying genital lesions and subclinical lesions caused by HPV. ALA-PDT is a valuable addition to our treatment options for non-melanoma skin cancer, urethral condylomata acuminate, erythroplasia of Queyrat and acne vulgaris.

O-6

PDT of skin HPV lesions and other viral infections

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Photodynamic therapy (PDT) has been used for eradicating non-malignant growths such as human papillomavirus (HPV). HPV skin lesions are very common and can present as foot, hand or genital warts and widespread distributed as epidermodysplasia verruciformis. The diagnosis is often straightforward, but the treatment is difficult and lengthy. Simple and currently employed medical treatments such as keratolytic agents, curettage, glutaraldehyde and invasive methods such as cryotherapy or electrosurgery and laser therapy show good therapeutic effect. But still, some warts remain resistant and especially for immunosuppressed patients or under immunosuppressive therapies new treatment modalities are needed. Some pilot studies demonstrate that PDT might be an additional option in the treatment of cutaneous and mucosal HPV-infection alone or combined to conventional modalities to reduce recurrence rates.

O-7

Pharmacokinetics and pharmacodynamics of aluminium phthalocyanine chloride in a murine non-melanoma skin cancer animal model

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Topical photodynamic therapy (PDT) is potentially useful for the treatment of non-melanoma skin cancer and other skin diseases. In this work, the biodistribution and photodynamic efficiency of aluminum phthalocyanine chloride (AlClPc) excited by a diode laser emitting at 670 nm, were examined after topical application on murine non-melanoma skin carcinomas. Skin biopsies 1—6 h after application, combined with chemical extraction of the photosensitizer from tumor examined by fluorescence spectroscopy revealed that AlClPc is a very selective photosensitizer. Its concentration in tumor was 40 times higher than in normal skin even 1 h after application. Moreover, the penetration depth of AlClPc was examined using laser scanning confocal microscopy. The in vivo efficiency of AlClPc was assessed as the percentage of complete tumor remission, the tumor growth retardation and the cosmetic outcomes. Seven different combinations of treatment parameters were adapted from those routinely applied in animal studies. The results underlined the importance of fluence rate in the final outcome of photodynamic therapy (PDT). Furthermore, they demonstrated the high in vivo effectiveness of topical AlClPc PDT and are very promising for the future application of this PDT scheme on skin non-melanoma cancer treatment.

O-8

Characteristics of fluorescence induced by ALA and MAL

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Superficial skin lesions can be treated effectively using PDT following application of 5-aminolevulinic acid (ALA) or methyl aminolevulinate (MAL). The purpose of this study was to investigate the characteristics of PPIX-induced fluorescence in normal skin following the application of ALA and MAL. Three aspects were studied: namely dosage, site of application, and duration of application. ALA and MAL were applied topically in dosage ranging between 0.01 ml and 0.09 ml in aluminium chambers which provided a 50 mm^2 area. The results show that fluorescence was not dose-dependent, within the range investigated. Four different body sites were investigated (inner forearm, outer forearm, lower leg and lower back). Inner forearm consistently showed the highest fluorescence, and the lowest fluorescence was noted on the outer forearm and lower leg. Application times from 1 to 6 h were studied and fluorescence monitored for periods up to 28 h. Maximum ALA-induced fluorescence for shorter application times (1—3 h) was noted at 7 h, compared to 24 h for longer application times (4—6 h). Peak MAL-induced fluorescence for all application times was noted at 7 h. ALA-induced fluorescence was significantly greater than MAL-induced fluorescence. These results help to define the characteristics of ALA- and MAL-induced PPIX fluorescence in normal skin, and thus provide a

good understanding of the kinetics of PPIX production in normal cells exposed to ALA/MAL PDT.

O-10

A new light source for cutaneous PDT

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Recent organic optoelectronic advances have enabled the creation of a new PDT light source. It utilises an organic light emitting diode to make a wearable and potentially disposable light source suitable for ambulatory treatment. The technology consists of a thin layer (2 cm diameter circular) organic semiconductor between glass contacts. Application of a voltage results in emission of LED light. The organic light emitting diode feature of particular interest is the fact that they are area light sources and not point sources. This enables an area irradiation effect.

We have conducted a pilot study in 12 patients with Bowen's disease (8) or superficial basal cell carcinoma (4), who were treated with ALA applied under Tegaderm for 3 h. The dose administered on two treatments separated by 1 month was 45—60 J/cm^2 red light (550—750 nm, peak 620 nm). All lesions were histologically confirmed. Follow up at six and 12 months revealed five non-responders at 12 months with a tendency for this to arise in the larger lesions where a peripheral margin failure was evident in four. Pain scoring using our standard methodology with comparison to pain data using standard inorganic LED Actalite source, revealed none of the study patients to have required pain relief and only mild pain recorded. In this open study, ambulatory photodynamic therapy using a low irradiance organic OLED patch device appears to be a safe, well-tolerated treatment.

I-5

Low dose Photofrin PDT improves clinical outcomes

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Photofrin has been commercially available for more than 25 years and allowed PDT to gain a worldwide audience. However, therapy with this photosensitizer still can be refined. Currently, the clinical standard is to employ 2 mg/kg for all indications. This results in a robust tumor kill but also significant normal tissue reaction. Clinically, this normal tissue effect can have profound negative ramifications that may lead to hospitalization. We have explored lower doses of this drug (0.8—1.2 mg/kg) in an attempt to better control the photodynamic reaction. This is based on the clinical concept of photobleaching. As Photofrin will preferentially accumulate in the diseased tissue as compared to surrounding normal tissue, it may be possible to clinically define a minimum photosensitizer dose that will still destroy tumor while simultaneously being in low enough concentration in normal tissue to prevent significant reaction. We have maintained outstanding tumor response with very limited normal tissue toxicity in our series of 50 patients. These include tumors of the head and neck, skin, and breast. Importantly our series included individuals who already had undergone maximum radiation therapy, surgery and chemotherapy and had no other therapeutic options available. Reliably, the lower drug dose refines the intensity of the reaction to better spare normal tissues and also improves selectivity within the illumination field. Lowering photosensitizer dosage is a simple, reliable means to improve clinical PDT with Photofrin.

O-11

The role of autofluorescence colonoscopy in diagnosis and management of solitary rectal ulcer syndrome

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Solitary rectal ulcer syndrome (SRUS) is a chronic disease of the rectum. Although SRUS is a benign condition there are studies which suggest that chronic ischaemia which occurs in the SRUS may lead to ''transitional mucosa'' that is similar to that adjacent to colorectal carcinomas and adenomas and may lead to colorectal dysplasia and carcinoma development. The exclusion of primary or metastatic malignancy is the most important aim in the differential diagnosis of SRUS. In our study we assess the possibilities of autofluorescence colonoscopy (AFC) in diagnosis and management of SRUS.

We performed white light colonoscopy first. The tissue samples were taken for pathological examination. When SRUS was histopathologically confirmed AFC was performed by means of Xillix OncoLIFE. During AFC numerical colour value (NCV) of autofluorescence of SRUS lesions was noted.

During 1946 colonoscopies eight persons were diagnosed as having solitary rectal ulcer syndrome. We did not observe autofluorescence increase in case of polipoid and flat ulcer lesions (NCV 0.39—0.67; mean .0.525) and little increase of autofluorescence in case of erythema lesion (NCV —0.94).

SRUS is a rare disorder of the rectum but it causes differential diagnosis problems. The most common reason for incorrect diagnosis is inadequate tissue specimens. AFC allows to reveal subtle areas within the lesions of more intense autofluorescence and localizes the potential cancer-transformating dysplasia. In this way the most representative area with highest risk of pre- or cancerous changes, for biopsy specimen is indicated.

O-12

Comparison of endoscopic photodynamic therapy (PDT) with surgical resection for early oesophageal cancerK. Moghissi[1], K. Dixon[1], M. Stringer[1,2]

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Objective: The aim of this study was to compare and contrast the efficacy and results of PDT with surgical resection in patients with ESOC.
Methods: Subjects: 40 consecutive cases of ESOC treated by endoscopic PDT (Group 1), and 40, treated by surgical resection from the senior author's (KM) archives (Group 2).

For Group (1) Photofrin was administered; IV, followed by endoscopic illumination of 630 nm laser light, 24—72 h later. For Group (2) oesophagectomy and reconstruction with gastric tube was performed.
Results: In Group 1; patients were older with higher co morbidity and there were more adenocarcinomas than in Group 2. There was no procedure related mortality in Group 1, and six episodes of non-fatal complications. Fifteen patients died between 2 and 100 months (mean 26.2 months) and 25 patients are alive between 10 and 110 months. In group 2 hospital mortality was one and three others had post-operative complications. Eleven died between 13 days to 48 months (mean 30 months). Twenty-nine were alive from 5 to 30 years.
Conclusions: There was significant difference in population characteristics between the two groups in this study as well as the staging (clinical in Group 1, pathological in Group 2). Actuarial survival

curves appears to suggest that with careful selection of patients, PDT can potentially achieve long survival comparable with that of resectional surgery.

O-13

PDT in metastatic brain carcinomas

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Introduction: Surgical resection still plays a role in the management of single brain metastasis particularly when the primary is unknown. However, eradication of the tumour is not possible because the basic oncological principle of resecting the tumour mass with a cuff of normal tissue cannot be applied to the brain. We reviewed our results of adjuvant PDT in brain metastases from carcinomas.
Methods: Case note review of prospectively collected data on patients who were treated with PDT at the time of surgery for brain metastases.

Patients were consented for the surgery and PDT. Patients were given 2 mg/kg body weight of Photofrin® 48 h before the surgery. Following resection of the tumour using microsurgical and image guidance techniques, the post-excision cavity is filled with a balloon using 0.32%. Intralipid solution and up to five consecutive PDT treatments were given using 100 J/cm^2 diode laser 630 nm.
Results: We have treated 14 metastatic brain cancers in 14 patients. The details of these patients and metastases are summarized in Table 1. Seven were lung in origin and the other seven were of variable origin. One patient with lung metastasis died of unrelated cause while the remaining six had remained free from brain disease till their death. Two of the remaining seven patients died of local brain recurrence; one bowel after 4 weeks and one of unknown primary after 70 weeks.

Table 1. Summary of 14 Brain metastases treated with repetitive PDT

Primary cancer	Mean age years (S.D.)	Total number (males %)	Mean survival in weeks (S.D.)	Cause of death
Lung	60.7 (13)	7 (29%)	33.35 (51)	Primary 6, unrelated 1
Breast	50 (2.8)	2 (0%)	19.5 (9.2)	Primary 2
Bowel	69 (12.5)	2 (0%)	30.5 (37.5)	Primary 1, brain met 1
Ovarian	75 (32)	2 (0%)	41.5 (3.5)	Primary 2
Unknown	60	1 (0%)	70	Brain met 1
Total		14 (14%)		Primary 79%, brain 14%

Conclusion: Adjuvant repetitive PDT seems to offer an excellent local control of metastatic brain carcinomas with about 79% of patients succumb to the primary and only two out of fourteen developed brain recurrence with the best results obtained in lung cancer.

I-6

PDT has come of age—time to see the world

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Photodynamic therapy has proven not only to be efficacious, but in certain constellations to be the treatment of choice (e.g. many cases of Bowen's disease in dermatology). Randomized controlled trials have been performed to provide the level of evidence needed to make PDT a licensed treatment option for a group of diseases. But from the lessons in dermatology we learned that published cure rates alone are not sufficient to promote a ''new'' therapeutic modality to a point that it is widely accepted in the medical community. Not offering PDT as part of the therapeutic spectrum in dermatology means depriving patients from the treatment option that might be the most suitable one in their constellation. A lot of effort has yet to be made to spread interest in, knowledge about, and experience with PDT in the medical world. Although cooperation with the pharmaceutical industry is indispensable it is important to make clear that PDT from a doctor's point of view should not be regarded as economical interest of a few companies, but that it is the treatment concept itself that is convincing. PDT is — to a certain degree — a therapeutic modality that is caught between two stools (surgical and non-surgical). Specialized PDT meetings are the right platform to exchange ideas between experts, but in many medical disciplines it is also necessary to step out oft his group of ''aficionados'' and try to position the PDT topic in the main sessions of relevant meetings.

I-7

Foscan® mediated photodynamic therapy (PDT) in head and neck cancer, The Netherlands Cancer Institute Experience

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Since the introduction of the so-called second-generation photosensitizers, like Foscan, and the availability of relatively cheap high power diode lasers, there has been a renewed interest in PDT.

Several studies have demonstrated its effectiveness.

Since 1996 PDT has been one of the treatment options for head and neck cancer in the Netherlands Cancer Institute; more than 250 patients have now been treated with PDT. It has been applied in primary cancer, second primaries or recurrent disease, and in cases where no other treatment options were available. All patients were treated with surface illumination (20 J/cm^2), given 4 days after 0.15 mg/kg Foscan intravenous injection. Drawback of this method is the limited depth infiltration (to 10 mm), which makes this treatment unsuitable for more bulky disease and difficult accessible tumours. Recently there has been increasing interest in other light applications like interstitial illumination. Research in this field has started, initially focusing on light dosimetry and implantation techniques. Nowadays this technique in our Institute is comparable with the standard procedures as used in brachytherapy, using transparent tubes implanted in the tumour area and light diffusers for internal illumination of the tumour area.

The use of PDT in nasopharyngeal cancer, with a specially designed light applicator, has been tested in local recurrent and/or residual disease of the nasopharynx after adequate therapy, i.e. (chemo)-radiation. In a feasibility study in Indonesia, this method has proven to be efficient and safe.

I-8

PDT and optical diagnostics in the head and neck

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Head and neck cancer was one of the first cancers to receive approval for treatment with PDT. However, there have been few studies designed to extend the application of this therapy and certainly no prospective randomised studies are currently planned. In the United Kingdom, PDT has been specifically mentioned in the 5 years cancer plan so it is hoped this situation will change. In the first part of this talk, the published studies will be reviewed and the predominantly investigator driven extended applications described. In particular, the matching of treatment to the total disease volume has seen the greatest challenges and the use of image guidance is taking on increasing importance.

In tandem with this has been the need for better assessment of disease volume, depth assessment and continuing surveillance. Optical techniques have shown great promise in addressing many of these problems. Surface mapping with fluorescence or elastic scattering spectroscopy has been shown to be effective and optical coherence tomography is being developed for depth assessment of early disease before it becomes visible on ultrasound. These techniques are among the most exciting approaches to disease volume measurement and have the added advantage of being non-invasive.

Finally, an update on new drug formulations will be presented and the use of photochemical internalisation and antibody fragment linking discussed.

O-14

The role of autofluorescence diagnosis in the oral mucosa diseases

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Introduction: Life induces fluorescence diagnosis (LIFE) can be used as an imaging system of precancerous and neoplasmatic lesions of oral mucosa. LIFE system utilize healthy and neoplasmatic changed tissue to autofluorescence, without using any fluorescence substance. Neoplasmatic lesions are seen in pseudo colours, healthy tissue in a green colour and abnormal tissue in red colour. All seen colours have different intensity. Colour intensity is relevant to grade of dysplasia, carcinoma progress and is called numerological value of color index (NCV).
The aim: The aim of our study was to find correlation between autofluorescence diagnosis with NCV assessment and type of histopathological diagnosis of specimen biopsy.
Patients and methods: Fourteen patients participated in our study. Lesions affected a variety of intraoral sites. The most common location was: buccal, gingival and mandibular mucosa. Patients were examined using life induces fluorescence diagnosis (400—750 nm wavelength) with numerological value of color index (NCV) using Onco LIFE system. Then the specimen biopsy from the lesion was taken and histopathological examination was performed.
Results: We have noted different NCV and dependence of NCV on histopathological findings.
Conclusion: Diagnose using white-light imaging with LIFE imaging is not only significant faster method and better diagnose of preneoplasmatic and neoplasmatic lesions, but also there are correlations between NCV and histopathological diagnosis. The further investigations are needed to prove these preliminary findings.

O-15

Treating laryngeal carcinoma using PHOTOFRIN photodynamic therapy

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Objective: To study the short-term effect of PHOTOFRIN-mediated photodynamic therapy (PDT) in patients with laryngocarcinoma.
Methods: Fifteen patients with squamous carcinoma in laryngeal regions were treated with PHOTOFRIN PDT in our department between 2003 and 2006. PHOTOFRIN was administered intravenously at the dose of 2 mg/kg bw. After 48 h, light irradiation was performed using a 630 nm laser through an optical fiber. The fiber was passed down through the biopsy channel of an electronic endoscope. The light dose ranged from 100 to 300 J/cm^2. Two days later, necrotic tissues were removed. The primary sites and other newly identified sites were subjected to a second irradiation. Endoscopy examination was performed 1 month after PDT. The quality of life was evaluated by Karnofsky score.
Results: PDT was a safe and tolerable procedure. The total effective rate was 90% in these patients. Among them, the complete response rate of vocal cords cancer patients was 75% (3/4). These patients showed significant improvement in voice function. PDT was very effective in ablating obstructive tumor and improving the quality of life. The Karnofsky score increased from 35 to 65. The 1-year follow up of two patients showed no sign of recurrence.
Conclusion: PHOTOFRIN PDT is effective in the treatment of laryngeal carcinoma. PDT may not only cure early stage malignancy, but also reserve the function of the organ and significantly improve the quality of life in laryngocarcinoma patients.

O-16

Interstitial photodynamic therapy in recurrent head and neck carcinoma

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Introduction: PDT has proven to be effective in the palliation of incurable recurrences of head and neck squamous cell carcinoma. For treating larger tumor volumes the illumination can be performed with interstitially placed fibers. Here we describe our initial results of interstitial PDT with a palliative intent in recurrent H&N squamous cell carcinoma.
Methods: Eleven patients were treated with Foscan-based PDT. The tumor recurrence was either incurable or the patient refused a total glossectomy. The initial tumor stageing, before primary treatment, varied from T1N0M0 to T4bN0M0. Tumor localisation was tongue and floor of mouth. Patients were injected with 0.15 mg/kg Foscan i.v., 96 h prior to illumination. Hollow transparent canules were placed in and surrounding the tumor. All canules were illuminated with a total dose of 30 J/cm^2 (100 mW/cm^2) using light diffusers.
Results: Tumor response varied from 50 to 100% established 8—12 weeks post-treatment by clinical evaluation or MRI. Four patients are alive with no evidence of disease 4, 12, 29, and 46 months after PDT. Four patients are alive with disease 4,4,4 and 9 months after PDT. Two patients died 4 and 19 months of their H&N tumor and one patient died of a lung carcinoma.

Conclusion: Interstitial PDT seems to have potential for palliation in H&N squamous cell carcinoma. Remarkably, some patients were cured from their recurrence after being diagnosed as incurable. A phase II study will be performed to evaluate the tumor response in a larger group of patients.

I-9

Photodynamic diagnosis und therapy in gynaecology

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Photodynamic diagnostic (PDD) and therapeutic methods (PDT) have been evaluated successfully in several malignant and non-malignant gynaecologic diseases.

Fluorescence diagnosis is currently employed in gynaecologic conditions such as cervical intraepithelial neoplasia (CIN), endometriosis and ovarian cancer. For the access to the abdominal cavity, laparoscopic devices are being used. In the field of endometriosis, PDD has been shown to be useful for the detection of active lesions. Regarding ovarian cancer, several studies show that PDD is more effective in the detection of implantation metastases then white light inspection alone.

In the recent years, it became evident that a genital infection HPV is the cause of most cervical and vulvar cancerous and precancerous lesions (CIN and VIN). In the detection of CIN and VIN lesions, PDD using 5-aminolevuninic acid has been examined successfully in some studies.

Surgical excision techniques for cervical intraepithelial neoplasia (CIN) may cause cervical incompetence with premature deliveries and low-birth-weight. PDT of CIN is an outpatient procedure which uses a specifically designed portio applicator for the simultaneous homogeneous illumination of the endo- and ectocervical canal. ALA-derivatives such as hexyl- (HAL) or methylaminolevulinate (MAL) are currently being investigated in preclinical and phases I—II studies with promising perspectives.

Vulvar intraepithelial neoplasia (VIN) is a precursor of squamous cell vulvar cancer. Vulvar intraepithelial neoplasia is often multifocal which makes VIN a difficult disease to treat with a high rate of recurrence. Surgical excision in patients with extensive disease is both mutilating and associated with persistent disease in up to 40% of the cases. ALA-PDT is a procedure which shows equivalent results to CO_2 laser vaporization. However, preservation of normal vulvar morphology was observed after ALA-PDT since it did not induce scar formation and healing times after PDT are far below those published for laser evaporation.

O-17

PDD guided CO_2-laser excision and pDT of AIN, VAIN and VIN

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Problem: HPV-induced lesions are an upcoming thread for health systems of Europe. Currently we observe a high prevalence of HPV infections and a growing tendency for late sequales. Excisional therapy is the gold standard, but often compromised by recurrencies.
Patients and methods: In this preliminary study all patients where referred from other hospitals or offices. Twenty-five consecutive patients with 30 lesions underwent PPIX-fluorescence guided CO_2-laser-ablation of diseased tissues and/or PDT with various photosensitizers (PS) or PS-combinations (chlorin-e6, 5-ALA, HPD) between years 2000 and 2007. Fifty-two treatments were performed. Twenty-two patients (48 treatments) were continuously followed up, at least every 6 months with histological examinations. The average number of treatments for each patient was 2.2 (min: 1—max: 6).
Results: Three patients developed lesions at new sites during the study. Recurrencies occured after 11 treatments, the grade of dysplasia was lowered in 16 and NED was achieved after 21 treatments with an average follow up of 25 months (max: 77). Patients with multifocal lesions showed a higher recurrence rate. Healing was complicated in 2, delayed in 5 and normal in 41 treatments, with good cosmesis. Currently 20 patients show NED, one each a lower grade dysplasia or recurrence.
Conclusion: PPIX-fluorescence guided CO_2-laser-excision of diseased tissues or PDT may offer additional options for the treatment of AIN, VIN and VAIN as good delineation of diseased sites, early detection of recurrencies or new tumour sites. Larger studies are requested to compare the overall benefits for patients as recurrence free interval or percentage of progressive diseases.

O-18

Photodynamic therapy (PDT) of virus-associated oncology pathology of uterine cervix

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PDT application for virus-associated oncology of uterine cervix, assessment of antitumoral and antiviral efficiency of Russian photosensitizers, namely, Photosens (mixture of sulfonated aluminium phtalocyanine) 0.3 mg/kg; 5-ALA (5-aminolaevulinic acid) 5 g. 20% cream.

Sixty-nine women aged 22—63, 2/3 of them being of a reproductive age, underwent PDT of uterine cervix from 2002 to 2007. PDT with photosens was applied in 35 women with pre-cancer lesions (DIII), and 12 women with non-invasive cervical cancer (Group 1), 5-ALA PDT was applied in 22 women after surgical amputation of cervix: 8 cases of pre-cancer lesions (DIII), 9 cases of non-invasive cervical cancer, and 5 cases of invasive cervical cancer (Group 2). Consequently, all women were tested for the occurrence of human papilloma virus (HPV) using polymerase-chain reaction (PCR). ''High-risk'' virus types (16, 18, 31, 33, 58) were found in 127 patients (90%); genotypes 16/18 were found in 95% of cases. Follow up was from 6 months to 5 years.

In group 1, complete regression of pre-cancer and non-invasive cervical cancer lesions was achieved in 33 and 10 cases. In Group 2, we only assessed the antiviral efficiency photosensitizers. After clinical recovery, 5 women got pregnant. Anti-viral effect of PDT was registered in 55 out of 65 cases. The longest HPV-free period observed was 5 years.

The obtained PDT results with Russian photosensitizers verifies its great potential and speaks in favour of its further investigation and clinical tests as an alternative method in an organ-sparing treatment, in particular for women planning their labour.

I-10

Applications of photodynamic therapy in the treatment of microbial infections

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Photodynamic therapy (PDT) is coming of age as an efficient alternative treatment for microbial infections, a problem which is presently aggravated by the increasingly widespread diffusion of antibiotic-resistant microbial strains. In particular, the use of red light-absorbing photosensitisers as photodynamic antimicrobial agents is characterized by various favourable features, including: (a) the broad spectrum of antimicrobial action of selected phenothiazines, porphyrins, and phthalocyanines, which promote the photosensitised inactivation of Gram(+) and Gram(−) bacteria,

fungi, mycoplasma, and parasites in both the vegetative and cystic stage by using one phototherapeutic protocol and mild irradiation conditions; (b) porphyrins/phthalocyanines display no appreciable toxicity in the dark at photochemically active doses (i.e. in the micromolar concentration range); (c) a therapeutic window can be identified which allows an extensive (>4 log) decrease in pathogen survival with no detectable damage to the host tissue; (d) microbial cell death is primarily a consequence of membrane photodamage through a typically multi-target process, which minimizes the risk of both the onset of mutagenic processes and the selection of photoresistant microbial strains; (e) such photosensitisers act with essentially identical efficiency against both wild and antibiotic-resistant strains, as shown by studies on both isolated cell cultures and a variety of clinical isolates; (f) the *in vitro*/*in vivo* photoactivation of antimicrobial photosensitisers can be achieved by using low cost non-coherent light sources, possibly couple with a bundle of optical fibers to pilot the light beam into organs such as the vagina or oral cavity; (g) a combination between antibiotic-based and photodynamic therapy is possible. At present, antimicrobial PDT appears to be especially convenient for the treatment of localized infections, such as oral candidosis, acne, periodontitis or chronic wounds. Initial clinical trials appear to yield very promising results.

O-20

Photodynamic treatment of the dermatophyte *Trichophyton rubrum* with a cationic porphyrin

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Background: Dermatophytes are fungi that can cause infections of the skin, hair and nails because of their ability to feed on keratin. Superficial mycoses (tinea) are probably the most prevalent of infectious diseases in all parts of the world. One of the most important restrictions of the current therapeutic options is the return of the infection and the duration of the treatment. Recently we demonstrated, using porphyrin photosensitizers, that in vitro a single photodynamic treatment (PDT) was sufficient to achieve a 100% fungicidal effect, viz. killing of the dermatophyte *Trichophyton rubrum*.
Purpose and method: To evaluate and optimize the photodynamic activity of the cationic porphyrin 5,10,15-tris(4-methylpyridinium)-20-phenyl-[21H,23H]-porphine trichloride (Sylsens B) towards T. rubrum in a situation that mimics the clinical situation of tinea, we investigated the PDT efficacy as a function of fungal growth and pH using an ex vivo model. Visualization of a lethal PDT-effect was performed by means of scanning electron microscopy.
Results and conclusion: It was demonstrated in this model that the susceptibility of T. rubrum to PDT was determined by the time of application in relation to the developing germinating microconidia. The microconidia appeared to be more susceptible than the mycelium. Furthermore, within fungal growth stage a pH effect was determined. A low pH (preferably <5.5) resulted in a better efficacy.

A lethal PDT caused different morphological wall alterations, like loss of the characteristic fibre-like structure and flattening of fungal elements. In case of sub-lethal PDT fungal re-growth was observed at mainly the hyphal tips. A different morphology could be responsible for this.

I-11

Photodynamics (PDD and PDT) for the management of bladder cancer. Development and current status

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The hexyl-derivative of 5-aminolevulinic acid (h-ALA, Hexvix®) has recently gained European approval for bladder cancer (BC) detection by fluorescence imaging. Clinical applicability of the procedure became feasible as soon as the instillation of 5-ALA proved so much superior in inducing tumor selective fluorescence compared to the systemic application of preformed porphyrins investigated before. The simplification of the excitation light source also contributed. Clinical data unambiguously prove significantly increased sensitivities for the intraoperative localisation of especially carcinoma in situ (CIS), a high risk lesion. Most studies also showed reduced rates of residual tumor, tumor recurrence and even a modification of treatment strategy.

PDT of bladder cancer potentially represents a very attractive additional treatment option for this most expensive oncological disease. Early clinical studies used 5-ALA via topical or systemic administration and dye-laser irradiation. Complete response rates of around 50% could be achieved. The most recent study used instillation of h-ALA and irradiation with a flexible catheter and a white light source. PDT was performed in 3 sessions, with 6 weeks intervals. The use of white light not only aims at simplification and cost reduction, but also at the exploitation of the phototoxic potential of any photoproducts induced. Light dose calculation aimed at a complete bleaching of the porphyrins throughout the whole bladder. This could be achieved in all cases. Most prominent side effects were postoperative urgency and bladder pain. Preliminary evaluation shows a complete response assessed 3 months after the third PDT-session in 8 of 10 patients after the first (6 patients), second (1 patient) or third (1 patient) session.
Conclusions: PDD of BC is an established procedure and now is awaiting approval in US.

White-light PDT with the special flexible catheter system proved technically feasible and safe. Future work is directed towards reducing postoperative symptoms and towards avoiding general anaesthesia.

O-21

TOOKAD-mediated vascular-targeted photodynamic therapy (VTP) for the treatment of prostate cancer—Preclinical study

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Objective: The VTP treatment modality using TOOKAD (Padoporfin; WST09) was investigated as an alternative approach for treating organ-confined prostate cancer.
Materials and methods: Under laparotomy, an interstitial, superficial or transurethral VTP of the canine prostate (normal or pre-irradiated) was performed by using a diode laser (763 nm) during or shortly after the IV injection of TOOKAD. The sensitivity of the adjacent tissues was investigated by directly irradiating the surface of the bladder, colon, abdominal muscle and pelvic plexus. The prostate and the adjacent tissues were harvested 1 week post-VTP and subjected to histopathologic examination. The effects of drug dose, light dose, light fluence rate and drug-light interval were studied for one and two VTP sessions. Pharmacokinetics were studied by HPLC assay. The feasibility of using perfusing CT or MRI scans for assessing the VTP-induced tissue lesions was evaluated.
Results: As true vascular-targeting photosensitizer, TOOKAD cleared rapidly from the circulation. The TOOKAD-VTP-induced acute

lesions in the various prostate models were characterized by marked hemorrhagic necrosis. The lesion size correlated well with the drug and light doses. Similar responses were also observed in canine prostates with spontaneous cancer. The adjacent tissues appeared to be sensitive to the TOOKAD-VTP, although the VTP-induced responses in these tissues were minimal, as compared to that observed in the prostate at the same dose level.
Conclusions: The TOOKAD-VTP performed in several canine models demonstrates it is safe and effective in ablating the prostate *via* the vascular effects.

Acknowledgment

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O-22

Preliminary results from a clinical study of mTHPC-mediated photodynamic therapy of prostate cancer with real-time light dosimetry

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We report on initial clinical experiences from the SpectraCure interstitial PDT system for light delivery and online dosimetry. A multicentre study for mTHPC-mediated PDT of low-risk prostate cancer is ongoing. Patient inclusion criteria are stage T1c, Gleason <7, PSA <10 ng/ml, and a prostate volume <40 ml. The aim of the treatment is to ablate the entire gland by delivering a dose of >5 J/cm^2 to all parts. Patients are injected with 0.15 mg/kg mTHPC 4 days prior to treatment. Pre-dose planning and fibre placement is performed by transrectal ultrasound guidance. Optical fibres are placed in the prostate through 18 needles, each fibre delivering a constant power of 150 mW at 652 nm. Real-time dosimetry adjusts the irradiation time of each individual fibre.

Results from the first patients show that the software calculates accurate dose plans for the individual patients and adapts the dose plans to the optical properties of the prostate as well as changes during treatment. The dose plans successfully discriminate between prostate and organs at risk. Treatment times are in the order of 10—30 min. MRI 2 weeks post-treatment reveals changes that correlate with calculated dose maps. No differentiation from baseline MRI is seen in tissues outside the prostate. Patients were catheter-free 2—3 days post-treatment and experienced mild urgency for up to a few weeks. No PDT-related adverse effects have been reported. PSA for the first patient (9 ng/ml pre-PDT) was down to 3.7 ng/ml 8 weeks post-PDT.

I-12

Contribution of photodiagnosis (autofluorescence bronchoscopy/AFB) and photodynamic therapy to the treatment of central type lung cancer

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Objective: To present the Yorkshire Laser Centre/UK Medical Laser Centre (YLC) experience and review the literature concerned with detection and treatment of central type lung cancer (endobronchial tumours) and to discuss the contribution that these two methods have made in lung cancer treatment.

Introduction: The standard treatment for lung cancer for the past 40—50 years has been surgery, radiotherapy and chemotherapy with surgery being the treatment of choice which, in early cases, achieves about 70% 5 years survival. However, 10—15% of patients are eligible for surgery. Some of the many inoperable cases are ineligible for operation on account of general condition. For the past 25 years bronchoscopic PDT has been used for some of these inoperable patients in 2 groups; advanced (group A) and/or early stage disease (group E). In the latter, AFB has been invaluable in diagnosing the extent of the disease, its multi-focality and occult cancer.
Method: We reviewed the published literature concerned with PDT in central type lung cancer including 200 patients of the YLC treated with bronchoscopic PDT. We also reviewed the YLC experience in AFB as well as the literature in order to evaluate the contribution of AFB.
Results: PubMed shows nearly 350 articles published on the subject of which only 30 accorded with our criteria to be included in the review. There is generally no procedure-related mortality related to bronchoscopic PDT. The most important adverse event is photosensitivity (5—40%). In group A, symptomatic relief is achieved and is objectively paralleled with X-ray clearance and improved pulmonary ventilation. In group E cases, 5 years survival of over 70% or more is achieved. AFB has added a new dimension in the diagnosis and sampling of early stage cancers including up to 20% multi-focal lesions for the majority of whom, surgery is not applicable.
Conclusion: AFB and PDT are making important contributions to lung cancer treatment; in advanced cases it achieves palliation of symptoms and survival benefit in some. In early cases, complete response and long term survival ($\geq$70%) is obtained. PDT is now an approved treatment in Japan for early lung cancer and in Britain the National Institute of Clinical Excellence (NICE) has given a guideline for its use. We urgently need a Phase III trial of PDT in early lung cancer. There are, however, financial and logistical difficulties to do so.

I-13

Potentials of photodynamic application in neurosurgery

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Malignant brain tumors are carrying a lethal prognosis despite all available treatment. Malignant gliomas WHO IV and WHO III demonstrate a median survival of 15 and 36 months, respectively. Sine the early 1990 photodynamic therapy has been added to the armentarium of treatment. Whereas the initial reports were promising with long-term survivors, one large controlled trial showed only small not significant benefit for Photofrin-mediated-PDT. ALA-mediated-fluorescence guided resection improved significantly the result of the resection but not the time to progression. The use of second-generation photosensitizers such as Foscan and the combination of PDD and FGR and simultaneous PDT improved the results in recurrent glioblastomas.

The proof of principle has been well demonstrated for PDD and PDT. Due to the infiltrating nature of the brain tumors the efficacy of PDD/PDT has to be further improved by using the knowledge, which has been gained in the laboratories. The combination of PDT with chemotherapeutic agents targeting special molecular signature of the tumor, or new techniques for delivery the photosensitizers such as PCI, transvection enhanced delivery or new encapsulated formulations of the sensitizers. There are also various attempts to use boron neutron capture strategies, multiple photon activation, metronomic PDT or interstitial fibre implantation to increase the phototoxic inactivation.

This presentation will report upon the clinical results and will high lighten the future potential of photodynamic applications in neurooncology.

O-24

Antimicrobial photodynamic therapy (APDT) in treatment of tuberculosis—The first clinical results

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The poor efficacy of treatment of tuberculosis, especially of multidrug-resistant forms is known. The method of a photodynamic therapy is used for rising efficacy of complex treatment we in flow at 5 years, utilizing following principles: (1) APDT for treatment of a tuberculosis is local and high-dose PDT; (2) the photosensitizer for APDT of a tuberculosis should have a high quantum yield of a ROS-generation; (3) If there is the cavern in lung formed, the transthoractic puncture under X-ray control for delivery of a photosensitizer and light we applied; (4) for infiltration forms of a tuberculosis the injection of a photosensitizer and light delivery throat fiberoptic bronchoscope we applied; (5) procedure is repeated from 3 up to 7 times.

Results of APDT-application are the prompt clearing of a tubercular cavern and drop of fastness of mycobacteria to antibiotics. The transformation of the open forms (MBT+) of tuberculosis to enclosed forms (MBT−) is marked in 74% of cases, as contrasted to 43% in control group of patients, receiving only conventional therapy in flow 6 months of treatment at the drug resistant forms of tuberculosis.

O-25

Autofluorescence bronchoscopy to detect bronchial epithelial changes associated with cigarette smoking amongst asymptomatic volunteers: A single centre prospective pilot study

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The link between cigarette smoking and lung cancer is well documented. The introduction of autofluorescence bronchoscopy (AFB) has allowed visualisation of pre-invasive neoplastic changes of bronchial mucosa. We evaluate the sensitivity of AFB to epithelial changes compared to that of white light bronchoscopy (WLB). Inclusion criteria for this study demanded heavy smokers (>20 pack years) at least 40 years old, asymptomatic, with clear chest X-ray and no history of cancer. Candidates completed a questionnaire and were interviewed to record medical history and smoking habit. Bronchoscopy was carried out under topical or general anaesthesia using a standard flexible fibre-optic bronchoscope for WLB and the Xillix LIFE Lung® system for AFB. The study included 93 subjects (57 male, 36 female) aged 40—75 years. AFB indicated positive images in the bronchial tree of 51 subjects: biopsies showed epithelial abnormalities in 27 (15 metaplasia, 12 inflammatory changes). WLB showed abnormality in 1 subject but with no pathological changes revealed by cyto-histology. Therefore, the sensitivity of AFB to metaplasia was 75% compared to zero for WLB. AFB yields positive predictive values for metaplastic and overall mucosal changes of 29.4% and 52.9%, respectively. Over 16% of asymptomatic smokers had metaplastic changes in their bronchial mucosa. AFB proved more sensitive in revealing early pre-neoplastic changes than WLB. Such individuals should be followed using AFB coupled with molecular genetic studies in order to better understand the significance of epithelial metaplasia in the context of smoking and to elucidate markers of early neoplastic changes.

I-14

PDT-generated cancer vaccines

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An important development in the exploitation of immune-activating potential of PDT is the invention of effective cancer vaccines generated by *in vitro* PDT treatment of tumor cells. Unlike standard clinical PDT where tumors *in situ* are exposed to light, in the vaccine protocols tumors are not directly PDT treated but the host receives a vaccine consisting of *in vitro* PDT-treated tumor cells or their lysate. The first report was by Drs. Gollnick and Henderson, who showed that lysates of Photofrin—PDT-treated mouse tumor cells can be exploited as a prophylactic vaccine because their injection protects mice against challenge with the same tumor. This effect was shown to be based on tumor-specific immune response (ineffective against mismatched tumor types) involving the induction of cytotoxic T cell response and associated with functional maturation of dendritic cells. We have established therapeutic autologous whole-cell cancer vaccines based on PDT-treated tumor cells or *ex vivo* tumor tissue, which reveals the prospects of using patient's own tumor for preparing vaccines tailored for individual patients targeting the person's malignancy-specific antigens. There are clear advantages of such whole-cell/polypeptide vaccination over targeting specific epitopes. Polyvalent vaccines secure greater coverage of potential/diverse tumor antigens (even if most of them are unknown), including the necessary determinants for helper T-cells, and are thus less likely to encounter ''tumor escape'' by downregulation of antigen expression. Autologous whole-cell vaccines are optimally conditioned to express antigens in a patient-specific manner and provide patient-matched MHC through which tumor peptides can be recognized. In comparison to other polyvalent vaccination strategies like whole cell RNA-mediated transfection, the advantage of PDT vaccine is in offering photooxidative changes-modified antigenic fingerprint characterized by greater immunogenicity and presenting it in the context of complex immunostimulating environment including unique molecular changes unfolding in cells dying from PDT-mediated oxidative stress.

O-26

The influence of photodynamic therapy (PDT) with delta-aminolevulinic acid (ALA) on J-774A.1 macrophage cell line

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Introduction: Despite direct necrotic and apoptotic ways of cell death after PDT, there is a variety of additional events leading to and magnifying the inactivation of tumor cells, connected with infiltrating immune cells, which release free radical species, and cytokines, leading to additional eradication of tumor cells.
The aim: Therefore, the role of the host immune system in contribution to tumor regression following ALA photodynamic therapy (PDT) was examined.
Material and methods: J-774A.1 cells were incubated with ALA at different concentrations and then irradiated with VIS (400—750 nm) at the dose of 5, 10 and 30 J/cm^2 delivered from the incoherent light source PDT TP-1. The effects of the application of ALA-PDT (using different energy doses) were evaluated on the basis of cell viability, lipopolysaccharide (LPS)-induced nitric oxide (NO), chemilumines-

cence, tumor necrosis factor-alfa (TNF-alfa) and interleukin-1 beta (IL-1beta) production by the J-774A.1 cells.
Results: The cell viability was comparable with control group at 5, 10 and 30 J/cm^2. At these doses of energy using different concentrations of ALA we have observed that the higher were the energy doses, lowering of the level of IL-1 beta production and decrease of NO release. At 10 J/cm^2 energy density increase of TNF-alfa release and free radical species release (chemiluminescence) was observed.
Conclusion: As PDT not only reduces tumor burden but also induces inflammation, it is proposed that recruitment of the activated macrophages to the inflammatory tumor lesions is the major factor for the complete eradication of tumors.

O-27

Metabolic profile of a peptide-conjugated chlorin-type photosensitizer targeting neuropilin-1: An *in vivo* and *in vitro* study

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Since angiogenic endothelial cells of the tumor vasculature represent an interesting target to potentiate the vascular effect of photodynamic therapy, we recently described the conjugation of a photosensitizer (5-(4-carboxyphenyl)-10,15,20-triphenylchlorin, TPC), *via* a spacer (6-aminohexanoic acid, Ahx), to a VEGF-receptor-specific heptapeptide (ATWLPPR), and demonstrated that TPC-Ahx-ATWLPPR binds to neuropilin-1. TPC-Ahx-ATWLPPR was stable *in vitro* in human and mouse plasma for at least 24 h at 37 °C but, following intravenous injection in glioma-bearing nude mice, was degraded *in vivo* to various rates, depending on the organ considered. TPC-Ahx-A was identified as the main metabolic product and pharmacokinetic studies suggested that its appearance in plasma mainly resulted from the degradation of the peptidic moiety into organs of the reticuloendothelial system. According to *in vitro* cell culture experiments, TPC-Ahx-ATWLPPR was also significantly degraded after incorporation in human umbilical vein endothelial cells. As we demonstrated that TPC-Ahx-ATWLPPR mostly localized into lysosomes, HUVEC were treated with the lysosomal enzymes inhibitor ammonium chloride, which resulted in a significant decrease of the peptide degradation. But, taken together, our results suggest that the low levels of degradation observed in plasma, tumor and skin, are not likely to have a negative impact on our tumor-targeting photosensitizer strategy.

O-28

Cell death mechanisms of glioblastoma cells during Hypericin PDT

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Hypericin has gained increasing interest as a potential PDT drug. Several studies have shown that Hypericin efficiently kills glioblastoma cells upon light irradiation. However, the mechanisms of cell death as well as intracellular localization of the drug are still a matter of debate. To clarify further the cellular response during Hypericin PDT U373MG glioblastoma cells were incubated for 1 h with different concentrations of Hypericin. The intracellular localization was proved by coincubating with organelle-specific markers for mitochondria, ER and lysosomes. In addition, the intracellular fluorescence decay of Hypericin was measured by FLIM. Cell death mechanisms during PDT were investigated by flow cytometry as well as intracellular detection of non-specific caspases due to D2R cleavage.

Mainly early apoptotic cell death was observed after irradiation between 1 and 10 J/cm^2 whereas higher light doses induced late apoptosis and necrosis. Very low light doses (below 1 J/cm^2) induced stimulation of cell growth. Localization of Hypericin was observed in the ER but not in lysosomes. Besides there was also found a strong localization in mitochondria. Low dose PDT-induced intracellular Ca^{2+} oscillations, probably by Ca^{2+} shuttling between ER and mitochondria, higher doses a sustained Ca^{2+} overload. To measure Ca^{2+} signals specifically in the mitochondria, cells were stably transfected with calmodulin tagged to YFP subcloned into a mitochondria-specific expression vector. Ca^{2+} overload was observed in the mitochondria after application of high light doses, a process which is known to originate cell death. Mitochondria seem to be the primary target in Hypericin PDT of glioblastoma cells.

O-29

Effects of hexyl 5-aminolevulinate and light in rat bladder cancer cells

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Introduction: ALA-based PDT combines the selective accumulation of the photosensitizer protoporphyrin IX (PpIX) in tumour tissue with visible light to produce reactive oxygen species (ROS). The relatively new ester derivate, hexyl 5-aminolevulinate (HAL), was used to stimulate intracellular photosensitizer formation in rat bladder cancer cells (AY-27).
Materials and methods: The effects of ALA-based PDT were investigated in AY-27 cells, and levels of PpIX and other porphyrins were determined fluorometrically using HPLC. The cells were treated with HAL (0—500 μM, 3.5 h) in absence of serum-free medium and irradiated with blue light (0—12 kJ/m^2) using a Actilite Lamp (Model CL-128, PhotoCure ASA). Cellular damage and viability were determined using fluorescence microscopy and standard MTT assay (response on mitochondrial dehydrogenase activity).
Results and conclusions: The accumulation of PpIX reached a maximum and stable level at 25 μM HAL after incubation (3.5 h), and the viability was reduced to about 50% after 1.5 min of illumination, and to 5% after a total illumination period of 6 min. The cell survival was not reduced in cells exposed to HAL or light alone. The sensitive reverse phase HPLC technique detected five different porphyrins in extracts from AY-27 cells after stimulations with HAL. The photoreactions of PpIX with light in AY-27 cells properly generate free radical hydroxyl and singlet oxygen and might be a combination of a type-I and type-II reaction.

O-30

Gene expression pattern of human carcinoma cells A-431 after PDT with hypericin

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Hypericin is a very powerful naturally occurring photosensitizer and is found in *Hypericum perforatum*, commonly known as St. John's work. In a concentration and light dose-dependent manner it is able to induce both apoptosis and necrosis. To analyze the fundamental molecular mechanisms leading to this photocytotoxicity in cancer cells, we studied the alteration of the gene expression pattern in the human squamous cell carcinoma cell line A-431 at different time points after photodynamic treatment with

hypericin by cDNA-array technique. Cells were incubated for 16 h with 200 ng/ml hypericin and irradiated with a fluence of 0.6 J/cm^2 resulting in 50% survival until 8 h post-treatment and an apoptosis rate of 80% compared to an UV-induced (200 mJ/cm^2) control. RNA was isolated at 1.5, 3, 5 and 8 h post-treatment as well as of three controls (untreated, light only and dark), radioactively labelled by reverse transcription with ^{33}P-dCTP and hybridized onto macroarray filters containing PCR products of 9738 genes of the Incyte Human UniGEM Microarray clone set. Verification of observed expression changes was carried out by quantitative real-time PCR. We found significant changes in expression levels of genes involved in apoptosis induction, oxidative stress response, proliferation, MAPK and RAS signalling pathways, energy metabolism and cell adhesion.

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Posters

P-1

PDT for extramammary Paget's disease

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Extramammary Paget's disease is rare with only a few hundred cases having been reported in the world literature. It is an in situ adenocarcinoma, but may be associated with invasive internal malignancy, or develop areas of invasive adenocarcinoma within the area of the Paget's disease. Although surgery is considered the standard treatment, there is a high recurrence rate of 40—50%. This is because the lesion frequently spreads widely and is often multifocal.

Others have reported using PDT post-surgery in an attempt to reduce the recurrence rate. In contrast, in November 2005, we published a single case report detailing the successful treatment of a patient with extramammary Paget's disease by means of a combination of topical and systemic PDT alone. Extensive surgery was thereby avoided. We will provide an update on this patient and his long-term response to treatment.

We will also present case studies on the treatment by PDT, as an alternative to major surgery, of a further four patients suffering from extramammary Paget's disease. Two patients had extensive disease of the groin and scrotal skin, one patient had disease of the perianal skin and lower anal canal, and one patient had widespread disease of the vulva.

All of our patients have had excellent short-term resolution of disease following PDT, suggesting that the approach has some promise. However, the follow-up times for three of our patients are still rather short, so it is too early at present to recommend PDT as a definitive treatment for all cases of extramammary Paget's disease.

P-2

Efficacy of topical ALA-PDT in the treatment of acne vulgaris

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Objectives: To determine the safety and efficacy of topical 5-aminolevulinic acid-photodynamic therapy (ALA-PDT) in the treatment of acne vulgaris.
Materials and methods: Forty-two patients with acne from 18 to 39 years old (16 men and 26 women) with different form of acne were enrolled in this study. PDT with the use of endogenous porphyrins — 20 (47.6%) patients and PDT with the use of exogenous protoporphyrin IX from 5-ALA — Alasens (FSUE ''SSC'' ''NIOPIK'') — 22 (52.4%) patients. In last case Alasens was used in the form of solution or cream with 10—20% concentration of 5-ALA as external applications under the occlusive bandage for 1.5—2 h. To determine the concentration of porphyrins and to control PDT the laser electro-spectral analyzer (LESA-01-BIOSPEC) was used. Irradiation was carried out with photodiodic videofluorescentic equipment UFF-630-01-BIOSPEC, shining non-stop light with the wavelength of 630 nm and energy density of 36 J/sm^2. The irradiation sessions were held once a week for the course of 4—10 sessions.
Results: In the process of PDT clinical remission was achieved in 34 (81%) patients and the rest are under experienced improvement. The following temporary side effects were noted, which independently subsided in 3—5 days: burning, itching, swollen, erythema.

Conclusions: Therefore, the effectiveness of PDT acne directly depended on the level of endogenous porphyrins—the higher the level, the better clinical results. In order to strengthen the photodynamic reaction Alasens may be used, which turned out to be highly effective in stabilization of process and allowed to achieve clinical remission with 81% patients.

P-3

Efficacy of photodynamic therapy of human papilloma virus associated diseases of female genital organs

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Aim: The aim of this study was to estimate the clinical efficacy and safety of 5-ALA-based medicinal formulation Alasens in PDT of HPV associated diseases of vulva and cervix. The kinetics of 5-ALA-induced protoporphyrin (PPIX) accumulation in above tissue after oral application of Alasens was studied by in vivo local fluorescence spectroscopy.
Materials and methods: The group of 34 patients included 21 women with pointed condyloma (62%), 6 women with pathology of cervix uterus (18%) and 7 women with both types of pathologies (20%). The PDT sessions were carried out 3—6 h after oral administration of Alasens (FSUE ''SSC'' NIOPIK) at the dose of 25 mg/kg bw. The energy density laser's light varied from 30 to 150 J/cm^2.
Results: The fluorescence contrasts between pathological foci and healthy vulva were detectable as soon as 1 h after Alasens administration and reached the maximum value 4 h after According the data of PCR diagnosis performed 3 months after PDT the complete antiviral effect was confirmed.

P-4

Cells resistant to ALA-photodynamic therapy: Decreased metastasic phenotype and cytoskeleton changes

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Photodynamic therapy (PDT) is a novel cancer treatment modality utilizing a photosensitizer, visible light and oxygen. PDT often leaves a significant number of surviving cells. Previously, we isolated and studied two PDT resistant clones from the murine mammary adenocarcinoma LM3 line. The isolated Clone 4 and Clone 8 exhibited a more fibroblastic, dendritic pattern and were larger than the parentals.

Here, we studied the metastasic potential of the two clones compared with LM3. We studied in vitro the adhesive and invasive characteristics, together with the proteolytic enzymes profile and changes in the expression of cytoskeletal and adhesion proteins. In vivo, we assayed the ability of resistant clones to induce spontaneous metastasis.

We found that 100% of LM3 were able to invade Matrigel, whereas Clones 4 and 8 only invaded. 100% of LM3 cells migrated towards a chemotactic stimuli whereas $38 \pm 8\%$ and $73 \pm 10\%$ of Clones 4 and 8 migrated. In vivo, 100% of LM3 injected mice developed spontaneous lung metastasis, whereas none of Clone 8 did, and only one of the injected mice with Clone 4. No differences were found in the proteolytic enzyme profiles among the cells. Anchorage-dependent adhesion was also impaired in vivo in the resistant clones, evidenced by lower tumor take, latency time and growth rates, although both clones showed higher binding to collagen I without overexpression of β1 integrin. Changes of the metastasis phenotypes are due to differences in expression of F-actin in both clones, and E-cadherin, β-catenin and vinculin specially in Clone 8.

P-5

Uptake and distribution of hypericin doped silica nanoparticles in human cancer cells for future therapy

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Introduction: The specific delivery of photodynamic agents to their desired targets with minimal systemic side effects is an important challenge in cancer therapy. With the aim to improve cancer treatment options, we here investigate the potential of combining a photosensitizer with nanotechnology.
Materials and methods: Silica-based nanoparticles had been synthesized using a water-in-oil microemulsion, and the photosensitizer hypericin was embedded in the silica shell. The human head and neck squamous carcinoma cell line UMB-SCC-745 was cultured under standard conditions and exposed to hypericin-labelled nanoparticles (5 μg/ml) between 30′ and 5 h. Thereafter, cells were fixed and processed for transmission electron microscopy (TEM). In plus, fixed cells were studied by immunohistochemistry using antibodies against the endosomal markers EEA1 and Rab7 (Abcam, Cambridge, UK) as well as the lysosomal marker Lysotracker (Invitrogen, Eugene, OR).
Results: Single nanoparticles were already detectable intracellularly after 30′ by TEM. However, after 5 h, a considerable accumulation of nanoparticles was observed in the cytoplasm. Over time, different stages of endocytotic processes were apparent, leading mainly to the formation of endosomes with multiple nanoparticles. With immunohistochemical methods we found that after 1 h labelled nanoparticles colocalised with early and late endosomes and with lysosomes.
Conclusion and outlook: Our first experiments with hypericin-labelled silica nanoparticles showed promising results. Due to the fast uptake, good biotolerance and flexibility these nanoparticles open a broad spectrum of applications in cancer treatment. Our next steps will include further experiments with cell lines, spheroids, tumour tissue and *in ovo* experiments.

P-6

Photodynamic therapy with intratumoral administration of lipid-based mTHPC in a model of breast cancer recurrence

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We have evaluated the use of an intratumoral injection of a liposomal formulation of mTHPC (Foslip®), followed by a red laser light irradiation ($\lambda = 652$ nm) at different times after injection, in an animal model of local recurrence of breast cancer. The photosensitizer fluorescence assessed by macrofluorescence in excised whole tumors (5 mm) was inhomogeneous and fluorescence intensity increased with time after injection. Model studies *in vitro* using polarization technique were employed to investigate the transfer of the dye to liposomes, acting as a model for cellular membranes. Maximal mTHPC fluorescence polarization was obtained at 24 h incubation in an excess of non-loaded liposomes, thus suggesting that at this time point mTHPC has migrated from its lipid-based formulation to non-loaded liposomes. This slow rate of mTHPC transfer is consistent with the progressive increase in intratumoral fluorescence intensity. It also correlates with the better PDT efficacy observed at 24 h after Foslip injection. Minimal damage was observed at skin level. Plasma levels of mTHPC decrease after 15 h so repeated PDT sessions might be favourable in terms of side effects and tumor response.

P-7

Delta-ALA-mediated fluorescence spectroscopy of gastrointestinal neoplasia

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Newly detected colorectal tumors are on the third place, stomach neoplasia is on fifth place in the list of newly developed cancer cases in Bulgaria. The situation in the other European countries is very similar—gastrointestinal neoplasia are on leading places in cancer incidence statistics.

Delta-ALA/PpIX is used as fluorescent marker for gastrointestinal dysplasia and tumor detection. ALA is administered per os six to eight (depending on the lesion location) hours before measurements at dose 20 mg/kg weight. High-power light-emitting diode at 405 nm is used as an excitation source. Special opto-mechanical device is built for the LED to use the light guide of standard video-endoscopic system. Through endoscopic instrumental channel a fiber is applied to return information about fluorescence to microspectrometer.

The fluorescence detected from tumor sites has complex spectral origins. It consists of autofluorescence, exogenous fluorophores fluorescence and re-absorption of chromophores accumulated in the tissue investigated. Features observed could be distinct as: 450—630 nm region, where tissue autofluorescence is observed; 630—710 nm region, where fluorescence of PpIX is clearly pronounced; 530—580 nm region, where minima in the autofluorescence signal are observed, related to re-absorption of oxy-hemoglobin in this spectral area.

The lack of fluorescence peaks in the red spectral area for normal mucosa is an indication for highly selective accumulation of 5-ALA/PpIX only in abnormal sites and gives high contrast when lesion are determined from clinicians during video observation in the diagnostic process. Very good correlation between fluorescence signals and histology examination of the lesions investigated is achieved.

P-8

Photodynamic medicine—results, activities and perspectives in Bulgaria

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Introducing of PD and PDT of tumors is an integral part of contemporary medicine. New high power and wide spectral range LEDs technologies allow developing of inexpensive convenient systems for cancer detection and treatment.

Main aim of the current presentation is to demonstrate the collaboration between physicists and physicians in Bulgaria in the field of development of equipment and methodologies for photodiagnosis and photodynamic therapy. A major aspect of these activities is the improvement of oncological diseases treatment effectiveness.

Specialized systems for fluorescent diagnosis and photodynamic therapy based on light-emitting diodes are developed. LEDs matrices emitting at 405 nm maximum are using for fluorescence visualization and detection of exogenous sensitizers' accumulation in tumor area. For therapeutic goals LEDs matrix (max at 635 nm, power density: 60 mW/cm^2) is developed. Delta-aminolevulinic acid is applied for PD and PDT.

These methods are introduced in Bulgarian medical centers for early detection and treatment of skin and mucosa neoplasia for non-melanin pigmented skin tumors and esophageal, stomach and colon neoplasia, respectively.

After fluorescent diagnosis of skin and mucosa tumors, photodynamic therapy procedures using red light irradiation are carried out. In the field of dermatology we already have short and long-term observations that prove high applicability of Delta-ALA-mediated PDT for non-melanoma cancer treatment.

As a part of our research activities in common collaboration with chemists, newly synthesized long-wavelength photosensitizers' properties and their applicability for PD/PDT, photodynamic inactivation of pathogenic microorganisms, as well as fluorescent markers for protein investigations are also investigated.

P-9

Photodynamic therapy with Photosens for classic subfoveal choroidal neovascularization

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Purpose: To examine the 36-month results for patients with choroidal neovascularization (CNV) who were treated with photodynamic therapy (PDT) with Photosens.

Material and methods: Eighteen patients with classic subfoveal CNV secondary to age-related macular degeneration (AMD) and 24 patients with CNV secondary to pathological myopia (PM) occurred at 12-month intervals were observed. Standardized protocol refraction, visual acuity testing, ophthalmologic examinations, color photographs, fluorescein angiography were used to evaluate the

results of photodynamic therapy with Photosens (produced by FSUE ''SSC ''NIOPIK'', Moscow—0.02% solution of mixture sulfonated aluminium phtalocyanine 0.05 mg/kg, intravenously). A diode laser (''Biospec'' Inc., Moscow) was used operating in the range of 675 nm. Need for retreatment was based on fluorescein angiographic evidence of leakage at 3-month follow-up intervals.
Results: At 3, 6 and 9 months 24 (42.9%) patients had significant improvement in the mean visual acuity. At the end of the 12-month minimal fluorescein leakage from choroidal neovascularization was seen in 11 (26.2%) patients and the mean visual acuity was slightly worse than 0.2 which was not statistically significant as compared with the baseline visual acuity. Patients with fluorescein leakage from CNV underwent repeated PDT with Photosens. At 36 months leakage decreased angiographically in 16 eyes (53.3%) and remained stable in 6 eyes.
Conclusions: The results of this study substantially confirm the safety of PDT for CNV secondary to AMD and PM. Photodynamic therapy with Photosens reduced CNV leakage and moderate visual loss did not develop during follow-up in any cases.

P-10

The effectiveness of photodynamic therapy in the treatment of exudative age-related macular degeneration

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Purpose: To establish the effectiveness of the photodynamic therapy (PDT) using modern methods of ultrasound diagnostics.
Method: The total number of patients was 53 (64 eyes). All the patients were treated with two sessions of PDT. We compared changes in the central retina area using modern methods of ultrasound diagnostics with the help of system ''VOLUSON 730 Pro'' (''Kretz'') linear probe SP 10—16 Hz before and after the treatment. To establish the changes in the central retinal area we used coefficient of ultrasound density *K2D*. The coefficient *K2D* we calculated according the relation: $K2D = (A^1 + A^2)/2/A^3$, where A^1 and A^2 were ultrasound density in the pathological area, A^3 was scleral ultrasound density.
Results: We determined ultrasound density in the pathological area in several points and compared the results. Scleral ultrasound density we determined as mean value after several investigations. If coefficient of ultrasound density *K2D* was more than 1.0 we predict sever central scar formation. If coefficient of ultrasound density *K2D* was less than 1.0 we predict formation of a flat scar in the central retinal area.
Conclusion: Modern methods of ultrasound diagnostics give the opportunity to predict the effectiveness of the photodynamic therapy.

P-11

Immunologic and ultrasound criteria in exudative form of age-related macular degeneration

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Purpose: To establish the role of immunologic and ultrasound criteria in the prognosis of choroidal revascularization development.
Method: The total number of patients was 53 (64 eyes). All the patients were treated with two sessions of photodynamic therapy. We compared changes in the central retina using ultrasound system ''VOLUSON 730 Pro'' (''Kretz'') and volume probe 10—16 Hz. We compared volume index of ultrasound density MG (mean grey), vascularization index (VI) and flow index (FI) before and after the treatment. Also we determined the level of transforming growth factor (TGF-β2) in blood serum.
Results: We determined ultrasound indices using 3D virtual model of the pathological area. If the level of TGF-β2 was equal or more than 1218, MG index was equal or more than 30.0, VI was equal or more than 2% and FI was equal or more than 22.0 we predict sever central scar formation. If the level of TGF-β2 was equal or less than 1218, MG index was equal or less than 30.0, VI was equal or less than 2% and FI was equal or less than 22.0 we predict formation of a flat scar in the central retinal area.
Conclusion: Immunologic and ultrasound criteria give objective information about choroidal revascularization development.

P-12

In vivo local fluorescence spectroscopy under multiple laser excitation for early stage cancer detection

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Objective: The paper presents the clinical experience of in vivo laser-induced fluorescence spectroscopy (LIFS) application for detection of early stage cancer.
Materials and methods: In vivo LIFS has been applied in 449 patients. The tumor localizations have included intraepithelial lesions of bronchus and larynx, esophageal and stomach cancer, superficial bladder cancer, as well as superficial basal cell carcinoma of skin. LIFS under multiple laser excitations have been investigated both for autofluorescence diagnosis and for fluorescence diagnosis of cancer with domestically produced photosensitizers from porphyrins, phthalocyanines and chlorine groups (Photogem, Alasens, Photosens, Radachlorin).
Results: The clinical investigations have proved that in vivo LIFS during fluorescence visualization allowed to minimize a number of false-positive fluorescence and to reduce a number of biopsy specimens which were commonly used.
Conclusion: A combination of AF and PDD imaging with in vivo LIFS improved quality and reliability of the diagnostic examinations and had a high potential for revealing occult pre-malignant lesions, intraepithelial and micro invasive primary cancers of various localizations, residual tumor or early recurrences after a previous treatment, as well as occult lesions of synchronous and metachronous primary-multiple cancer.

P-13

Development of bacteriochlorophyll *a*/cyclodextrins supramolecular systems of PDT applications

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The encouraging results of recent studies [1,2] on the photochemical and photophysical properties of the supramolecular systems, chlorophyll *a*/cyclodextrins (CDs), in vitro and in vivo have prompted us to extend the study even to an other photosynthetic pigment, the bacterichlorophyll *a* (BChl *a*), as potential supramolecular photosensitizers in PDT applications.

This pigment has a good solubility in organic solvents having a poor biocompatibility and is almost completely insoluble in water where has a high tendency to aggregate reducing its availability and effectiveness as photosensitizers. In order to overcome these limitations we have coupled the BChl a with CDs, developed a solubilization procedure and studied the behaviour in aqueous solution.

The CDs used are 2-hydroxypropyl-α-CD, 2-hydroxypropyl-β-CD, 2-hydroxypropyl-γ-CD, heptakis (2,6-di-*O*-methyl)-β-CD, heptakis (2,3,6-tri-*O*-methyl)-β-CD. The interaction BChl *a*/CDs has been studied by means of different spectroscopic technique performed at room temperature and at 5 °C in presence and in absence of endogenous oxygen.

P-14

Lethal photosensitisation of *Staphylococcus aureus* using a tin chlorin e6-gold nanoparticle

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The growing resistance of bacteria to conventional antimicrobial agents necessitates the development of alternative approaches to preventing and treating infections. One such approach is the use of light-activated antimicrobial agents (LAAAs) such as tin chlorin e6 (SnCe6). Upon exposure to light of a suitable wavelength these reagents produce free radicals and reactive oxygen species that cause non-specific damage to the bacterial cell which leads to death. Gold nanoparticles have unique physical and chemical properties that make them attractive as molecular scaffolds and which we have hypothesised may potentiate the effectiveness of LAAAs. Here we show that covalently coupling SnCe6 to a gold nanoparticle through a glutathione linker forms a potent antimicrobial agent when exposed to white light or light from a helium–neon laser. This novel LAAA had potent antimicrobial activity towards a range of Gram-positive bacteria, including both methicillin-resistant and methicillin-sensitive strains of *Staphylococcus aureus* and *Streptococcus pyogenes*.

P-15

Targeted photodynamic therapy with recombinant antibody fragments multiply loaded with photo-sensitisers

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Introduction: Current photodynamic therapy (PDT) of cancer is limited by inefficiencies involved in specifically targeting photosensitisers to tumours. Although antibodies are being explored as targeting vehicles, they present significant challenges, particularly in terms of pharmacokinetics and drug-coupling.
Materials and methods: We describe a novel, effective system to covalently attach multiple photosensitiser molecules (pre-clinical pyropheophorbide-a, and clinically approved verteporfin photosensitisers) to single-chain antibody fragments (scFvs).
Results: Not only do the resulting photoimmunoconjugates (PICs) retain photophysical functionality, they are more potent than free photosensitisers, effectively killing tumour cells in vitro and in vivo. Further, treatment of human breast cancer xenografts with a PIC comprising an anti Her-2 scFv linked to eight molecules of pyropheophorbide-a, leads to complete tumour regression.
Conclusions: These results give important insights into the features that make scFvs good carriers for PDT drugs; and provide proof of concept of our unique approach to targeted photodynamic therapy (tPDT). This promises significant improvements over current photodynamic therapy for the treatment of cancer.

P-16

Update on the current indications and results of photodynamic therapy (PDT) for lung cancer treatment

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Objectives: This presentation aims to highlight the current indications and results of photodynamic therapy (PDT) in lung cancer based on an updated review of literature and Yorkshire Laser Centre (YLC) experience.
Method: Review of the publications cited by Pub-Med with 10 patients or more on PDT in lung cancer. This included 200 patients treated at YLC.
Results: Twenty-eight articles (1288 patients) were identified relating to PDT for endobronchial tumours. Some 20 patients with peripheral lung cancer received PDT. Two groups were identified: 636 had advanced inoperable disease (A group) and 652 had early stage (E group) disease. Recent experience had lead to the formulation of the following: Group A indications are; extensive exophytic endobronchial, obstructive tumour in a symptomatic and inoperable patient. The aim is palliation by clearance of local endobronchial tumour. Only patients with good performance status and those without extra thoracic metastases are expected to have survival benefit. Group E indications are tumour confined to bronchial mucosa with no lymphadenopathy or metastases. The aim is cure and long-term disease free survival. Aims in both groups of patients are achievable.
Conclusion: PDT is making important contribution to lung cancer treatment. In order to have optimal effect the indications have to be carefully adhered to. The future programme of research should concentrate on early detection of cancer at the intraepithelial stage (photodetection) which indicates pre-invasive lesion which are most suited to PDT with a curative intent.

P-17

Intraoperative optical identification of pituitary adenomas

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Introduction: Identification of pituitary adenomas, particularly secretary microadenomas, is of paramount importance in the successful eradication of the adenoma and preserving pituitary functions. Several methods have been employed over the years with variable success, leading some surgeons to perform total hypophysectomy in many patients. We have examined an optical biopsy system that can be used intraoperatively to localise the adenoma. This report summarises our findings to date.
Methods: A prospective observational study design.
Technique: Patients were given 20 mg/kg body weight 5-aminolevulenic acid (ALA) mixed in non-fizzy orange juice to take orally 3 h before surgery. Surgery was performed in the supine position, under image guidance, through the right nostril using Storz 0 degree endoscope assisted with microsurgery as required. The endoscope was attached to photodiagnostic filters allowing switch-

ing the light from white to blue at the flick of a foot paddle (PD). After the dura of the floor of the sella was incised a laser probe was inserted into the pituitary lesion/gland to identify the ALA-induced protoporphyrin IX spectroscopy at 632 nm using an Optical Biopsy System (OBS, Fig. 1). Once the adenoma was identified by the OBS or fluorescence it was removed and its type was confirmed by histopathology.

Patients: Thirty consecutive patients were studied, fourteen were non-functioning adenomas (NFA), two were prolactinomas (ProA), three were Cushing's (ACTH secreting), two were Acromegaly (GH secreting), five were gonadotrophin secreting (GnTA) and four non-adenomas. Twenty-four of these were examined by the OBS and PD systems. The true positive (sensitivity) of the PD and OBS systems were (21/26) and (21/22) respectively. The true negative (specificity) of PD and OBS were (4/4) and 2/2), respectively. The false negative rate of PD was (5/26) and for OBS was (1/22), while the false positive rate for PD was (1/5) and for OBS was 0. The breakdown of sensitivity and specificity for both systems in different subtypes of adenomas is summarised in the following table.

System	PD	OBS
NFA specificity	11/14 ( )	12/12 (100%)
Pro A specificity	0.5 (50%)	2/2 (100%)
ACTH A specificity	2/3 (66.66%)	2/2 (100%)
GH A specificity	0.5 (50%)	0.5 (50%)
GnT A specificity	5/5 (100%)	4/4 (100%)

Conclusion: Intraoperative optical identification of pituitary adenomas is feasible and reliable way to localise pituitary adenomas during transsphenoidal surgery and it may lead to improved cure rate and preservation of normal pituitary functions.

P-18

Multi-course photodynamic therapy (McPDT) of esophagus and stomach cancer

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We started the development of McPDT in 1992. McPDT means multiply sessions of PDT with repeated injections of photosensitizer and light irradiation. The aim of McPDT is a partial tumor destruction and symptomatic therapy in patients with cancer stage III-IV and complete regression of tumor stages I—II.

Up to now, 66 patients with primary and recurrent malignant tumors of esophagus (35 patients/35 tumors) and stomach (31/32) have been treated by McPDT (2—18 courses). Esophagus and stomach cancer stages I—II was in 20 cases, esophagus and stomach cancer stages III—IV was in 46 cases. Squamous cell carcinoma was observed in 29 cases, adenocarcinoma in 37 cases. For PDT we used Russian photosensitizers (Photogem (hematoporphyrin derivative), Photosens (aluminum sulphonated phthalocyanine), Radachlorin (E6 chlorin), Alasens (5-aminolevulinic acid)), Russian diode lasers (Crystall) with different wavelengths and endoscopic equipment by Olympus (Japan). In 19 patients with esophagus cancer stage III-IV PDT was performed through esophageal stent.

Complete regression of the tumor was achieved in 4 (20%) out of 20 patients with esophagus and stomach cancer stages I—II, partial regression and stabilization of tumor growing in 16 (80%). Partial regression and recanalization of tumors stenosis was observed in all cases with cancer stages II—IV.

Results of our study showed that a multi-course PDT method seems to be perspective in treatment of advanced malignant tumors of esophagus and stomach.

P-19

Intraoperative photodynamic diagnosis (PDD) and photodynamic therapy (PDT): Its role in surgical treatment of superficial bladder cancer

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In Moscow Oncology Institute named after Hertzen the photodynamic therapy of superficial bladder cancer has been used since 1994. For the last 4 years we have developed and began to use method that embodies TUR with PDT.

From 2004 to present 75 patients with superficial bladder cancer T1 G2—G3 were treated. PDD and PDT were applied with Fotogem (derivation of hematoporphyrin) and Alasens (5-aminolevulinic acid, FSUE ''SSC ''NIOPIK''). Fotogem was injected intravenously 48 h prior to treatment. Alasens was injected endovesicality 1.5—2 h prior to the treatment. During operations, PDD was performed with Alasens by fluorescent Karl Storz optics followed by TUR of all visualized tumors and fluorescent focuses. PDT performed with a diode laser (635 nm) and was the last stage of treatment.

In the course of the combined treatment by the developed method, no toxic reactions or induced postoperative cystitis were registered. Fifty-two patients of 75 were followed up from 6 to 24 months. Cancer recurrence was registered in 18 (24%) cases.

The developed method has increased the efficiency of treatment of superficial bladder cancer. The efficiency of TUR rises due to resection of fluorescent foci—hidden cancer. Principles of ablastics are also kept with the help of intraoperational PDT (using two types of photosensitizers with different variants of interstitial distribution).

P-20

Uptake, cytotoxicity and phototoxicity of Chl *a*/cyclodextrins complexes on Jurkat cells

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Recently we have proposed as sensitizers in PDT the combined use of a natural chlorine, chlorophyll *a* (Chl *a*), with cyclodextrins (CD), cyclic oligomers of glucose widely used in pharmacological applications as delivery systems of drugs having a poor solubility in water.

In order to test this hypothesis the aggregation status of chlorophyll *a* (Chl *a*) and the ability of four cyclodextrins, hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin, to solubilize the pigment in the complete cellular medium RPMI 1640 have been investigated. Afterwards the cytotoxic and phototoxic activity of these complexes towards human leukemia T-lymphocytes (Jurkat cells) have been tested and compared to results obtained delivering the pigment by means of ethanol. In order to understand the mechanism involved in the process induced by light, experiments aimed to discriminate between the Chl *a* located in the membrane protein fraction and in the cytosolic fraction have been performed. The results, herein reported, indicate: the pigment interacts with cyclodextrins in the cellular medium differently to that observed in water, HP-β-CD is the cyclodextrins having the lowest cytotoxicity and the highest phototoxicity and the preferential localization of Chl *a* in cells depends on the incubation time.

P-21

Phthalocyanine nanoparticles as potential antitumor agents with use of powerful laser irradiation

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Earlier we have reported tumor growth inhibition using powerful laser irradiation of carbon nanoparticles in tumor [1,2]. In present work an antitumor efficiency of nanoparticles of phthalocyanines (aluminum, zinc, copper, non-metal) under laser irradiation in tumor blood vessels has been studied.

In vivo experiments were performed on mice with C26 colon adenocarcinoma and S37 sarcoma. Aqueous suspensions of phthalocyanine nanoparticles (average size of 200—300 nm) were injected into tail vein in doses of 7—30 mg/kg. Q-switched ruby laser has been used for tumor irradiation (wavelength of 694 nm, maximum energy density per pulse of 0.6—0.8 J/cm^2, maximum fluence per session of 60—80 J/cm^2).

Irradiation right after injection result in maximum antitumor effect increasing with increase of nanoparticles dose, energy density per pulse, fluence per session. Phthalocyanine nanoparticles provide better effect in comparison with carbon nanoparticles. Control continuous laser irradiation with the same fluence per session result in much lower efficiency.

We believe the antitumor mechanism is damage of tumor blood vessels as result of nanoparticles ''microexplosions'' under irradiation by short powerful laser pulses.

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P-22

Cu—phthalocyanine nanoparticles as photosensitizer for PDT

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Earlier we have reported antitumoral effect *in vivo* of unsubstituted aluminium phthalocyanine (AlPc) nanoparticles (NPs) as photosensitizer for PDT [1]. Effect was, because hydrophobic AlPc molecules are dissolved in biosubstrata of tissue partially, though in low concentration, and provide conventional PDT.

Here we have studied *in vitro* photooxidation of substrata in aqueous suspension of Al-, Zn- or Cu—phthalocyanine NPs (average size of 200—300 nm). Human serum albumin and furfuryl alcohol were used as substrata for oxidation by singlet oxygen. Laser source (670 nm, 200 mW) was used for excitation of NPs. Oxygen consumption rates in suspension were measured by oxygen tension sensor based on quenching of triplet states of phosphorescent dye (palladium *meso*-tetra-(phenyl)-tetrabenzoporphin) by oxygen in polystyrene film [2]. Highest oxidation rate was in case of CuPc NPs though CuPc molecule is not photosensitizer unlike AlPc or ZnPc molecules. Addition of singlet oxygen quencher (sodium azide) into suspension result in inhibition of photooxidation. Study of mechanism of singlet oxygen generation by Cu-phthalocyanine NPs is required.

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P-23

PDT technique with photosensitizer triplets saturating

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Shortcoming of conventional PDT is essentially nonuniform spatial distribution of the photodynamic dose (PD) in tissue. This is due to nonuniformity of the light dose distribution owing to absorption of light by tissues. The tissue layers (including intact tissue near tumor) located closer to a light source, receive too high PD, much higher than necessary one. This entails pain and excessive damage of intact tissue. PDT techniques with PD saturating can eliminate these problems. One of these techniques uses PS triplet states saturating (TSS). Here theoretical consideration of features of TSS technique is presented. Requirements to light sources and photosensitizers are discussed. Possibility of essential reduction of duration of PDT session is shown.

P-24

5-ALA PDT in the treatment of superficial basal cell carcinoma—4 years of experience

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In January 2004, we have started topical photodynamic therapy in the treatment of epithelial precancerous and cancerous lesions. Lesions were treated by topical application of 20% 5-aminolevulinic acid (5-ALA) dissolved a proprietary oil-in-water emulsion. The ALA containing emulsion was applied under occlusive dressing 5—6 h before illumination with noncoherent red light (wavelength 630—700 nm, light dose 100—150 J/cm^2). We have treated 59 patients with solitary or multiple superficial basal cell carcinomas (BCC). In more than 90% of BCC complete clinical and histopathological regression was achieved after one to three treatments. The number of required treatments increased with lesion diameter.

P-25

Efficacy of topical ALA-PDT in the treatment of acne vulgaris

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Objectives: To determine the safety and efficacy of topical 5-aminolevulinic acid-photodynamic therapy (ALA-PDT) in the treatment of acne vulgaris.

Materials and methods: Forty-two patients with acne from 18 to 39 years old (16 men and 26 women) with different form of acne were enrolled in this study. PDT with the use of endogenous porphyrins — 20 (47.6%) patients and PDT with the use of exogenous protoporphyrin IX from 5-ALA — Alasens (FSUE ''SSC ''NIOPIK'') — 22 (52.4%) patients. In last case Alasens was used in the form of solution or cream with 10—20% concentration of 5-ALA as external applications under the occlusive bandage for 1.5—2 h. To determine the concentration of porphyrins and to control PDT the laser electro-spectral analyzer (LESA-01-BIOSPEC) was used. Irradiation was carried out with photodiodic videofluorescentic equipment UFF-630-01-BIOSPEC, shining non-stop light with the waive length of 630 nm and energy density of 36 J/sm^2. The irradiation sessions were held once a week for the course of 4—10 sessions.

Results: In the process of PDT clinical remission was achieved in 34 (81%) patients and the rest are under experienced improvement. The following temporary side effects were noted, which independently subsided in 3—5 days: burning, itching, swollen, erytema.
Conclusions: Therefore, the effectiveness of PDT acne directly depended on the level of endogenous porphyrins—the higher the level, the better clinical results. In order to strengthen the photodynamic reaction Alasens may be used, which turned out to be highly effective in stabilization of process and allowed to achieve clinical remission with 81% patients.

P-26

Feasibilities of photodynamic therapy with cationic photosensitiser in the treatment of bacterial keratitis: an experiment study

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Objectives: Infectious keratitis is an important cause of ocular morbidity. There are some common pathogens that cause acute bacterial keratitis. Streptococcal species, staphylococcal species, pseudomonas and enterobacteriaceae make up the four most common classes of infective agents.
Materials and methods: We determine the efficacy of photodynamic therapy (PDT) with Cholosens (a new cationic photosensitiser—$ZnPcCholin_8$) in the treatment of bacterial keratitis in a rabbit eye model. Corneal ulcer was induced in 14 rabbits using a culture of Staphylococcus aureus. In all animals, a corneal infiltrate developed of 5—8 mm diameter. After 120 h, all eyes showed a similar corneal ulceration. Pathologic changes were determined by slit lamp examination and histopathologic analysis. Irradiation was performed after 40 min application of Cholosens in dose 2.0 mg/ml. A diode laser (''Biospec'', Inc., Moscow) was used operating in the range of 675 nm. Its energy was calculated as 40 J/cm^2.
Results: The histological investigation by method of semithin sections revealed that PDT effectively destroys bacterial cells. The resolution of inflammatory signs one could see after 10 days of PDT.
Conclusions: These results provide evidence that bacterial ulcers resolve with PDT treatment.

P-27

Visualization of eyelids and conjunctival tumors by fluorescence diagnosis using 5-aminolevulinic acid hydrochloride

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Introduction: In recent years, the need for new methods of early cancer detection has stimulated the rapid development of non-invasive optical diagnostic techniques. Fluorescence imaging has been shown to be a potential complement to visual inspection for demarcation of basal cell carcinoma (BCC), which is the most common type of skin cancer. The majority of conjunctival processes have a similar clinical picture in consequence of the expressed inflammatory response that essentially complicates diagnostics of these conditions. Fluorescence imaging has been shown to be a potential complement to visual inspection for demarcation and visualization of eyelids and conjunctival tumors.
Materials and methods: A total of 22 pathologically verified eyelids BCCs and 32 pathologically verified conjunctival neoplasm (conjunctival lymphoma, squamous cell carcinoma, pterigium, pyogenic granuloma and pingveculum) in 43 patients were investigated. Patients per os received 5-aminolevulinic acid hydrochloride (Alasens), produced by FSUE ''SSC ''NIOPIK'') in the dose of 15 mg/kg of the body mass. Source of excitation were illumination fluorescent lamps (433 nm). Fluorescence was imaged using a single chip CCD red/green/blue (RGB) color camera (combined with a slit lamp). Digital images acquired were analyzed using image analysis software.
Results: Maximum of fluorescence tumor contrast occurred in the group of patients with malignant tumors. Except for delimitation of the BCC the difference between Red-channel ratio in the tumor and in the surround tissues was calculated ($\triangle R$%). $\triangle R$ in BCC of eyelids is fluctuated in the range of +12.4% to +24.3%. In 22 patients with conjunctival neoplasm, tumors were subjectively better distinguishable from their surroundings through an enhancement of 5-ALA-induced PPIX fluorescence than by ordinary inspection.
Conclusions: By using a CCD camera system together with digital imaging, the contrast of the acquired fluorescence images can be significantly enhanced and allows the determination of a threshold of eyelids and conjunctival tumors, which can be utilized either for a directed biopsy or for preoperative planning.

P-28

The possibilities of improvement the sensitivity of cancer fluorescence diagnostics by computer image processing

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Background: The principle of autofluorescence is the natural ability of tissue to fluoresce when exposed to a specific wavelength of light. The difference in fluorescence between normal and progression to cancer allows the physician to see subtle areas of abnormality for biopsy and follow-up.
Aim: The aim of our study was to evaluate whether computer image processing of images obtained during examination using ONCOLIFE can improve the effectiveness of autofluorescence diagnostics.
Methods: Function of image $f(x,y)$: $R^2 \rightarrow R^3$ was transformed from original color space RGB to space in which vector of 46 values refers to every point labeled by defined xy-coordinates—$f(x,y)$: $R^2 \rightarrow R^{46}$. By means of Fisher discriminator vector of attributes of concrete point analyzed in the image was reduced according to two defined classes defined as pathologic areas (foreground) and healthy areas (background). As a result the highest four fisher's coefficients allowing the greatest separation between points of pathologic (foreground) and healthy (background) areas were chosen. In this way new function $f(x,y)$: $R^2 \rightarrow R^4$ was created in which points x, y correspond with vector Y, H, a^*, c_2.

In the second step using *Gaussian Mixtures* and *Expectation-Maximisation* appropriate classificator was constructed. This classificator enables determination of probability that the selected pixel of analyzed image is a pathologically changed point (foreground) or healthy one (background). Obtained map of probability distribution was presented by means of pseudocolors or as a 3D image function.
Results: Image processing techniques improve quality and sharpness of original fluorescence images.
Conclusion: Computer image processing enables better visualization of suspected areas.

P-29

Photodynamic therapy and fluorescent diagnostics: Equipment, technique and clinical use

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Methods of optical diagnostics and photodynamic therapy are actively used in medical and biological researches. However wide application of PDT in clinics is limited to capabilities of the existing equipment.

This paper presents the equipment developed in Laser Biospectroscopy Lab of A.M. Prokhorov General Physics Institute recently.

Among the new developed therapeutic devices is a family of medical lasers for the photodynamic therapy, intended for use with new photosensitizers. General principles of construction of medical laser systems are presented. Key parameters of the developed lasers are given. Therapeutic lasers work in a red and near infrared wavelength range, depending on application and photosensitizer. Output optical power of lasers is from 1.5 up to 30 W CW.

Frequently LED sources are enough for an irradiation of a skin, while they are much cheaper than laser sources. A family of such devices, LED fluorescent system for a skin treatment, is presented at this paper. Devices allow to carry out therapeutic procedures and also to make a fluorescent diagnostics using various photosensitizers.

New opportunities of application of spectral systems LESA are considered also.

Equipment is created on industrial base and with participation of experts from JSC ''Biospec''.

P-30

Multi-purpose USB optical power meter for therapeutic lasers

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Carrying out of exact measurements of output optical power of medical therapeutic laser systems with a fiber optic output is an actual problem in the field of photodynamic therapy.

New device PM-3-Biospec for the control of irradiated power of medical lasers with various types of optical fibers and fiber tips has been developed. The device allows carrying out measurements on optical fibers with a direct output and with cylindrical diffusers with 5, 10 and 20 mm length.

PM-3-Biospec is intended for work in a range from 532 nm up to 760 nm. Relative error of measurement does not exceed 2%. Maximal value of measured power is 3 W. Device has USB output.

Optical power meter is realized on the basis of the digital signal processor and an analog-digital converter with the expanded dynamic range.

For display and record of results of measurements the special software has been developed. The software displays the current value of optical power, time chart, etc. The software allows changing an interval of data read-out from the device, to trace limits of a predetermined range, to smooth the data in time, to take into account features of measurement depending on type of a fiber and irradiation wavelengths and to save results of measurements.

Created device has been used for definition of optical characteristics of various biological tissues in a visible wavelengths range.

P-31

Significance of photodynamic therapy in dermatology

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Topical photodynamic method is useful as diagnostic as well as therapeutic options in dermatology. Photodynamic therapy (PDT) involves the topical application of aminolaevullinic acid (ALA) or methilaminolaevullinate (MAL), occluded for 3—6—15 h and then irradiated, preferable with red light. Dougherty et al. (1978) first reported the clinical use of PDT as an effective treatment modality for non-melanoma skin cancer, but Smetana et al. (1997) for viral infections as non-oncological indication using ALA-PDT. ALA-PDT can be a safe alternative therapeutic modality of recurrent and torpid condilomata accuminata in particular immunosuppressed patients when traditional therapy failed. The indications for PDT in non-melanoma skin cancer are actinic keratoses (AK, solar keratoses), superficial basal cell carcinoma (BCC), Bowen's disease (BD), and for non-oncological dermatoses: scleroderma, warts, acne, psoriasis. We have been using ALA PDT and MAL PDT at the University Department of Dermatology and Venereology.

P-32

DNA and RNA damage in human cancer cells after photodynamic therapy with a new chlorine-based photosensitizer

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Introduction: *Meso*-tetra-hydroxyphenyl-chlorine (mTHPC) is one of the most powerful photosensitizers available for photodynamic therapy (PDT). We here focus on changes at DNA and RNA levels after treatment with the novel mTHPC derivative Foslipos.
Materials and methods: PC-3 cells were treated with 5 μg/ml Foslipos (Biolitec, Jena, Germany) for 5 h and irradiatiated with 5 J/cm^2 (PDT). Controls included omission of both Foslipos and irradiation (CO), treatment with Foslipos only (FOS) and irradiation only (IRR). Cells were counted directly (TP0) or after 1 h (TP1), 2 h (TP2), 5 h (TP5) and 24 h (TP24). Nucleic acids were extracted and RNA degradation was assessed by the RNA integrity number (RIN), while DNA damage was estimated by its number of abasic/apyrimidinic sites (DNA damage kit, MBL, Woburn, MA).
Results: In PDT, the number of living cells was significantly lower and of dead cells significantly higher compared to controls from TP0 on. Over time, PDT treated cells showed a significant increase of dead cells, leading to only 23.3% of the initial cell number and 9% of CO after 24 h. RIN was significantly lower after PDT versus controls. Numbers of abasic/apyrimidinic sites after PDT were significantly higher compared to controls at TP0. Following a decrease at TP1 and TP2, number of sites subsequently rose in PDT.
Conclusions: PDT effects of Foslipos were characterized by a two-step process. A significant initial damage of nucleic acids was already observed within the first 15 min after irradiation, followed by a second boost after 5 h. The data will help to better understand molecular photodynamic effects.

P-33

The advance treatment with Zn(II)—phthalocyanines and red light of farm-fishes pathogenic bacteria

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Although the spread of microbial diseases though the water and food has been reduced by several hygienic and antibiotic-based procedures, it is of importance invention of a human-health favorable method so that to avoid the antibiotic loading of the table fishes.

The propose approach is based on photodynamic action of cationic phthalocyanines and red light on gram-negative bacteria *Aeromonas hydrophila*, which is causing illness to farm fishes. The cationic phthalocyanines with four functional groups namely, *methyl*pyridinium, *propyl*pyridinium, *hexil*pyridinium and *dodecyl*pyridinium, show preferable binding to microbial cells. At illumination of drug-incubated bacteria (10^6 cell/mL) with a red light (LED, 630 nm) the generation of highly cytotoxic oxygen species is occurred. The assessment of the photodynamic effect of phthalocyanines showed that the effect increase with hydrophobicity. An exception was tetrakis(4-*methyl*pyridinium)phthalocyanine with a complete bacterium inactivation even at mild treatment conditions (2 μM and 50 mW cm^{-2} for 15 min). The effect was full for tetrakis(4-*dodecyl*pyridinium)phthalocyanine for 0.9 μM drug at the same irradiation protocol. The obtained results concluded that cationic phthalocyanines plus red light can be an excellent treatment for farm-fishes illness.

P-34

PDT: Treatments of eyelid, nose and ear skin carcinoma

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Photodynamic therapy (PDT) is considered to be a minimally invasive treatment modality which shows great promise in premalignant and malignant dermatologic conditions. The principle of the treatment is a photochemical reaction initiated by light activation of a photosensitizer, which causes the dead of the exposed tissue. The technique is simple, can commonly be carried out in outpatient clinics, and is highly acceptable to patients. Photodynamic therapy is now shown to achieve equivalent or greater efficacy than standard treatment of premalignant and malignant lesions especially those localized on eyelid, nose and ear where surgery could cause disfigurement.

P-35

New efficient near-IR photosensitizer for photodynamic therapy of malignancies based on bacteriochlorin *p* derivative

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The work is devoted to investigation of photosensitizer on a base of bacteriochlorin *p* macrocycle—bacteriochlorin *p* *N*-methoxycycloimide oxyme methyl ester which absorbs at 790—795 nm. This photosensitizer seems to be promising for PDT spread tumors, due to low intrinsic absorption of tissue in this spectral range, and can also be considered for PDT of pigmented tumors, such as melanoma.

Due to hydrophobicity of photosensitizer it was intravenously administered in cremophor dispersion. In vivo studies were performed using F_1 mice bearing intramuscular Erlich tumor and BDF_1 mice bearing intramuscular B16 melanoma. Dynamics and selectivity of sensitizer accumulation in tumor and normal tissue were estimated from absorption spectra of sensitized tissue in vivo measured using fiber-optic spectroanalyzer LESA-01-Biospec.

Photosensitizer shows moderate selectivity of accumulation in tumor which achieves 1.5—2 in the therapeutic time range. Maximum concentration of photosensitizer in tumor is observed in the range of 10—90 min after administration. Photosensitizer quickly clears from the normal tissue, its content in skin decreases down to detection threshold by 8—10 h after administration. PDT was performed using 797 nm laser irradiation with power density of 300 mW/cm^2 for 20 min, starting 10—15 min after sensitizer administration, light dose density achieved 360 J/cm^2. Tumor growth inhibition for Erlich tumor achieved 80%.

Efficiency of photosensitizer was also tested on BDF_1 mice bearing B16 melanoma under the same treatment conditions. In this case, the treatment caused the increase of animal lifespan of 61% with tumor growth inhibition exceeding 92%.

P-36

New efficient near-IR photosensitizers for photodynamic therapy based on nanostructural dispersions of phenylthio- and alkylthio-derivatives of phthalocyanines

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Photosensitizers of the near-IR range (700—850 nm) are considered to be the most promising for PDT of spread tumors, due to low intrinsic absorption of tissue in this range. The work is devoted to investigation of two new classes of photosensitizers based on phenylthio- and alkylthio-derivatives of phthalocyanine macrocycle and represented respectively by aluminum hydroxide tetra-3-phenylthiophthalocyanine [$(PhS)_4PcAlOH$] and zinc octa-4,5-decylthio-octa-3,6-chlorphthalocyanine [$(DcS)_8Cl_8PcZn$]. Photosensitizers have efficient absorption in a range of 715—740 nm and can be considered promising for PDT of tumors.

Both active substances are hydrophobic and were solubilized in form of nanostructural dispersions to investigate their properties *in vivo*. $(PhS)_4PcAlOH$ was solubilized in liposome dispersion on a base of lecithin, cholesterol and mPEG2000-PE. Particle size was reduced and unified using high-pressure homogenizer. $(DcS)_8Cl_8PcZn$ was solubilized in micellar form based on Proxanol-268. Nanostructural properties of dispersions were studied by correlation laser spectrometry, using LCA-3 spectrometer.

In vivo studies were performed using F_1 hybrid mice bearing Erlich tumor. Accumulation of photosensitizers in tumor and normal tissue were estimated from fluorescence and absorbance spectra *in vivo* obtained using fiber-optic spectroanalyzer LESA-01-Biospec.

In vivo studies have shown that both photosensitizers selectively accumulate in tumor achieving their maximum concentration at

5—24 h after administration. Photosensitizers rather quickly clear from the normal tissue, their level in skin decreases down to detection threshold by 6—8 days after administration. For PDT, tumors were irradiated using 732 nm laser with power density 100—400 mW/cm^2 for 20 min, starting 5 h after administration of photosensitizers. Tumor growth inhibition exceeded 80%.

P-37

The effect of environmental oxygenation on lethal photosensitization of wound-associated organisms using indocyanine green and near-infrared light

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The current worldwide increase in antibiotic-resistant bacteria reduces our ability to treat infected wounds. A promising alternative to antibiotics is photodynamic therapy. Numerous factors affect the antimicrobial effectiveness of PDT; one of these factors is tissue oxygenation, which can limit the singlet oxygen yield of a photosensitizer.

The effect of environmental oxygenation on the lethal photosensitization of *Staphylococcus aureus* and *Streptococcus pyogenes* using indocyanine green and near-infrared laser light was investigated.

Bacterial suspensions were irradiated for 5 min with a fluence rate of 1 W/cm^2 under both aerobic and anaerobic conditions and the survivors were enumerated. Under anaerobic conditions the killing efficiency for both organisms was reduced. Lethal photosensitization under aerobic condition, using an initial bacterial load of 10^7 cfu/ml resulted in 99.56% kills of *S. aureus* and 99.96% of *S. pyogenes*. Under anaerobic conditions, the kills were reduced to 96.77% and 71.62% for *S. aureus* and *S. pyogenes*, respectively.

These findings imply that the level of tissue oxygenation is an important factor to consider during the eradication of bacteria from wounds.

P-38

Experience of outpatient skin cancer treatment by means of photodynamic therapy with chlorine derivatives

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Nowadays the most widespread photosensitizers are hematoporphyrin derivatives (Photofrin, Photohem). Photosensitizers of the first generation, however, have got a number of disadvantages: a low penetration of exciting light, a long-lasting skin phototoxicity; a low energy of light absorption.

For the last 10 years there has been an intensive development of photosensitizers of the second generation with a short-time term of elimination and a bigger wavelength of exciting light, which provides a deep penetration into biological structures, as well as corresponds to other main requirements to photosensitizers. Such photosensitizers are Russian-made chlorine derivatives Radachlorin and Photoditazine, as well as Belorussian compound Photolon, which have got corresponding permissions for clinical usage.

We are experienced in using photosensitizers of the second generation from the group of chlorine derivatives for the performance of PDT in order to treat skin cancer under outpatient conditions. The doses of Radachlorin and Photoditazine were 0.6—0.8 mg/kg, the drug-light interval was from 1 up to 4 h, the dose of input light energy was 100—300 J/cm^2. The dose of Photolon was 1.2—1.5 mg/kg, the drug-light interval was 3 h, the dose of input light energy was 150—200 J/cm^2.

PDT gets a 100% therapeutic efficiency. Among 178 patients we have received complete resorption for 162 (91%) patients, and partial resorption for 16 (9%) patients.

The results of PDT with chlorine derivatives are estimated to be good and perfect, due to the preservation of the collagenous structure of tissues and the healing of a tissue defect after resorption of a tumor by type of reparation, but not scarring.

PDT with application of chlorine derivatives under outpatient conditions is a comfortable and effective method for the treatment of skin cancer that provides good functional and cosmetic results under high-quality economic efficiency.

P-39

PDT off-label therapies

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Reports about the applications of topical photodynamic therapy (PDT) with metylaminolevulinate (MAL) or aminolevulinic acid (ALA) in dermatology cover common skin cancer types and also certain benign skin disorders. PDT with local application of a standardized preparation containing MAL (Metvix®), the only photosensitizers approved in European Union (EU) at a concentration of 20% in a O/W emulsion followed by red light exposure, has been shown to be efficacious in the treatment of actinic keratosis, superficial and nodular basal cell carcinoma and more recently has been registered for the treatment of Bowen's disease.

Open studies have provided promising results in oncologic dermatology for the treatment of cutaneous lymphoma, actinic cheilitis and squamous cell carcinoma but the use of MAL-PDT in these indications must follow all the rules and authorizations specified by the local Ethical Committees.

Topical photosensitizer (MAL and ALA) have been used also for the treatment of inflammatory skin disorders such as psoriasis or acne vulgaris, for HPV viral disease, for granulomatous skin disorders (localized scleroderma, necrobiosis lipoidica, etc.) and more recently for photorejuvenation. These conditions are under clinical investigation with different and contradictory results but with excellent future perspectives. However studies with longer follow-up are required to arrive at conclusive remarks about the usefulness of PDT. Our presentation reviews the recently published data on clinical MAL-ALA based off-label PDT.

P-40

The mechanism of combined photodynamic and catalytic action of cobalt phthalocyanine in molecular and nanodimensional state in vitro and in vivo

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The mechanisms of cobalt phthalocyanine transition from molecular to nanodimensional state in tumor tissues were investigated in our work. Cobalt phthalocyanine in molecular form was intravenously injected to mice with transplanted tumor. Then complementary introduction of the preparations resulting in cobalt phthalocyanine nanoparticles formation in mice tissue was realized. The nanoparticles were exposed to laser irradiation at the wavelength of 675 nm. The transition from non-water-soluble nanodimensional to molecular form was observed. The latter has catalytic activity and hydrogen peroxide is generated at interaction with ascorbic acid. So only irradiated tissue is destructed.

P-41

Synthesis of halogenated bacteriochlorins and their potential use as infrared sensitisers

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The quest for sensitisers with stronger absorption where biological tissues are more transparent to radiation and yet this radiation is sufficiently energetic to promote singlet oxygen sensitisation, lead us to synthesise partially water-soluble halogenated chlorosulfonated chlorin and bacteriochlorin derivatives. It is known that bacteriochlorins of natural origin are very labile, especially under irradiation. We found that halogenated chlorosulfonated tetrakisphenylbacteriochorins are much more stable to irradiation in solution than their natural counterparts. The present work explores the properties of such compounds and their potential use in photodynamic therapy. The properties surveyed include solubility, photostability, absorption and emission properties, lifetimes of triplet states, singlet oxygen quantum yields and the mechanism of production of other reactive oxygen species (superoxide and hydroxide). It is shown that the properties found for these sensitizers approach closely the properties that should be expected for an ''ideal'' photosensitiser with a strong absorption in the infrared.

P-42*In vivo* targeting of human malignant melanoma by photochemical internalization (PCI) of the recombinant antibody fusion toxin scFvMEL/rGel

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Intracellular sequestration of therapeutic agents and the subsequent degradation in endocytic vesicles is a major obstacle for several drug-based therapies of cancer. The purpose of the present study was to establish *in vivo* photochemical internalization (PCI) of a tumor-targeting immunotoxin (IT) for enhanced tumor selectivity, cytosolic delivery and anti-tumor activity. PCI of the recombinant anti-gp240 (HMW-MAA/NG2) antibody fusion toxin scFvMEL/rGel containing the powerful ribosome-inactivating plant toxin gelonin (rGel) was tested against human non-pigmented gp-240 positive melanoma cell line A-375 and gp-240 negative bladder carcinoma T-24 cells *in vitro*, and in a xenograft with A-375 injected s.c. PCI *in vitro* was performed by light activation of cells co-incubated with scFvMEL/rGel and the endo-lysosomal targeting photosensitizers $AlPcS_{2a}$ or $TPPS_{2a}$. Cells were chased in drug-free medium 4 h prior to light exposure. Cytotoxicity was evaluated by the MTT assay 48 h post-light. Mice with 50—100 mm^3 A375 tumors got one i.p. injection of 5 mg/kg AlPcS2a 48 h and 2 mg/kg scFvMEL/rGel administered once i.v. 24 h prior to 670 nm laser light exposure. Fluence rate of light was 100 mW/cm^2 and the total fluency given was 20 J/cm^2. Tumor growth was measured twice per week until tumors reached 1000 mm^3. PCI of scFvMEL/rGel demonstrated synergistic cytotoxic effects in A-375 cells *in vitro*, while PCI of the non-conjugated rGel was less toxic than the IT. There were no differences in toxicity between the IT and the toxin in T-24 control cells. *In vivo* it was demonstrated a complete response in 4 of 12 animals (33% CR) that achieved PCI of the IT. Fifty percent of the mice had tumors <800 mm^3 at day 110, while 1 out of 14 animals (7%) got CR after PDT only. This is the first *in vivo* demonstration of PCI of a tumor cell-targeting macromolecule-based agent. PCI of immunotoxins may be a potent drug delivery strategy which warrants further pre-clinical evaluation.

P-43

Sapphire applicators for FD and interstitial PDT

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Sapphire is a very promising material for a light delivery system in FD and PDT applications. The material reveals a large optical transmission band coupled with unique mechanical, optical and physics—chemical features, what allows expecting prevalent application of light delivery systems, as well as implants and introducers, based on a biocompatible and chemical passive sapphire material.

We have recently started with sapphire capillary needle (SCN) preparation employing shaped crystal growth by the Stepanov technique.

There are many medical branches where SCN could be applied as a main element of light delivery system for PDT along with FD. We fixed out attempts first of all on SCN preparation for IPDT of liver and prostate. Concerning PDT of prostate lesions with SCN help it is very desirable and to compose the delivery system based on the placement technique, which has been already established in brachytherapy under ultrasound guidance.

Preliminary SCN samples were tested with 2.5 W irradiation of LPhT-670-01-BIOSPEC laser system in order to find out an interaction with erythrocyte mass. The tests carried out have shown, that SNC fully put inside the erythrocyte mass did not undergo any changes at irradiation dozes up to 2.3 kJ, though coagulation and evaporation of liquid components have been observed.

P-44

Sapphire scalpels with simultaneous opportunity of fluorescent diagnostics

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Sapphire has a favorable combination of excellent optical and mechanical properties, together with high chemical inertness and biocompatibility it makes it an attractive structural material for medicine.

There are made sapphire surgical instruments to perform incision and simultaneous laser coagulation with the blade working as a wave guide concentrating radiation in the edge area. We developed surgical tools with an opportunity of a simultaneous incision and diagnostics of a resected tissue.

Sapphire blades with putting distal ends of fibres (irradiating and accepting) direct to a cutting edge of the blade have been made and tested for a light registration from a tissue contacted.

Experimental model of Ehrlich carcinoma tumour imparted intramuscularly to two white mice was used. The mice were intravenous given an injection of the hydroxialuminium trisulfophtalocyanine (PHOTOSENS) 1 day prior to operation, so its concentration at each mouse was up to 5 mg/kg. The 633 nm continuous semiconductor laser with power of 50 mW was used.

Analysis of fluorescence spectra during resection with a scalpel has shown that the attitude of a maximum of fluorescence inside a tumour in relation to scalpel in a healthy muscle is not less than 3. (Spectroscopy system LESA-01-Biospec).

It shows availability of the works on a diagnostic scalpel tools development.

P-45

Impedance as a dosimetric quantity for PDT evaluation

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We used electrical impedance spectroscopy (EIS) as surrogate *implied* metrics during the course of PDT to correlate the acquired data with depth of necrosis.
Methods: We propose to compare the efficacy of electrical impedance spectroscopy to obtain real-time and clinically relevant quantitative measurements during photodynamic therapy (PDT), and investigate if the data might be useful in reducing variability of treatment during clinical therapy.

The liver of male Wistar mice weighting 200 g was used as the PDT therapeutic site. The mice were from the Faculdade de Medicina de Ribeirão Preto-USP (FMRP).
Experimental design: We used 15 mice divided in five group with 3 animals in each group. The animals were maintained without food for 12 h, but with water. Next they were weighted and anesthetized with Vetanarcol®, with 0.08 ml/100 g associated with Coopazine® 2 g, with 0.04 ml/100 g. A median incision was done and the liver right lobe was removed from the abdominal cavity and isolated with cotton gauze with saline solution.
Conclusion: Preliminary data indicates that impedance changes with PDT and could be correlated to the depth of necrosis. Additional experiments are planned to further investigate EIS as a dosimetric quantity for PDT evaluation.

P-46

Determination of threshold dose using superficial necrosis in photodynamic therapy

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The concept of photodynamic therapy (PDT) involves the photoinduction of the cytotoxicity of proliferating cells using a photosensitizer (PS) agent, a light source with proper wavelength and molecular oxygen. This work evaluated the correlation between light penetration and depth of necrosis as well as surface light illumination and width of necrosis. Using a normal rat liver model the depth and width of necrosis induced by photodynamic therapy was investigated using hematoporphyrin derivatives (Photogem®, Photofrin®, Photosan®) and chlorines photosensitizers (Foscan®, Photodithazine® and Radachlorin®). Different photosensitizer concentrations and light doses were used. The depth and width of necrosis model analysis allows us to determine the threshold dose in different clinical conditions.

The threshold dose can be determined from the width of necrosis measurements and is the same within experimental errors when compared with depth of necrosis measurements.

The use of simple models to understand basic PDT features may contribute to the solid establishment of PDT dosimetry enhancing its use in the clinical management of cancers and others lesions.

P-47

Determination of optical parameters of liquid phantoms for photodynamic therapy purposes

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Background and objectives: Photodynamic therapy lacks accurate dosimetry. Tissue variability and patient-specific pathologies make it difficult to apply techniques utilized in ionizing radiation planning and treatment. Although a specific light dose can be prescribed for desired effect, inconsistent tissue optical properties can lead to over or under light dosage to the lesion. We present the tools and methods to effect a photon dosimetry for PDT.
Materials and methods: Measurement of dose versus depth on liquid phantoms had been done. Virtual tissues were constructed with geometry obtained from CT/MRI imaging. The goal of this study is to determine the optical properties of human tissues i.e. skin, prostate, bladder. ASAP a Monte Carlo transport code was used to reproduce the same design as the experiment. For realistic approach we divided this study into three parts. First we modeled the light beam coming out from a laser 630 nm. Second the beam was applied to liquid phantoms, and then derived the optical properties. Third, apply the same approach to human tissues.
Results: The results obtained in part one of the study shows an agreement between simulated and experimental light beam and the difference was less than 10%.
Conclusions: This work is still under investigation, the only conclusion we can make at this point is that the method mentioned above to extract the optical properties of tissues is feasible and the laser beam obtained from simulation agrees well with the experiment.

P-48

Optical phantom for PDT using polystyrene and nigosine

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Purpose/introduction: To study the optical properties of polystyrene microspheres (PM) and nigosine to devise optical phantoms for use in photodynamic therapy (PDT).
Materials and methods: Nigosine dye (10 wt.% dispersion in water) and polystyrene latex microsphere were used in the phantom composition and to simulate the absorption and scatter properties of typical tissues, respectively. Nigosine was diluted in ChromAR® water to produce 8.8, 22 and 44 μg/mL and their absorption coefficients (μa) were determined by spectrometric mean using a 1-cm path length cuvette for wavelength (λ) varied from 400 to 800 nm. The MiePlot code was used to modeling the scatter coefficient (μs) from the PM diluted in water in concentrations varying from 1×10^5 to 5×10^{10} mL^{-1}. Curves between μa versus concentration of nigosine and μs versus concentration of polystyrene were plotted and analyzed.
Results: The relationship between μa versus concentration of nigosine and μs versus concentration of polystyrene were linear. The slopes of μa versus concentration of nigosine varied from 0.0298 to 0.0179 cm^{-1}/μg/mL for λ ranged from 400 to 800 nm, respectively. The slopes of μs versus concentration of polystyrene ranged from 2.86×10^{-8} to 1.37×10^{-8} cm^{-1}/mL^{-1} for λ ranged 400—800 nm, respectively.
Conclusions: Theoretical and experimental results have shown the feasibility of nigosine and PM to devise PDT' phantoms. A composition made of 15.5 μg/mL and 1.06×10^{10} mL^{-1} may be assembled to simulate human bladder irradiated with 630 nm light beam.

P-49

The PDT using for treatment of inflammatory parodontium tissue diseases

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Background: At the present time different investigation are testing the photodynamic therapy (PDT) effect with various photosensitiser for inflammatory parodontium tissue diseases.

The aim of this study was investigation of accumulation of Alasens (5-ALA) and PDT effect in parodontium tissues of patients with chronical parodontitis light stage.

Materials and methods: Four patients with chronicle parodontitis light stage were sensitized 3 mg/kg Alasens (FSUE ''SSC ''NIOPIK'', Moscow, Russia) by mouth and 2 h after were treated with He—Ne laser at 632.8 nm, 30 mW/cm^2, light dose $^-$7.2 J/cm^2. The fluorescence intensity ALA-induced PP-IX were measurement before oral administration Alasens, 2 h after, and after lasing by LESA-01-''Biospec'' (produced by Biospec Ltd., Moscow, Russia). In control group, four patients with chronicle parodontitis light stage, were treated with He—Ne laser at 632.8 nm, 30 mW/cm^2, light dose —7.2 J/cm^2, without Alasens.

Before oral administration Alasens and after lasing, bacterial samples were obtained from parodontium pocket and were analyzed microbiologically. The microbiological investigation was done for patients from control group before and after lasing.

Results: Oral administration Alasens contributed higher fluorescence levels. During PDT we were monitoring a photo bleaching of ALA -induced PP-IX.

The PDT with Alasens resulted in a bacterial reduction in comparison a laser light only.

Conclusions: PDT using Alasens for inflammatory parodontium diseases is effective method for treatment these patients, but the next investigations are necessary for optimization PDT regime.

P-50

Combined photodynamic therapy and hyperthermia in oncodermatology

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Introduction: Hyperthermia is a type of cancer treatment in which body tissue is exposed to high temperatures (from 42 °C up to 44 °C) to damage and kill cancer cells. Hyperthermia is almost always used with other forms of cancer therapy. Since 2004 in our clinic we have been started clinical researches of combined photodynamic therapy (PDT) and hyperthermia (HT). The aim of our research is to evaluate the toxic effect and efficiency of the combined photodynamic and hyperthermia therapy with Photosens and Alasens as photosensitizers agents.

Materials and methods: From 2004 to 2007 combined PDT and HT was performed in 30 patients with recurrent basal-cell carcinomas (BCC) of skin after surgery, radiotherapy and PDT. As photosensitizers, we use domestic drugs Photosens (mixture of sulphonated Al-phthalocyanine, FSUE ''SSC ''NIOPIK'') and Alasens (5-aminolevulinic acid, FSUE ''SSC ''NIOPIK''). We used intravenous injection of 0.3 mg/kg of Photosens or topical application of 20% Alasens, diode laser (670 nm) and gold vapor laser (628 nm). Hyperthermia at 42—43 °C (laser 1069 nm) was carried out immediately after or simultaneously with PDT.

Results and conclusions: Complete regression rate was in 69% of recurrent BCC of skin in all patients. Toxic reaction to that treatment is competitive to that of a conventional PDT course. A combined treatment of superficial recurrent BCC with Alasens has an excellent cosmetic effect due to the absence of scars in the place of tumor.

P-51

Cytoreduction surgery with intraperitoneal photodynamic therapy (PDT) in case of malignant tumors of abdominal cavity

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Cytoreduction surgery with intraperitoneal PDT was applied to 30 patients with locally spread and disseminated types of stomach cancer (19), colon cancer (4), pseudomyxoma and mesothelioma of peritoneum (6) and melanoma metastases (1). In 23 cases, the surgery suggested resection of the target-affected organ with peritonealectomy. In six observations, the mentioned type of surgery was performed in case of the recurrence or persistent growth of the tumor.

Two photosensitizers were used for intraperitoneal PDT: Photogem (2.5—3.0 mg/kg i.v. 48 h prior to the operation), and Radachlorin (0.6—0.8 mg/kg i.v. in the course of the operation 3 h prior to PDT session).

Diode laser (635 nm) and copper-vapor laser with dye cell (660 nm) and were used for PDT session. Laser irradiation of all the peritoneum areas was performed with successive 14—16 expositions using a cylindrical diffuser. Energy density varied from 3 to 10 J/cm^2.

In 9 out of 30 patients, intraperitoneal PDT was initiated after the fluorescent laparoscopy and in the course of the abdominal cavity investigation. In other patients, massive invasion of serous membrane was revealed alongside with occurrence of malignant cells in peritoneal liquid and echo-sounding evidence of peritoneum destruction and penetration into the adjacent cellular tissue.

In the course of the surgery and in the immediate post-operative period, no complications, related to PDT, were registered. Maximum follow up with no signs of disease progressing after cytoreduction surgery with intraperitoneal PDT in patients with stomach cancer and dissemination of peritoneum was 12 months. We plan to proceed with the clinical trials using intraperitoneal PDT.

P-52

Laparoscopic intraperitoneal photodynamic diagnosis (PDD) and photodynamic therapy (PDT) in oncology

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For the period from 2001 to 2007, fluorescent laparoscopy with local spectroscopy in the combination with laparoscopic operation and PDT was performed in 63 patients aged from 18 to 72.

In 51 patients we used Alasens (5-ALA) with intra-abdominal or oral dosing; in 12 patients we used Photogem. Fluorescent laparoscope D-light of Karl Storz system was used for PDD. Multiple view regular laser irradiations (635 nm) of all the peritoneum areas was carried out with energy density of 5—10 J/cm^2.

The first group was composed of patients with stomach cancer stage III (28); the second one embraced patients with mesothelioma and pseudomyxoma of peritoneum (6); the third, patients

with ovary cancer after combined treatment or repeated surgery (29). Laparoscopic operation (19 cases) in the combination with PDT of peritoneum was performed in 12 patients: after gastrectomy (6), with malignant peritoneum mesothelioma (4), and in 2 patients with peritoneum pseudomyxoma.

In 14 (22.2%) patients PDD has revealed extra occult tumor microdissemination of peritoneum (verified morphologically). That made it possible to specify the stage of the disease and change the program and method of treatment. After laparoscopic PDT, 7 out of 12 patients showed complete and partial regression of tumor foci dissemination in the peritoneum alongside with tumor growth stabilization and extinction or considerable decrease in ascetic fluid volume. Maximum period of tumor growth stabilization is 4.5 years. No toxic or allergic reactions caused by photosensitizers were registered in any case. Post-operational period was without any complications in all the patients.

P-53

Photodynamic therapy (PDT) of primary multiple basal cell carcinoma (BCC) of skin

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PDT of skin BCC has been applied in P.A. Hertzen Moscow Research Oncology Institute since 1992 and great clinical experience (217 patients) has been gained for 15 years. We have worked of PDT application to BBC with Photosens, Radachlorin, Photogem and Alasens.

Results of PDT in 68 patients with primary multiple BCC (462 tumors) are of a special concern. The number of tumors in one patient varied from 3 to 70. Superficial BCC was in 52% of cases, and nodular type was in 48% of cases.

Photosensitizer and PDT technique choice was determined by tumor locations and their number, geometry and BCC invasion depth. Prolongated and multicourse PDT with several photosensitizers was used in 58% of cases.

In case of superficial BCC, a complete regression with good cosmetic effect was obtained in 100% of patients. Recurrence and new foci of primary multiple BCC were diagnosed in 7 (20%) out of 35 patients with the follow up from 1 to 12 years. Repeated PDT was a success.

Complete regression of all the tumor foci was obtained in 23 (70%) out of 33 patients with skin nodular BCC. Recurrence-free follow up is 8 years. Patients with partial tumor regression were treated by a multicourse PDT. Complete regression was obtained in 4 out of 10 patients.

We believe that individual approach to the choice of a photosensitizer and treatment pattern proved to be the optimal one in PDT of primary multiple BCC. We could recommend technique of a topical or systemic multicourse PDT.

P-54

Mucosectomy and photodynamic therapy of early esophageal cancer

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According to world statistics, the number of esophageal cancer cases as well as mortality of patients with these diseases has been constantly increasing during the last decades. Malignancies are diagnosed in people older than 50—60 years old more frequently than in younger people. That is why the percent of elderly patients with pronounced concomitant diseases and early forms of malignant tumors of this localization is rather high. These patients cannot be cured using surgical methods because of their concomitant diseases, so new treatment methods preserving organs and sparing their functions should be developed.

In P. Hertzen Moscow Research Oncology Institute methods of mucosectomy (ME) and photodynamic therapy (PDT) have been used for treatment of patients with stage I (Tis-I N0M0) of esophageal cancer since the 1992.

In 1992—2007, ME and PDT were performed in 48 esophageal cancer patients (48 lesions). For treatment we used endoscopic equipment by Olympus (Japan). For ME we used endoscopic loops by Olympus (Japan). For PDT we used Russian photosensitizers (Photogem (hematoporphyrin derivative), Photosens (aluminum sulphonated phthalocyanine), Radachlorin (E6 chlorin), Alasens (delta-aminolevulinic acid)), Russian diode lasers (Crystall) with different wavelengths.

As a results of ME and PDT complete regression were in 77% of esophageal cancer lesions. The follow-up period was 3—11 years. Median of survival was in 4.59 years.

The developed methods of ME and PDT are successful for treatment of early esophageal cancer and can be an alternative to surgical treatment especially of patients with serious accompanying pathology.

P-55

Initial experience of photodynamic therapy (PDT) in prostate cancer (PC) patients

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The aim of our study was to develop method of PDT in PC patients and evaluate side effects of this procedure. PDT was performed in nine patients with verified PC. Four patients have undergone PDT prior radical prostatectomy, in five patients PDT was performed after external beam radiation therapy. Median PSA level was 21 ng/ml. Gleason score was 5—6 in 4 patients, 7 in 4 and 8—10 in 1. Mean patient's age was 59 ± 7.6 years. In all patients trunsrectal ultrasound imaging (TRUS) was done. Median prostate volume was 30 cm^3. Two hours before PDT photosensitizers (Radachlorin: five patients and Photosens: four patients) were injected of intravenous infusion of was performed. TRUS-guided installation of 4—5 needles (18 Gauge) was performed into the prostate gland to deliver optical fibers. The number of radiation points varied from 4 to 15. The needles and fibers were moved simultaneously to expose the whole volume of prostate gland. Light irradiation (662 and 670 nm, energy on one position: 120—200 J) was used during PDT.

No complications were observed. The median PDT time was 46.8 min. Mean time of hospital stay was 4 ± 1 days. Urethral catheter was removed on the 3rd day after PDT. There were no post-operative complications in patients who had undergone radical prostatectomy after PDT.

PDT is a microinvasive method of PC treatment with potentially few numbers of side effects. In preliminary results obtained from a small number of patients we can conclude, that PDT could be an alternative method of treatment in PC patients not eligible for surgery or radiation therapy.

P-56

Photodynamic therapy for papilla of Vater cancer and common bile duct cancer

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During the period from 1992 till 2007 over 1500 patients were treated with Photodynamic therapy (PDT) in the State research Center for Laser Medicine.

Successes of photodynamic therapy in treatment of a cancer both external and internal localizations have given the basis for application of this method in treatment of bile duct cancer and a cancer of periampular zone.

In present time we have experience of treatment by PDT of 17 patients with a papilla of Vater cancer and a common bile duct cancer with extension to wall of duodenum and to head of pancreas at some patients. Photosensitizer (Photosense and chlorine derivatives) was entered intravenously to all patients.

Depending on localization of tumor, its extension and previous medical procedures we used some ways of leading of light.

The diagnosis of all patients is histologically proven: 14 patients had adenocarcinoma of various differentiation grades, 3 had cancer (without additional definition). Seventeen patients have been leaded 22 PDT treatments (5 PDT treatments have been leaded repeatedly in connection with the continued growth of the tumor in 2, 4, 6, 7 and 21 months). In this group of 17 patients 12 survived and are being followed-up in terms from 6 months to 5 years; 4 patients died of tumor progression, 1 patient died of accompanying infection in 3.5 months after PDT. At 7 patients treated more than 1 year ago, survival time ranged from 14 up to 60 months. Medial survival time is 2.9 years.

PDT reduces cholestasis, improves quality of life and elongates survival time of the patients. Results of PDT on life expectancy quite comparable with radical operations also exceed those for palliative operations. Delay of growth rates of tumors after PDT and increase in life expectancy in patients with residual tumors, most likely, is caused by the vascular mechanism of PDT action therefore blood supply of a tumor is damaged, as provides long stabilization of process.

P-57

Photodynamic therapy of unconvenient locations in skin cancer

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The traditional methods of skin cancer treatment are surgical excision and close-focus X-ray treatment. However, in case of unconvenient locations, e.g. skin cancer on a face, auricles, the hairy part of a head, and also in case of multiple foci, these methods are not applicable or bring about complicated functional and cosmetic defects. In this regard, unconvenient locations are eyelids, the tails of eyes, the wings of a nose, nasolabial folds, auricles, an external aural canal. The problem consists of that from 70 up to 80% of skin cancer is localized on the head and neck, and 30—40% of foci may be regarded as unconvenient locations.

We studied 104 patients with skin cancer of unconvenient locations (on eyelids or periorbital areas, on a nose, in the area of nasolabial folds, on auricles and on the skin of external aural canal). Primary cancer was diagnosed in 47 patients. 57 patients had recurrent cancer after the treatment applied to them earlier (surgery, close-focus X-ray treatment, cryodestruction, electro- and laser coagulation).

For photodynamic therapy (PDT) we used Russian-made photosensitizers (Photohem, Photosense, Photoditazine and Radachlorin), as well as Photolon made by the Belorussian ''Belmedpreparaty'' company, and Foscan (temoporfin) made by the German ''Biolitec AG'' firm (Jena, Germany). For irradiation of tumors we used lasers with a wavelength that corresponded to the absorption peak of a photosensitizer: for Photohem it was 630 nm, for Photosense — 670 nm, for chlorine photosensitizers (Photoditazine, Radachlorin, Photolon, Foscan) it was 662 nm.

The therapeutic effect was achieved in all patients who had been treated. A complete tumor resorption was observed in 77 patients (74%), whereas 27 patients (26%) showed a partial tumor resolution. A complete resorption of primary tumors was up to 91.5% (43 out of 47 patients), and of recurrent cancer—up to 59.6% (34 out of 57 patients). Due to the healing of a tissue defect after resolution of a tumor by type of reparation, but not scarring, absolutely most patients have got good cosmetic results.

PDT is a good alternative method of treatment for skin cancer localized on a face, auricles, the hairy part of a head, and on a neck, which makes it possible to achieve recovery with good and perfect cosmetic results in most patients.

P-58

An integrated system for light delivery and online light dosimetry for interstitial photodynamic therapy

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The SpectraCure interstitial PDT system uses up to 18 optical fibres, coupled to a bank of interchangeable laser diodes, for light delivery. Using an optical switch, the system rapidly changes from light delivery mode to spectroscopic monitoring mode utilizing the same optical fibres. Dosimetry calculations are carried out by the integrated software, interactive dosimetry by sequential evaluation (IDOSE). IDOSE consists of four core computation modules: (1) generation of a 3D tissue geometry; (2) an optimizing algorithm to establish fibre positions; (3) a tissue optics module to determine the optical properties of the tissue; and (4) a dose planner, based on Cimmino's method, to find the optimal irradiation times. The first two modules are part of the pre-dose planning only, while the latter two modules are also used in real-time during treatment to update the dose plan to account for changes in the tissue.

The system may be adapted to suit different medical indications and photosensitizers. In the case of PDT of prostate cancer, pre-dose planning is performed by transrectal ultrasound guidance. Once optimal fibre positions are established by IDOSE, fibres are inserted aided by a needle template attached to the ultrasound stepper. A first spectroscopic monitoring sequence is performed, and the dose plan is updated to account for patient-specific optical properties. Light delivery is then initiated, and is interrupted at regular intervals for intermediate monitoring to enable online adaptation of the dose plan by IDOSE. A description of IDOSE and dose planning results will be presented.

P-59

Inactivation of staphylococcal virulence factors using light-activated antimicrobial agents

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One of the limitations of antibiotic therapy is that even after successful killing of the infecting organism, virulence factors may still be present and cause significant damage to the host. Light-activated antimicrobials show potential for the treatment of topical infections; therefore if these agents can also inactivate microbial virulence factors, this would represent an advantage over conventional antibiotic treatment. *Staphylococcus aureus* produces a wide range of virulence factors that contribute to its success as a pathogen by facilitating colonisation and destruction of host tissues. In this study, the effect of the light-activated antimicrobial agent methylene blue in combination with laser light of 665 nm on the activity of staphylococcal virulence factors was investigated. Virulence factors were exposed to laser light in the presence of methylene blue and their activities determined. The activities of V8 protease, alpha-haemolysin and sphingomyelinase were shown to be inhibited in a dose-dependent manner, suggesting that photodynamic therapy, as well as killing *S. aureus*, may also be able to reduce its virulence potential.

P-60

Photocytotoxicity of mTHPC (temoporfin) loaded polymeric micelles mediated by lipase catalyzed degradation

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Meta-tetra(hydroxyphenyl)chlorine (mTHPC, temoporfin) is a second-generation photosensitizer, which is currently clinically used as a formulation in ethanol and propylene glycol (Foscan®). It is registered for the palliative treatment of advanced head and neck squamous cell carcinoma. In this report, we describe a novel mTHPC formulation in biodegradable polymeric micelles composed of the penta(ε-caprolactone) ester of monomethoxy poly(ethylene glycol) (750 Da) containing a terminal benzoyl group at the hydrophobic block. Physically stable mTHPC loaded micelles were prepared by hydration of a mixed film of the photosensitizer and the polymer, with an extremely high drug loading capacity of up to 30% (w/w), resulting in a solubilisate of 3 mg/mL. mTHPC-loaded micelles at a relatively high polymer concentration (0.5 mg/mL polymer) above the critical aggregation concentration (CAC) did not display photocytotoxicity up to an mTHPC concentration of 2 μM, nor any mTHPC-uptake by UM-SCC-14C cells at 10 μM mTHPC, in contrast to free mTHPC and liposomal mTHPC (Fospeg®). Interestingly, after dilution of the loaded micelles below the critical aggregation concentration (CAC), or after incubation with lipase causing micelle degradation, photocytotoxicity and cellular uptake of mTHPC were restored. In conclusion, the high loading capacity of the micelles, the high stability of mTHPC-loaded micelles above the CAC, and the lipase-induced release of the photosensitizer makes these micelles very promising carriers for photodynamic therapy *in vivo*. Biodistribution and therapeutic efficacy studies are currently performed in animal tumor models.

P-61

MAL-PDT of in situ, microinvasive and invasive squamous cell carcinoma

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Background: Photodynamic therapy (PDT) with methyl-aminolevulinate (MAL) is an approved non-invasive treatment option for actinic keratosis (AK) and Bowen's disease (BD), two precursors of squamous cell carcinoma (SCC).
Objective: To assess efficacy, prognostic features, tolerability and cosmetic outcome of MAL-PDT for the treatment of SCC.
Patients/methods: A total of 112 biopsy-proven BD and SCC in 55 subjects were treated in an outpatient setting. MAL cream (160 mg g^{-1}) was applied for 3 h prior to illumination with a light-emitting diode source (LED) (wavelength range: 635 ± 18 nm; light dose 37 J cm^{-2}). A second MAL-PDT session was given 7 days later. Complete response rate at 3 months after the last treatment, recurrence rate at the 24 months follow-up, and cosmetic outcome were recorded.
Results: The overall complete response rates were 73.2% at 3 months and 53.6% at 2 years. Clinical thickness, atypia and lesion depth were significant predictors of the response at 3 months when using a univariate analysis ($p < 0.001$). Cell atypia was the only statistically significant independent predictor of the treatment outcome at 3 months.
Conclusion: MAL-PDT may represent an effective and well-tolerated treatment option with good cosmetic outcome for superficial, well-differentiated (Broders' scores I and II) BD and microinvasive SCCs. In contrast, its use for superficial SCCs with a microinvasive histological pattern and for nodular, invasive lesions, particularly if poorly differentiated keratinocytes are present (Broders' score III and IV), should be avoided.

P-62

Treatment of photodamaged facial and scalp skin using MAL-PDT

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Efficacy and tolerability of photodynamic therapy (PDT) with aminolevulinic acid (ALA) in the treatment of photodamaged skin, so-called photorejuvenation, have been reported in a few recent studies. However, results are hardly comparable because there were evaluated only on a clinical basis and the treatment protocols, e.g. formulation and concentration of the cream, application time, spectrum and dose of the activating light, number and frequency of treatments, varied widely.

In the present study, 20 patients with pronounced photodamage and actinic keratoses (AK) of the face and scalp were treated with two monthly treatments with a proprietary preparation containing 160 mg/g of methylaminolevulinate (MAL) (Metvix®, Galderma, F) according to the standard treatment protocol that is approved by the European regulatory authorities for the treatment of AK. In brief, Metvix® was applied under occlusion for 3 h before exposure to 37 J/cm^2 of red light that was delivered by a LED source (Aklilite® CL 128, Photocure, N).

Improvement of different clinical signs evaluated separately according to a clinical scale and, in addition, photodamaged skin has been evaluated with high-resolution 20 MHz B-mode ultrasound scanner before and after the last treatment.

Treatments were well tolerated with only a mild and transitory pain and burning. Soon after the treatment, the skin showed diffuse erythema and oedema that were more pronounced with crusting in

the AK areas. These changes resolved spontaneously without scarring or pigmentary alterations within 5 days.

MAL-PDT could represent a novel, effective and well-tolerated non-invasive treatment of photodamaged skin.

P-63

Set-up for fluorescent diagnosis and PDT of brain tumors in combination with surgery

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To date PDT along with a fluorescent diagnosis is being studied extensively under clinical conditions as an adjunctive treatment in the neuro-oncological field. In the clinical trials implicated we have applied laser system, fluorescent devices and other equipment produced by Biospec Company.

LED light source UFPh-630/675-01-BIOSPEC allows to provide a fluorescent monitoring simultaneously combined with surgical operation, while an integrated high sensitive imaging system gives opportunity to observe fluorescent image of superficial tumors on the display screen and determine borders of tissue areas with high PPIX accumulation. As a photosensitizing drug we used aminolevulinic acid (5-ALA) to induce protoporphyrin IX, which has been orally administered in 2 h prior to operation.

The variable power range of the laser system LPhT-630-01-Biospec enables effective photodynamic therapy of different kinds of tumor. This laser system emits 630 ± 1 nm red light with the maximum power output of 1.5 W. PDT administration proposed to apply polymeric fibers of a 450 μm diameter with cylindrical diffusing distal ends tailored in such way that one or two fibers could be reserved near tumor localization. These polymeric fibers have been subsequently used as a delivery system for repeated PDT administration in 1 or 2 days.

Alternative to fluorescent video scope a spectroscopic system LESA-01-Biospec has been substituted in some cases. For example, a fluorescent image did not give a noticeable contrast under LED light source excitation of a melanoma malignant growth in contrast to a spectroscopic in situ study simultaneously with surgical removal of the tumor.

Ongoing clinical trials are fulfilling in Moscow Hertzen Research Oncology Institute, in department of professor V.V. Sokolov. All clinical results and its analysis in full measure will be presented elsewhere.

P-64

Multifibre application in PDT of a prostate

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We have tested PDT of prostate lesions with 670 nm laser light and aluminium phtalocyanine derivative as a photosensitizing drug. All experiments and clinical trials presented have been performed using equipment produced by Biospec Company. There have been developed an original laser system for photodynamic therapy LPhT-670-01-Biospec designed especially for prostate irradiation. The laser system consists of seven channels, one of which has been advisedly constructed to control any single channel and the total output power of 670 nm light up to 2.8 W. For PDT administration in prostate lobes typically four quartz 600 μm optical fibers with bare distal ends compose the delivery system based on the placement technique, which has been already established in brachytherapy under ultrasound guidance. PDT is best achieved by irradiating at the time of maximum concentration in the vasculature, which typically occurs in 24 h following intravenous injection of the aluminium phtalocyanine derivative. Patients were treated with the drug dose ranging from 0.3 to 0.6 mg/kg. In the first trial five fibers were placed in the prostate with the primary goal to demonstrate safety of PDT, so that the power supply per channel did not exceed 200 mW. In subsequent trials a light dose and a fluence rate were increased depending on a prostate volume and a number of channels involved.

Since the cancer is typically localized within the organ, the treatment can be based on irradiation the whole of the prostate rather than trying specifically to target tumor tissue. That is why seven-channel laser is more suitable in this case. Such whole-organ treatment is preferred, since there are no clinically adverse effects and it is also difficult or impossible to determine reliably all the tumor regions using current imaging techniques. Clinical trials are taking place now in Hertzen Research Oncology Institute, in department of professor V.V. Sokolov.

P-65

Fluorescent image analyzer for FD and in PDT of stomach cancer

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A laparoscopy in stomach tumor diagnosis is a minimal invasive and promising method, which could be combined with a laser fluorescent spectroscopy. We have designed an analyzer of fluorescent image adapted to an ordinary laparoscope. Such analyzer consists of a CCD camera, an adapter and a filter.

In order to excite the fluorescence of a targeted tissue we used a laser 635 nm red light emitted by LPhT-635-01-Biospec laser system. For this excitation wavelength fluorescence can be observed nearby 700 nm spectral range. Laparoscopic study of stomach cancer from a peritoneum side has been provided in 2 h after aminolevulinic acid (5-ALA) administered orally. We proposed to use high sensitive video camera for fluorescent image registration, and another one to observe target in white light. The video camera switching takes about 2 or 3 s by means of a device for changing filters.

Ongoing clinical trials are taking place in I.M. Sechenov Medical Academy, Moscow. Twenty patients with stomach cancer have been well studied with video fluorescent scope. This method allows to display an invasion of serous tunic of stomach wall, a metastatic lesion of peritoneum and regional lymphatic nodes accessible for inspectation.

The video fluorescent laparoscope designed gives opportunity to a physician more carefully appoint the borders of the tumor and its extent, what leads to an improvement of laparoscopic inspectation efficacy.

P-66

Topical 5-ALA photodynamic therapy for the treatment of urethral condylomata acuminata

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Objective: To investigate the effect of photodynamic therapy (PDT) with topical 5-aminolevulinic acid (ALA) on urethral condylomata acuminata and to examine the histological changes in lesions of condylomata acuminata after ALA-PDT.

Methods: One hundred and sixty-four urethral condylomata patients were given topical ALA followed by intraurethral PDT through

a cylindrical fiber. Histological changes were evaluated by light microscopy and electron microscopy on biopsy specimens collected after ALA-PDT from 16 penile and vulval condylomatous lesions in 11 patients.
Results: The complete response rate for urethral condylomata by topical ALA-PDT was 95.12% and the recurrence rate was 5.13% after 6—24 months follow-up. Keratinocytes in middle and upper layers of the epidermis with marked vacuolation and some necrocytosis were detected 1 and 3 h after PDT. Necrosis in all layers of the epidermis was noted 5 h after PDT by microscopy. In electron microscopy of kerationcytes, distinct ultrastructural abnormalities of mitochondrion, endoplasmic reticulum and membrane damage were observed. Apoptotic bodies were detected 3 h after PDT and a large number of the keratinocytes exhibited necrosis 5 h after PDT by electron microscope.
Conclusions: Results suggest that topical ALA-PDT is a simple, effective, relatively safe and comparatively well-tolerated treatment for urethral condylomata acuminata, characterized by a low recurrence rate. The mechanisms might be that ALA-PDT could trigger apoptotic process and necrosis in the HPV infected keratinocytes.

P-67

Study of protoporphyrin IX (PpIX) pharmacokinetics after topical application of 5-aminolevulinic acid in urethral condylomata acuminata

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Objectives: To investigate the pharmacokinetics of ALA-induced protoporphyrin IX (PpIX) in lesions of urethral condylomata acuminata.
Methods: Sixty patients with urethral condylomata acuminata were divided randomly into five groups to receive different concentrations of ALA solution. The ALA solution was applied topically to the lesions for a different length of time. Biopsy was performed at predetermined time and specimens were subjected to histological, PpIX fluorescence and HPV DNA typing analyses. The fluorescence intensity in corneous layer, epidermis, dermis, rete Malpighii, and koilocytes layer were quantified using an imaging analysis software.
Results: ALA-induced PpIX fluorescence was dominantly distributed in the epidermis and rete Malpighii regions. The maximal fluorescence intensity was detected at the 5 h of incubation. Higher ALA concentration (e.g. 5 and 10%) produced stronger intensity. In contrast, only the minimal amount of PpIX fluorescence was detected in the dermis. DNA typing indicated that all patients were positive for the low risk-HPV DNA and among them 18.3% patients harbored the high risk-HPV DNA.
Conclusions: The results suggest that the topical application of 5—10% ALA solution for 3—5 h are the optimal conditions for ALA/PpIX-mediated photodynamic therapy for the treatment of urethral condylomata acuminata.

P-68

5-Aminolevulinic acid photodynamic diagnosis in the diseases caused by HPV

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Objective: To investigate *in vivo* the PpIX fluorescence diagnosis after topical application of 5-aminolevulinic acid (ALA) in condylomata acuminata and *Bowenoid papulosis*.
Methods: Photodynamic diagnosis (PDD) was performed on the lesions, subclinical lesions and latent HPV infection of 36 patients with condylomata acuminata (clinical diagnosis) after 2 h application of ALA. And acetic acid test, histopathologic examination and HPV DNA measurement by gene array technique were employed in this study.
Results: The diagnosis of condylomata acuminata in 30 patients, Bowenoid papulosis in 5 cases and keratosis seborrheica in one case (rejected) were made by histopathologic examination. All the lesions and subclinical lesions were presented PpIX fluorescence and harbored HPV DNA. Some areas of latent HPV infection also presented PpIX fluorescence. Nonspecific PpIX fluorescence easily was found in the areas of mucosa, inflammatory infiltration and erosion lesions.
Conclusion: ALA-PDD could be employed for the diagnosis of the lesions, subclinical lesions caused by HPV. Some areas only harbored HPV also could be observed by ALA-PDD. However, we do not commend ALA-PDD for the lesions located on mucosa, inflammatory infiltration and erosion lesions.

P-69

The role of MAPK signalling in photodynamic therapy with a photosensitizer relevant for photochemical internalization

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Photochemical internalisation (PCI) is a method for release of endo/lysosomally trapped drugs into the cell cytosol. PCI is based on photosensitizers that accumulate in the membranes of endosomes and lysosomes. Light exposure generates reactive oxygen species that cause membrane rupture and drug release. PCI is a combination therapy of photodynamic therapy (PDT) and the administrated drug. The present work reports on MAPK signalling after PDT with the endocytically located photosensitizer $TPPS_{2a}$ (*meso*-tetraphenylporphine with 2 sulfonate groups on adjacent phenyl rings) as used for PCI in two cancer cell lines. ERK and p38, is activated immediately after PDT in both cell lines. Expression of the ERK phosphatase MKP-1 is increased 2 h after PDT but is not the cause of ERK dephosphorylation observed simultaneously. A transient activation of JNK was also observed after PDT, but only in the NuTu-19 cell line. Using suitable inhibitors we show that the p38 signal is a death signal, while JNK rescues cells after PDT. No connection was observed between the PDT-induced ERK activation and toxicity of the treatment. The present results document the importance of the MAPKs in $TPPS_{2a}$-PDT-induced cytotoxicity.

P-70

Systematic approaches to preclinical study of novel dyes and based on them officinal preparations for PD and PDT of malignant tumors

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The absence of standard methodic approaches complicates the comparison of antitumor photosensitizing efficiency of different substances and preparations and their preclinical study.

Basing on experience in study of above 200 dyes of different classes we developed three-stage multiparameter program of photosensitizers study including screening *in vitro* and *in vivo* (I stage),

in-depth study *in vivo* (II stage), and preclinical study (III stage).

The screening includes study of spectral-luminescent properties of dyes in aqueous media (containing salts, surface active compounds, proteins etc.), photo-induced and ''dark'' phototoxicity *in vitro* on mammalian cells, antitumor activity *in vivo* on mice with standard transplantable malignant tumors of different histogenesis.

As model *in vitro* a suspension or fastened cell cultures of at least three cellular lines are used varying parameters: seeding cell concentration, incubation time, light dose (5, 10 and 20 J/cm^2). Growth inhibition was evaluated using IC_{50} and IC_{90}.

In vivo biodistribution of dye (from 15 s till some days), retention duration in tissue and antitumor activity depending on dose, drug-light interval, regime and parameters of irradiation were assessed. As a criteria of efficiency serve tumor growth inhibition (TGI), life span increase (LSI) and recovery coefficient (RC).

Preclinical study: of perspective substance includes development of its clinical and consumer forms and optimal treatment regimes. The efficient photosensitizer would have RC = 80—100%, TGI $\geq$ 50%, LSI $\geq$ 80% during 20 days, low skin toxicity. PDT mechanism considering dye distribution in tissue and cells is also studied.

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P-71

''Hexasens-lyo''—New preparation for PDT based on 5-aminolevulinic acid hexyl ester

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One of the most known photosensitisers explored in fluorescent diagnostics and photodynamic therapy (PDT) is endogenous protoporphyrin IX. The main way of its accumulation in the cancer tissue is the stimulation of its production by 5-aminolevulinic acid (ALA) or its alkyl esters[1]. The latter ones as more lypophilic than ALA more effectively intercalate into a cell and could be more attractive source of protoporphyrin IX production in high concentration. We report here our preliminary results of investigation of the lyophilized ALA hexyl ester hydrochloride — ''Hexasens-lyo'' — in PDT. Starting ALA hexyl ester was prepared by esterification of ALA according to Ref. [2].

Evaluation of ''Hexasens-lyo'' specific activity in PDT both *in vitro* and *in vivo* exhibited its high therapeutic efficacy. Topical administration of ''Hexasens-lyo'' on the skin of animals with transplanted sarcoma S-37 and subsequent PDT results in long-term (over 14 days) antitumor effect (tumor growth inhibition of 71—100%).

The intravesical administration of ''Hexasens-lyo'' into rabbit bladder leads to effective induction of endogenous protoporphyrin IX synthesis in bladder epithelium, and minimal effective concentration of aqueous solution of ''Hexasens-lyo'' is much less than for ALA—0.0001% and 0.03%, correspondingly.

Pre-clinical investigations of ''Hexasens-lyo'' have been conducted to evaluate its toxic level at parenteral and topical (in bladder) administration. It was found that ''Hexasens-lyo'' is not toxic for peripheral blood and inner organs of animals in the wide range of the doses.

Acknowledgement

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P-72

Investigation of heat transfer in deep tissue layers under laser irradiation

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It is very important to control the temperature during the photodynamic therapy (PDT) in order not to achieve thermal tissue destruction. The heat transfer in biotissue is greatly influenced by blood flow, thus it is rather hard to estimate the temperature of the inner layers of living tissue. The purpose of this work was to investigate the deep temperature field in the living tissue with a dense vessel net during laser irradiation.

The experiment was carried out on a rabbit ear, which was about 3 mm thick and possessed a dense blood vessel net. The ear was irradiated by a laser irradiation of 810 nm wavelength. The light power density varied in the range from 0.5 W/cm^2 to 2.3 W/cm^2 while the ear was heated up to 54 °C. While irradiating the ear 2D thermo-images in the spectral range 3—5 μm of both sides of the ear were taken, using two infrared cameras. The dynamics of heat transfer due to blood flow was analyzed and the temperature fields from both sides of the ear were compared. Basing on the images of surface temperature distribution the heat transfer inside the irradiated tissue was reconstructed. The results could be used for light dosimetry optimization during PDT.

P-73

Photodegradation of hematoporphyrin in solution: Anomalous behavior at low oxygen concentration

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The photoactivation of a photosensitizer is the initial step in photodynamic therapy (PDT) that causes photochemical reactions, resulting in the production of reactive oxygen species and eventually cell death. In addition to oxidizing biomolecules, some of these photochemical reactions lead to photosensitizer degradation at a rate dependent on the oxygen concentration among other factors. We investigated photodegradation of Photogem a hematoporphyrin derivative, at different oxygen concentrations in aqueous solution.

The degradation was monitored by fluorescence spectroscopy. The degradation rate (M/s) increases as the oxygen concentration increases when the molar ratio of oxygen to Photogem is greater than 1. At lower oxygen concentrations an inversion of this behavior was observed. The data was fit to a kinetic model employing second-order dependence with the photosensitizer concentration and numerical modeling of the time dependent photodegradation reproduces well-obtained data. The importance of oxygen concentration on the photosensitizer degradation is discussed in this paper.

P-74

Optical fluorescence diagnostic for cancer and other lesions

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The use of fluorescence spectroscopy as an alternative technique applied biopsies is one of the most technologically innovative since optical biopsy is not an invasive technique. This technique presents high sensibility, simplicity and speed. Optical biopsy is an alternative for the characterization of biological tissues, evaluation of physiological conditions and diagnosis of malignity of organs and tissues (cancer, tooth, cirrhoses hepatic and others) cancer diagnostic:

The laser-induced fluorescence can provide information of the biochemical composition and the tissue architecture, in this way, each biological tissue shows a spectrum signature. This technique also called point spectroscopy evaluates the re-emitted light collected from the target tissue after the light excitation. Differences in tissue components and structure alter the fluorescence emission, its intensity and also behavior, so the normal and the cancer tissues can be discriminated using fluorescence analysis.

P-75

Prediction of cervical intraephitelial neoplasia response to photodynamic therapy using in vivo fluorescence imaging

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The aim of this study was to evaluate an ability of in vivo fluorescence imaging to predict the response of photodynamic therapy of the cervical intraepithelial neoplasia (CIN-PDT).

PDT of exocervix was performed 3 h after i.v injection of Photolon (water-soluble drug composed of a chlorin e6 and polyvinylpyrrolidone) in dose 2—2.5 mg/kg of body weight. The diode laser (670 nm) had been used for irradiation. The colposcopic and fluorescence images were registered before and immediately after PDT by the upgraded colposcop, equipped with LED light source for fluorescence excitation and a high sensitivity CCD camera for fluorescence registration. Images were collected from the cervix of 10 female patients (age 19—35 years) with verified CIN grades II—III. PDT response was studied by analysis of colposcopic images of necrosis, registered 3 days after treatment, and post-PDT fluorescence images.

The fluorescence intensity registered before PDT either by fluorescence imaging measurements or by point-by-point spectroscopic measurements directly from the tissues displaying similar trends of distribution. After PDT fluorescence intensity at the irradiated area becomes significantly lower than before treatment due to photosensitiser photobleaching. Necrosis area correlates well with borders of photobleached region on fluorescence images.

Fluorescence imaging allows to predict a CIN-PDT response by estimation of the irradiated area photobleaching. Application of fluorescence imaging in clinical practice may be used to improve efficiency of treatment and reduce the side effects and recurrences.

P-76

Photodynamic therapy of women with high-grade cervical intraepithelial neoplasia using Photolon®

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The objective of this study was to evaluate the outcome of the photodynamic therapy with ''Photolon'' (composed of chlorin e6 and polyvinylpyrrolidone) for treatment of the associated with human papillomavirus (HPV) high-grade CIN.

Fifty-seven patients with CIN (9 with CIN-II, 41 with CIN-III) and 7 with carcinoma in situ were undergone the PDT. Irradiation was performed 3 h after i.v. drug injection at a dose of 2.0—2.5 mg/kg by diode laser (670 nm). Cervix was irradiated superficially with light dose 50 J/cm^2 (5 patients) or 100 J/cm^2 in other 52 cases. Endocervix was irradiated by fiber optic with cylindrical diffuser and the light dose 100—300 J/cm diffuser length. The PDT outcome was estimated every 3 months after treatment by the expanded colposcopy, morphological and polymerase chain reaction tests.

Thirty patients have been observed for less than 1 year, 17 patients between 1 and 2 years and 10 patients for more than 2 years after PDT. Complete response to PDT, proven by cytological and histological tests, was observed in case of 53 patients (93%). HPV was detected in 48 out of 57 observed patients (84%). Despite 3 months after PDT HPV detected on only 12 patients (25%), 6 month after PDT the number of HPV positive patients increased to 25 cases (52%), possibly, due to reinfection.

PDT with Photolon is an effective method for the treatment of high-grade cervical intraepithelial neoplasia.

P-77

Use of the ^{67}Ga radionuclide imaging for PDT-response observation in experimental model of peritoneal carcinomatosis

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Peritoneal carcinomatosis is generally an incurable condition with a poor prognosis. Palliative treatment is based on tumor-debulking surgery combined with either intraperitoneal chemotherapy or hyperthermic peritoneal perfusion. Intraperitoneal photodynamic therapy (i.p. PDT) is a promising method because of its superficial treatment effect. The aim of this study was to develop an experimental model of peritoneal carcinomatosis in rats and evaluate this method for non-invasive estimation of response to i.p. PDT by radionuclide imaging.

The experimental model of intraperitoneal carcinomatosis was developed by intraperitoneal injection of carcinoma RS-1 cell suspension. When tumors developed in peritoneal surface of abdomen, two rats were subjected to the i.p. PDT with ''Photolon'' sensitizer during laparotomy by laser with emission wavelength 670 nm. Light dose was 30 J/cm^2 for tumor and 5 J/cm^2 for bowel. To assess non-invasively the tumor response to PDT we performed radionuclide imaging of rat after gallium [^{67}Ga] citrate injection before and after i.p. PDT. ^{67}Ga uptake was quantified using a region-of-interest (ROI) method resulting in tumor:background (T:B) ratios. The observed average T:B ratio after PDT was significantly lower than T:B ratio before treatment (1.18 vs. 2.54, respectively), probably, because of developed ischaemia of tumor with subsequent necrosis. Thus ^{67}Ga radionuclide imaging allows to estimate the i.p. PDT response in animal model of peritoneal carcinomatosis.

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