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ANNOUNCEMENT

1st German–Russian Oncology Symposium, Munich, Germany; 25–26 June 2010

On 25th and 26th June 2010, the 1st German–Russian Oncology Symposium for Oncology was held at the University Clinic Munich, Großhadern. Presenters from Germany and Russia gave an insight into scientific updates from basic to clinical research. The main topics of this symposium were medical laser applications such as photodynamic therapy, laser applications in surgery, urology and gynecology, tissue imaging and hyperthermia. In addition to these presentations, participants had the opportunity to discuss topics with Russian and German specialists at special round table events in order to initiate new contacts and possibilities for future cooperation. An exhibition showed new commercial products and developments from the sponsors. In this context, the organizational committee would like to thank all partners for their dedicated support during the organization and realization of the symposium.

We would also like to extend our very sincere thanks to all the participants who helped to make the 1st German–Russian Oncology Symposium a productive, successful, and enjoyable conference where colleagues and friends could meet each other.
Yours,
Michael Fedorov
Symposium Chair

Symposium Chair
Dr. Michael Fedorov, Munich, Germany

Organizational Committee
Dr. Reinhold Baumgartner, Munich, Germany
Felix Schade, Munich, Germany
Christian Steiner, Munich, Germany
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Abstracts

Basics

[1] Biophotonics in oncology

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Looking back at the development of lasers and the influence they have had on oncologic surgery, the first step was made with the introduction of the CO_2 laser by Isaac Kaplan. The development of power transmitting fibers was the second step, optimally synchronized with the development of endoscopic surgery and promoted by gynecologists, urologists and pediatric surgeons alike. The era of the thermal lasers had begun and numerous surgical techniques were developed, some of which are now routine procedures and others which have been disregarded. The concept of *in situ* destruction of diseased tissues by interstitial laser coagulation offered a minimal invasive option and is still used today in oncology for the destruction of liver metastases. In the course of time, the less surgical but more elegant photodynamic therapy (PDT) has been re-invented, delivering a new and enlightened option for cancer treatment and is constantly gaining in importance. Major future developments are to be expected both in this field and diagnostics. Fluorescence spectroscopy, fluorescence imaging and optical coherence tomography (OCT), to name just a few, are currently being investigated for their use in oncologic imaging and surgical feedback systems. New technical concepts in lasers are being discovered and may lead to new surgical systems with specialized applications of a high precision, being less bulky and more affordable compared to current surgical systems. Multiphoton effects, nanomedicine and photointernalization also offer new solutions for old problems. Drug–light interaction may not be limited to the current strategies.

[2] Network for optical technologies in photodynamics

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Tissue light interaction in the visible or near infrared spectral range enables precise and effective therapeutic modalities to diagnose or cure numerous diseases. In photodynamics, light is absorbed in different tissue chromophores and the corresponding energy can be converted either to heat and fluorescence, or can generate reactive oxygen species.

The major issues are fluorescence diagnosis to assist tumor detection, fluorescence life-time imaging to non-invasively measure oxygenation of tissue, laser medicine, and the destruction of either tumor cells or bacteria by means of reactive oxygen species. These supposedly different issues are based on the same mechanisms that are called photodynamic processes.

Currently photodynamic processes are widely applied in different medical fields that still show a high potential for generating new and innovative procedures. In addition, photodynamic processes are not necessarily restricted to medical applications.

In order to exploit the innovative potential of photodynamics effectively, different research institutions should collaborate with companies and the users of such new products and procedures. To facilitate this collaboration and to accelerate the process of innovation in photodynamics, a new network has been recently founded in Bavaria, called ‘Optische Technologien in der Photodynamik’ (Optical technologies in photodynamics, (OTPD)) (see also: http://www.sensorik-bayern.de/?lang=de&site_id=504). The OTPD network consists of two renowned photodynamic groups in Munich and Regensburg, as well as several companies from all over Germany.

Photodynamic therapy (PDT)

[3] New developments in photodynamic diagnosis and therapy

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From March 2009 to April 2010, the number of clinical studies on “photodynamics”, listed in “clinicaltrials.gov” increased from 158 to 206. Photodynamics is thus gaining more and more clinical acceptance. The broadest fields are the treatment of choroidal neovascularization (AMD), currently in combination with antiangiogenic drugs, and skin lesions. However, 23 more areas of application are addressed by photodynamic therapy (PDT), including non-oncologic conditions such as acne, bacterial infections, benign prostate hyperplasia and even hair removal. A total of 22 different types of photosensitizers were listed in 2009. Diagnostic applications of photodynamics are not representatively covered by this search term, as is evident when scanning for “aminolevulinic”, where, for example, the studies dealing with fluorescence-guided resection of malignant glioma only then appear in the listing. An update on this search will be given in the conference.

Clinical PDT in most cases is still performed according to simple protocols and drugs and light dose parameters are prescribed without considering patient specific characteristics of drug accumulation and optical tissue parameters. Many groups are eagerly exploring possibilities to individualize

treatment regimes in order to obtain more favorable and stable clinical outcomes. With the same aim, much preclinical work is being performed to improve the efficacy of PDT-related treatment effects, in which the role of the immune system appears to play a major role. A subjective selection of recent research on these topics, which appears much more promising will be presented.

[4] Immune stimulation and tumor cells escape – The two sides of the coin of protoporphyrin IX-based photodynamic tumor therapy

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Objective: Photodynamic therapy (PDT) uses the combination of a photosensitizing drug and light to cause selective damage to solid tumors. For early or localized disease, PDT can be a curative therapy with many advantages over available alternatives. Curative therapy is usually not possible for more advanced or metastatic disease. However, there is increasing evidence that PDT can also induce systemic antitumor immunity, indicating that PDT could become a rational therapeutic option, even if not all tumor cells are primarily eliminated by PDT. One prerequisite to minimize tumor relapse in these settings is to counteract repair mechanisms of PDT-induced damage and to aid PDT-triggered immune stimulation.

Materials and methods: We have characterized the immune-stimulatory and rescue response of human prostate cancer (PC-3, Du145) and glioblastoma cells (U87, U373) *in vitro* and in murine TRAMP-C2 prostate tumors grown subcutaneously in albino C57BL/6-Tyr^{c-2J} mice exposed to sublethal low dose PDT after 5-aminolevulinic acid-induced protoporphyrin IX (PpIX) sensitization at the transcriptome level using Affymetrix oligonucleotide microarrays. Cells and tumors were irradiated with laser light at a wavelength of 635 nm adjusted to an irradiance of 100 mW/cm^2 with irradiations varying between 0.5 and 3 J/cm^2 and 75–100 J/cm^2 respectively. The normalized expression data were analyzed by comparing matched samples and by gene set enrichment analysis (GSEA). Selected cytokines secreted by PDT-treated PC-3 cells were quantified using a bead-based immunoassay (CBA).

Results: The early response was characterized by the up-regulation of early stress response genes like FOS, JUN, EGR1, ATF3, DUSP, heat shock protein (HSP) genes as well as histone and metallothionein genes, therefore, resembling the early response of tumor cells to high dose PDT but without signs of irreversible cell damage. Twenty-four hours after PDT the cells still express high levels of early response genes but additional probe sets/genes were significantly up-regulated. The most prominently up-regulated genes belong to gene families encoding HSP 40-related proteins and aldo–keto reductases, the latter being probably involved in detoxifying processes and might aid tumor cell survival. In terms of a possible anti-tumor immune response it is noteworthy that also a multitude of chemokines and interleukin genes, including CXCL2, CXCL3, IL1A and IL6, were up-regulated by the tumor cells upon PDT. Most of them are involved in granulocyte attraction and activation and indeed, the most significantly up-regulated sets of functionally coupled genes belong to inflammatory and granulocyte and mast cell activation pathways.

Conclusion: In conclusion, the global molecular characterization of the response to PDT of tumor cells indicates that PDT, besides inducing repair mechanisms, rather favors anti-tumor immune responses than tumor immune escape reactions. Therefore combining PDT and immunotherapy seems to be an attractive direction for the establishment of novel multimodal tumor therapies.

Acknowledgement: The work was supported by the Deutsche Krebshilfe (107320).

[5] Photosensitizers for PDT

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An assortment of photosensitizers for photodynamic therapy (PDT) and fluorescence diagnostic (FD) of oncologic and non-oncologic diseases was developed and some of them have found clinical application. The presentation gives an overview about some of them, namely:

- Photosens is composed of di- to tetra sulphonated aluminum phthalocyanines dissolved in distilled water. Photosens was registered on June 2001. It has intense

absorption in the red spectral region with maximum at 676 nm. Since 1994, PDT with Photosens has been conducted on around 4000 patients with tumors of different types and localization.

- Phthalosens is composed of sulphonated non-metallic phthalocyanines. Phthalosens is a water-soluble photosensitizer of anionic type, absorbing at 690–695 nm and markedly exceeds Photosens in tumor growth inhibition (TGI) *in vivo*. Its lyophilized form "Phthalosens-lyo" is sterile, apyrogenic, and possesses high photodynamic activity. It is currently in the final stages of preclinical tests.
- Cholosens is a water-soluble photosensitizer of cationic type absorbing at 675 nm. It is indicated for PDT, antimicrobial PDT and photodynamic water disinfection. PDT on mice with a dose of 1.0 mg/kg resulted in full tumor resorption. Use of the lyophilized form for local administration is less phototoxic than Photosens and fluoresces at 696.7 nm with high contrast. The photosensitizer is currently being investigated in preclinical studies.
- Thiosens (hydroxyaluminum tetra-3-phenylthiophthalocyanine) in liposomal compositions can be used for intravenous administration based on a lecithin–cholesterol mixture with absorption in sensitized biological tissue at 710–740 nm. It is currently tested in a preclinical study.
- Octasens (octa-4,5-decylthio-octa–3,6–chloro-PcZn) is a nanostructural photosensitizer solubilized in micellar form using non-ionic Pluronic-like surfactant with an absorption maximum at 730 nm. It selectively accumulates in tumors. TGI for Ehrlich tumor exceeds 80%.
- ALAsens (5-aminolevulinic acid, registered on 2000) has been used since 1999 for FD and PDT in more than 10,000 patients with tumors of different types and localization.
- Hexasens-lyo (5-aminolevulinic acid hexyl ester) is now in final stage of preclinical tests.

Acknowledgement: This work was supported by Moscow City Government.

[6] The photo-induced antitumor efficiency of some synthetic bacteriochlorins

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A series of bacteriochlorin derivatives have been prepared, and some of them (i) tetra-3-pyridylbacteriochlorin tetra iodomethylate, (ii) tetra-3-pyridylbacteriochlorin tetra tosylate, and (iii) tetra-(4-pyridiniumbutoxyphenyl) bacteriochlorin tetra bromide have been studied as photosensitizers for photodynamic therapy (PDT).

The investigated compounds (i)–(iii) are stable in solution and *in vitro* possess high photo-induced activity (irradiation wavelength $\geq$640 nm) toward human larynx cancer (HEp-2) cells. The half maximal inhibitory concentration (IC_{50}) is 1.5 ± 0.1, 0.79 ± 0.08 and 2.60 ± 0.11 μM, respectively.

In vivo (i) and (ii) accumulate in tumor tissue. The maximal fluorescence intensity is achieved 15 min after application and is retained for 15 min with fluorescent contrast of 2.0 ± 0.3 to 3.2 ± 0.2 au (tumor/skin), and 2.0 ± 0.3 to 3.0 ± 0.3 au (tumor/muscle).

Photo-induced antitumor activity of (i) and (ii) was studied on mice in Lewis lung carcinoma (LLC) tumors at doses of 2.5 and 5.0 mg/kg. PDT was conducted on day seven of tumor growth using laser and light diodes with a wavelength of 754 ± 14 nm as irradiation sources (power density 90 J/cm^2).

In our opinion, synthetic bacteriochlorins have high photo-induced activity *in vitro* and *in vivo* and may be used in the future as near-infrared photosensitizers for PDT of malignant tumors.

Acknowledgement: This work was supported by Moscow City Government.

[7] Equipment for FD and PDT

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The report presents new and modified versions of the previously developed devices and instruments for fluorescent diagnostics (FD) and photodynamic therapy (PDT) of cancer. Particular attention is paid to light delivery systems and estimation of the tissue absorbed light dose. Methods are discussed for the measurement of photosensitizer concentration in different tissues depending on the type of tissue and geometric parameters. A method is presented to measure the degree of hemoglobin oxygenation in the microvasculature. The possibility is discussed of using pulsed light sources for PDT, diagnostics and painkillers for irradiated tumors.

Acknowledgements: The work was performed under the Moscow government program "Development and practical introduction of new methods and means of prophylaxis, diagnostics and treatment of oncologic, infectious and other dangerous diseases for healthcare".

[8] Photodynamic therapy using Photosens and ALAsens – An experience-based report at a municipal polyclinic

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Objective: Photodynamic therapy (PDT) is a highly effective, organ-preserving method to treat oncologic and non-oncologic diseases. It is based on the selective accumulation of a photosensitizer in pathologic tissue, followed by a photochemical reaction, which develops under the influence of laser irradiation, and leads to the destruction of atypical cells. The PDT method differs favorably from other treatment methods due to the preservation of the collagenous skeleton of the tissues, and the healing of a tissue defect by means of tumor resorption without scarring. Thus, PDT can provide good cosmetic results. Nowadays, this method is widely used to treat oncologic and non-oncologic diseases, but is limited to large oncologic research centers and hospitals.

Materials and methods: At the Municipal Polyclinic No. 84 in the South-West Administrative District of Moscow, a treatment room for PDT and high-technology methods was equipped to treat oncologic and non-oncologic diseases. Ambulant PDT was applied in the treatment of patients with skin cancer, oropharyngeal cancer, precancerous diseases of the female reproductive system and for persistent wounds. PDT was performed with the Russian photosensitizers Photosens and ALAsens (NIOPIK, Russia). The irradiation laser systems used were LPhT-675-01-BIOSPEC and LPhT-630-01-BIOSPEC (Biospec Ltd., Russia), working in a continuous irradiation mode.

Results: Up to now, we have treated 163 patients, 97 patients with various localizations of primary and recurrent basal-cell skin cancer, 17 patients with metatypical skin cancer, 23 patients with squamous-cell skin cancer, 5 patients with squamous-cell cancer of the upper and lower lips, and 21 patients with persistent wounds. For the oncologic treatment, Photosens was introduced intravenously; the corresponding drug dose was in the range of 0.5–0.7 mg/kg body weight. The drug-light interval was 12–24 h. The light dose was 150–250 J/cm^2. In the case of persistent wounds, we also used Photosens dissolved in sterile physiological salt solution at a concentration of 250 μg/ml. An eight-ply gauze wad was then soaked in the solution to a saturation of 0.5 ml per 1 cm^2, and applied directly onto the wound surface. The drug light interval was 2–4 h and the light dose was 30–60 J/cm^2.

The PDT of the female reproductive system diseases was conducted with 20% ALAsens cream. The drug light interval was 6 h and the light dose was 150 J/cm^2.

Results: The efficiency of treatment of oncologic patients was estimated according to the criteria laid down by the World Health Organization. In 138 of 142 oncologic patients full tumor resorption could be observed after PDT, in four patients a partial resorption was reached.

In the 21 patients with persistent wounds, a therapeutic effect was achieved for 18 patients and no effect was observed in 3 patients. In the case of virus-associated gynecologic diseases, a therapeutic effect was achieved in all 25 patients as a result of treatment.

The PDT method of oncologic diseases provided excellent cosmetic results for skin cancer treatment, especially at inconvenient locations.

Compared to surgery and close-focus X-ray treatment, both of which are used more often to treat oropharyngeal cancers, the PDT method significantly reduces the duration of treatment, decreases the number of complications, restores the ability to work for certain age groups and reduces the duration of the patient's disability.

PDT of persistent wounds helps to quickly clears wounds of purulo-necrotic masses, reduces swelling, improves the flow of blood in microvessels and speeds up wound repair.

Treatment of precancerous diseases of uterine cervix by means of PDT is an effective but preserving method of treatment, which helps to avoid scarring and preserve the anatomic functional continuity of the uterine cervix and the cytoarchitectonics of cervical canal.

Conclusion: The PDT treatment room at the municipal polyclinic provides the facilities for a quick, effective, economic and alternative kind of treatment.

Acknowledgement: This work was supported by Moscow City Government.

[9] Experience of using photodynamic therapy in oncologic diseases at various localizations at the St. Petersburg I.I. Mechnikov State Medical Academy (SPSMA)

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Clinical experience with photodynamic therapy (PDT) is presented in the therapy of tracheobronchial cancer with respiratory impairment and bile ductules occlusion.

Two groups of patients were involved in the study. The first group included nine patients (age range: 42–72 years) with inoperable malignant tumors of the trachea and main bronchi; the second group was made up of four patients with cholangiocellular cancer.

Second generation photosensitizers (photoditazene, radochlorine, and photolone) and semiconductor Atcus-2 (JSC Semiconductor Devices, Russia) and Latus-2 laser systems (MILON, Russia) with a wavelength of 662 nm were used for PDT.

As a result, using PDT the speed of trachea and main bronchi tumor growths could be significantly reduced. The survival of two-thirds of the patients with cancer of trachea and main bronchi was prolonged and the quality of life was better in cases where other contemporary methods had

not proved effective. Control X-rays of treated patients with cholangiocellular cancer showed a reduction in tumor growth intensity and their survival time increased compared to similar patients who had not had PDT.

[10] Photodynamic therapy of malignant tumors of external location

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Introduction: Organ preservation treatment is an upcoming trend in modern oncology. Photodynamic therapy (PDT) is a possible therapy option for patients having malignant tumors in the initial stages of cancer. PDT is based on the selective destruction of tumor, whilst preserving normal tissue structures. Therefore, PDT shows only rare side effects making the procedure suitable for patients with contra-indications to surgical, chemotherapeutical and radiotherapeutical treatment due to age and somatic status.

Materials and methods: PDT usually has a penetration depth of 4–6 mm. At Moscow Oncology Clinical Dispensary No. 1, a method of non-invasive contact PDT has been developed which increases the penetration depth of the laser light to 10–15 mm. A combination of remote and contact PDT expands the possibilities of the method. The PDT method was used in the dispensary to treat more than 1400 patients suffering from different oncologic pathologies. More than 70% received outpatient treatment. 49% of patients were older than 70 years. In 53% of the cases, the patients refused conventional treatment because of concomitant pulmonary and cardiac diseases, and the advanced cancer status.

The Russian photosensitizers Photosens and ALAsens were used for PDT. Laser exposure was carried out in 1–10 sessions by remote or by contact method. PDT was applied to patients having malignant tumors of the following entities: skin cancer, initial skin melanoma, metastatic lesions of skin due to melanoma, metastatic lesions of skin due to mammary gland cancer, Kaposi's sarcoma, vulvar and vaginal cancer, cervical cancer, oral mucosa cancer.

Results: Complete regression was observed after PDT treatment of patients with skin cancer (T1-3, N0, and M0) for 85% of the patients with basal and metatypical cell carcinoma and 74% with flat cell skin cancer. In case of initial skin melanoma (T1-3, Nx, and M0), complete tumor regression occurred in 86% of the cases. PDT applied in patients with skin metastases of breast cancer was efficient in 66% of the cases. A combination of gamma therapy and PDT increased the efficiency of treatment in this group of patients up to 86–90%. In 80–93% of patients with vulvar cancer (T0, Nx, M0; T1, Nx, M0; T2, Nx, M0) a complete tumor regression could be observed. Usually, patients suffering from cervical intraepithelial dysplasia CIN II–III and cervical carcinoma *in situ* were conservatively treated by surgery. For organ preservation PDT can be applied in an out-patient procedure in one session. Complete regression of the suspicious areas occurs in 80–90% of the cases. Eradication of human papilloma virus according to clinical research amounts to 94% of all cases.

Conclusion: Photodynamic therapy is an effective and safe method of organ preservation method in the treatment of patients suffering from malignant tumors of external location, and is a promising trend in clinical oncology.

[11] Fluorescence diagnosis and photodynamic therapy of pterygia using 5-aminolevulinic acid

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Background and objective: Pterygia can vary from small, atrophic quiescent lesions to large, aggressive, rapidly growing fibrovascular lesions that can distort the corneal topography and, in advanced cases, obscure the optical center of the cornea. At present, no reliable medical treatment exists to reduce or even prevent pterygium progression. The aim of the presented study was to assess the possibility of 5-ALA-induced PpIX fluorescence guidance for photodynamic therapy (PDT) in pterygia.

Materials and methods: A total of 8 pterygium lesions in 7 patients were investigated. All patients received 20 mg/kg of body weight of ALAsens (NIOPIK, Russia) orally 4 h prior to PDT. PpIX fluorescence was detected using a non-invasive spectroscopy system. Fluorescence emission spectra were measured under 632.8 nm excitation. Additionally, fluorescence images were used for the assessment of the features of autofluorescence and 5-ALA-induced PpIX fluorescence in different parts of pterygium. A blue light source (390, 415 and 433 nm) was used for the fluorescence imaging. For PDT, pterygia were illuminated with narrow-band red light (635 nm) at a light dose of 50–80 J/cm^2.

Results: We identified 2 groups of pterygia with high or low intensity of 5-ALA-induced fluorescence. Low values were observed in pterygium with slow growth and no signs of activity. In these cases, there was predominance of blue-green fluorescence in pterygium body. All patients experienced a partial regression and reduction of vessel caliber in pterygium fibrovascular tissue in 7–10 days after PDT.

Conclusion: PDT with ALAsens can be used as a treatment option for patients with pterygium lesions.

Acknowledgement: This work was supported by Moscow City Government.

[12] Optical control of fat cell lipolysis and apoptosis by photodynamic/photothermal treatment in tissues

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Introduction: A lipoma is a benign tumor comprised of fat cells that manifests itself in the subcutaneous tissues. Lipomas are usually very slow growing, and may not be noticed until they are rather large. Currently the only practical treatment for lipomas is surgical excision. The basic advantages of photodynamic and photothermal methods are their minimal invasiveness. These methods have been approved in numerous studies; however they require further development and adaptation to specific applications such as treatment of lipomas and other tumors containing fat cells. The goal of this work is to design a new technology for the photodynamic and photothermal treatment of fat cells in tissues sensitized by a water-soluble tricarbocyanine infrared dye, indocyanine green (ICG), in free and encapsulated states, and to find the optimum laser radiation dose required to induce fat cell lipolysis and/or apoptosis in tissues.

Materials and methods: For the *in vitro* experiments, adipose tissues slices 100–150 μm in thickness were used. Water–ethanol solutions of ICG and aqueous solutions of ICG capsules, 1 mg/ml and 2 mg/ml in concentration respectively, were used for staining the fat tissue. A continuous wave (cw) diode laser (ACCULASER, 810 nm) was used for irradiation of tissue slices at power densities of 250, 375, 500, and 625 mW/cm^2.

Results and discussion: Cell lipolysis was seen as an optical clearing effect of the upper cell layers of the photodynamically/photothermally modified fat tissue slices. As a result of lipolysis, due to light-induced cell membrane porosity, the intercellular content of the cell (free fatty acids) percolates through these temporal pores into the interstitial space. Thus the refractive index of the interstitial fluid (initially equal to $n_i = 1.36$) becomes close to the refractive index inside the adipocytes (fat refractive index, $n_a = 1.44$) and due to the refractive index matching effect the optical medium becomes optically homogeneous and more transparent to light.

Conclusion: The usage of a combination of photothermal and photodynamic treatment allowed us to provide effective lipolysis of adipocytes and to effect a manageable change of cell morphology (indicated as apoptosis) in tissue samples.

Laser surgery

[13] Trends in laser surgery

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Clinical medical laser application using high power laser light offers the opportunity to destroy both malignant and benign soft and hard tissue. Innovative developments in clinical endoscopic techniques allow the delivery of high power laser light of up to 200 W in continuous wave (cw) mode or 2 J/pulse with single fibers. In urology, high power cw laser application with side-firing laser fibers are in use for treating benign prostatic hyperplasia (BPH) symptoms. Urological stone fragmentations are performed with pulsed infrared (IR)-laser applications. Endoluminal laser treatment of incompetent great saphenous veins of patients suffering from varicosis is a promising new procedure which has fewer side effects compared to conventional surgical stripping methods. In each of these procedures the combination of a suitable laser wavelength and laser power provides the basis for the laser-tissue interaction and its effects to achieve satisfactory treatment parameters for the benefit of the patient.

Neurosurgery

[14] Photodynamic therapy as an adjuvant therapy of cerebral gliomas

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Background: The morbidity rate of primary and recurrent brain tumors is 10.9–12.8 per 100,000 of the population. About half of cases are glial tumors, most of them having supratentorial localization. The occurrence rate of high-grade gliomas makes up to 50% of all glial tumors. The accepted standard of treatment includes surgical treatment aimed at maximum possible tumor resection and histological diagnosis verification, radiotherapy (summary total dose being 50–60 Gy), and chemotherapy.

Considering the frequent recurrance of high-grade gliomas, in spite of complex treatment, the elaboration of new treatment methods is a highly topical problem. Photodynamic therapy (PDT) has a direct cytotoxic effect upon the tumor tissue, as well as possessing a number of

photochemical effects amongst which are anti-angiogenic, anti-inflammatory, immunologic and antiviral properties.

One of the potential advantages of PDT is its selective effect on tumor tissue, which depends on the difference between photosensitizer concentration in tumor and normal tissue, as well as on the limited light penetration into biological material

Patients and methods: Thirty-one patients with high-grade glial tumors of supratentorial localization underwent PDT in our department, (age range: 23–69 years, sex: 18 male, 13 female). The second generation Russian drug Photoditazin of e6 chlorine group was used at a dosage of 50 mg/kg body weight as photosensitizer. The follow-up period was 1 month to 4 years.

The first group included 16 cases of newly diagnosed tumors; the second 15 cases of recurrent gliomas (glioblastomas: $n = 21$, anaplastic astracytomas: $n = 7$, anaplastic oligodendrogliomas: $n = 3$). During anesthetic induction, the patients received Photoditazin intravenously.

Complete tumor tissue resection was performed to the greatest extent possible. Ultrasound navigation was used to control the resected tumor volume. Test model of semiconductor laser "Latus-2.5" was used as irradiation source (power: up to 2.5 W; radiation wavelength: 662 nm). After resection, a multi-fiber flexible light guide from the laser was placed into the tumor bed, the wavelength corresponding to the maximum spectral absorption of Photoditazin. The tumor bed was irradiated with a photo dose of 180–400 J/cm^2.

Results: All the patients discharged from the hospital had a compensated status. No complications resulted from the administration of the photosensitizers and further intraoperative irradiation was revealed. In the postoperative period all the patients with newly diagnosed glial tumors had radiotherapy (summary total dose: 54–60 Gy). The level of the quality of life during the hospital stay was 70–90 according to Karnofsky index. The length of the recurrence-free period for the patients with primary malignant gliomas was 18.5 ± 3.8 months; for the patients with recurrent malignant gliomas 10.7 ± 2.3 months. Within the recorded follow-up period, two patients were still alive, one having been newly diagnosed with glioblastoma multiforme of the right parietal lobe (4 years catamnesis), and the second one had recurrent glioblastoma of the right frontal and parietal lobes (6 months catamnesis).

Conclusion: PDT is a relatively safe method which allows intra-operative impact on the residual tumor cells volume located in perifocal zone. The short period required for the medication washout allows minimization of the isolation regime restriction in the postoperative period as well as making it possible to start radiotherapy 3–4 weeks after surgery.

PDT use in complex treatment of high-grade glial tumors of supratentorial localization allows the recurrence-free period to be prolonged for this category of patients.

[15] Fluorescence guidance with microscope and endoscope in cranio-cerebral tumors

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Background: According to published data, intra-operative fluorescence-guided resection allowed a two-fold increase in the six-month survival and radical removal, but did not influence overall survival of patients with glioblastoma. Absence of the accumulation of 5-aminolevulinic acid (5-ALA) by brain tissue may theoretically increase specificity up to 100%.

Patients and methods: The series included patients with gliomas of different grades and cerebral metastases, and malignant skull base tumors: carcinoma, olfactory neuroblastoma and chordomas. All patients were given 25 mg/kg body weight orally of ALAsens (NIOPIK, Russia) prior to surgery and all of them had previously signed an informed consent. We performed 28 tumor resections and 2 endonasal biopsies. We used Carl Zeiss OPMI Pentero microscope and a special Karl Storz device for endoscopic fluorescence-guided resection. The decision to use an endoscope was based on the necessity to visualize hidden and obscured areas inaccessible for the microscope. Immediate surgical results were evaluated by contrast-enhanced MRI in the 24 h after tumor removal.

Results: Positive fluorescence was observed in glioblastoma, metastases of carcinoma and low-pigmented melanoma. Negative fluorescence was typical for esthesioneuroblastoma and black (pigmented) melanoma. In all cases dural invasion and fibrous stroma of tumor were negative. Two cases of clival chordoma were also operated on: in the first case we observed strong positive fluorescence, and in the second case no fluorescence was visible. Interviews with the surgeons who had applied endoscopic-assisted fluorescence-guided resection indicated that the overall impression was positive. Negative moments were associated with timing, additional instrumentation and the necessity to correlate endoscopic and microscopic views. We found that a hemostyptic agent, Tachocomb, does not oxidize hemoglobin and provides a clear view of tumor remnants without additional high signal areas.

Fluorescent-guided resection allowed the operator to differentiate the tumor tissue or borderline zone from normal tissue of the ischemic brain, and multiple biopsies confirmed the high specificity of the technique.

Conclusion: We are convinced that fluorescent guidance should be applied in every case of glioblastoma multiforme to determine tumor borders and if necessary, to know where

to stop. Obviously, only a small number of 'nice' removable tumors may be maximally respected without danger to a patient. We believe that in the future it will be necessary to use all available methods – neuroimaging, electrophysiological and metabolic ones. This combined approach may be the best choice.

[16] Intra-operative fluorescent navigation (IFN) and intra-operative photodynamic therapy (IPDT) for the treatment of brain metastases

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Introduction: One of the most difficult localizations for treatment is metastases of the brain. There are no statistics about the incidence of brain metastases in Russian Federation, however, it is estimated that on average 14–16 new brain metastases per 100,000 of the population occur yearly. In 15–20% of the patients, the source of brain metastases are extra-cranial malignant neoplasms, mainly lung cancer, breast cancer, melanoma, renal cell carcinoma, and colorectal cancer.

At present, the median survival of brain metastasis without treatment is approximately 1 month, after additional treatment with corticosteroids about 2 months, with whole-brain radiotherapy about 4–6 months and after application of local therapies such as surgery or radio surgery, combined with whole-brain radiotherapy, 12–14 months. Disadvantages of surgery are (1) the impossibility of radical brain-tumor resection with localization in functional zones of brain, and (2) the high frequency of postoperative extended growth.

All the above mentioned facts encouraged us to search for new technologies to improve the patient treatment results, without affecting the quality of life. Intra-operative photodynamic therapy (IPDT) in combination with intra-operative fluorescent navigation (IFN) with 5-aminolevulinic acid may be one possible therapy option.

Materials and methods: ALAsens (NIOPIK, Russia) was used for both IFN and IPDT, because ALAsens easily penetrates through the blood–brain barrier (BBB). ALAsens was given at a dose of 20 mg/kg body weight dissolved in 100 ml of sterile water 3 h before starting fluorescence diagnosis. Most of the radical tumor resection was done under IFN control, followed by IPDT with irradiation of the tumor cave in order to destruct tumor microfossils.

Efficiency of treatment was controlled within the first 24 h after the operation by postoperative CT with contrast enhancement, and every 2–3 months after therapy by MRI.

Results: Up to now, at the P.A. Hertsen Moscow Oncology Research Institute, 20 patients with brain metastases have been treated with IFN-controlled surgery combined with IPDT; and 30 patients without IFN and IPDT (control group). The median observation time was 14 months.

Conclusion: The first experiences of applying surgical treatment with IFN and IPDT to metastatic brain tumors demonstrates the perspective of this technology.

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Gynecology

[17] Diagnostic efficacy of optical coherence tomography (OCT) in the management of CIN

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Objective: Optical coherence tomography (OCT) is a non-invasive high resolution imaging technique that permits characterization of microarchitectural features up to 2 mm in depth in real-time. The purpose of this study was to evaluate the feasibility of OCT in the characterization of pre-invasive and invasive cancer of the uterine cervix.

Patients and methods: We conducted a single-institution, board-approved, prospective study on the use of OCT in women with suspected cervical intraepithelial neoplasia (CIN). The images were evaluated immediately, and independently by two investigators. In addition they were compared to the corresponding histology. Sensitivity and specificity of the new technique were calculated.

Results: We compared 189 images with corresponding histology in 106 women undergoing colposcopy for suspected CIN. With 130 (127) true positive, 22 (23) true negative, 34 (33) false positive and 3 (6) false negative results, the sensitivity calculated for both investigators with the threshold at CIN 1 was 98% and 95% respectively. The specificity was 39% and 41% respectively.

Conclusion: OCT is a rapid, easy-to-use modality that provides real-time microarchitectural information of the cervical epithelium. A potential role can be envisaged for this new technique in the management of pre-invasive and invasive cancer by identifying pre-malignant states and the depth of tumor invasion.

[18] Photodynamic therapy in women with vulvovaginal candidiasis

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Objective: In the past decade, a sharp increase in resistance of many strains of bacteria and fungi to antibiotics and antimycotics has led to an increased search for alternative therapy options. The aim of this study was to estimate the clinical efficacy and safety of the 5-ALA-based medicinal formulation ALAsens in PDT of vulvovaginal candidiasis in women.

Materials and methods: Women with complaints of itching, burning, flushing and swelling of external genitalia were selected. To achieve the objectives of the study, the investigations were conducted in two steps. In order to determine the accumulation of ALAsens-induced protoporphyrin IX (PpIX), 5 patients with vulvovaginal candidiasis underwent fluorescence spectroscopic study using the spectral fluorescence system Spectr-Cluster (Cluster Ltd., Russia) with an excitation wavelength of 405 nm.

In the second phase, 15 patients (age range: 22–59 years, average: 33 years) with vulvovaginal candidiasis received PDT. The LED device "APS" (Polironik Ltd., Russia) with a wavelength of 400 ± 10 nm was used for irradiation. Four sessions of PDT were performed on each patient with an interval of 7–8 days between treatments. Before and after each treatment Gram-stained smears were examined microscopically and estimated, and after bacterial cultivation in nutrient media, the bacterial colonies were counted and a definition of Candida species of pathogens was made.

Results: During the spectroscopic investigations, an intense fluorescence of PpIX in the red spectrum could be observed in the mucosa of the vulva and vagina within a specified time after drug administration of ALAsens.

In all 15 PDT-treated patients, itching and burning disappeared or significantly decreased immediately after the first PDT. In 5 cases (33%) bleeding occurred after the 3rd PDT session.

Immediately after PDT in all patients, a 3-fold reduction of Candida, bacteria and leukocytes were observed. In 9 patients (60%), an increase in lactic acid bacteria in the smears was determined after the PDT sessions, demonstrating the positive effect of PDT on the vaginal microflora, as lactic acid bacteria are involved in the formation of an environmental barrier and provide resistance to the vaginal biotope.

The observed side effects were minor and did not require medical treatment. Six patients (40%) experienced discomfort in the vagina after the application of 5-ALA solution. Three patients (20%) complained about a slight burning at the treated site after the PDT session.

Conclusion: Analyzing the results, we can conclude that PDT is a suitable method for the treatment of patients with vulvovaginal candidiasis. This is shown by the disappearance of complaints from the patients (itching, discomfort, isolation), and the positive microbiological effect (disappearance of fungi of the genus Candida). Additionally, PDT positively influenced the vaginal microflora, resulting in a decrease in the number of leukocytes and an increase in lactic acid bacteria in the smear. All patients reported a good tolerance of the procedure and disappearance of the severity of symptoms after the first session of PDT. To increase the effectiveness of the method it would seem appropriate to reduce the interval between the PDT sessions from 7–8 to 2 days.

[19] Photodynamic therapy of non-oncologic diseases of female genital organs

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Background and objective: More then 50% of members of the sexually active population are infected by human papilloma virus (HPV) during their lifetime. Diseases such as genital condylomata, cervical intraepithelial neoplasia (CIN) and malignancies of cervix, vulva, vagina, and perianal zone are associated with a HPV virus infection. Surgery, laser ablation and cryodestruction are usually used as treatment methods. Photodynamic therapy (PDT) is an alternative and minimal invasive method in the treatment of HPV-associated diseases.

The aim of this study was to estimate the clinical efficacy and safety of the 5-ALA-based medicinal formulation ALAsens in the PDT treatment of HPV-associated diseases of the vulva and cervix. The kinetics of 5-ALA-induced protoporphyrin IX (PpIX) accumulation in the above-mentioned tissues, after oral application of ALAsens, was studied *in vivo* using local fluorescence spectroscopy.

Patients and methods: The study group of 34 patients included 28 cases with pointed condylomata and 13 cases with pathology of the cervix uteri. The PDT sessions were carried out 3–6 h after oral administration of ALAsens with a dose of 25 mg/kg body weight. For tissue irradiation, a diode laser with an emission wavelength of 635 nm (Biospec Ltd., Russia) was used. The average duration of the PDT session was 27 min. The applied energy density varied from

30 to 150 J/cm^2 (90 J/cm^2 on average). The clinical efficacy of PDT treatment was evaluated visually, and on the basis of data from the polymerase chain reaction (PCR) diagnosis, colposcopy, cytology and histology.

In vivo local fluorescence spectroscopy was applied to all patients before and every hour after oral administration of ALAsens. The fluorescence measurements were performed using a spectral fluorescence system Spectr-Cluster (Cluster Ltd, Russia) with a working range of 410–1000 nm and tissue fluorescence excitation at 405 and 532 nm.

Results: The fluorescence bands of 5-ALA-induced PpIX, which were measured from the vaginal portion of cervix in all patients, peaked at 635 and 705 nm and were detected against the autofluorescence background in all spectra. Thereby, the atypical epithelium generally exhibited more intensive 5-ALA-induced PpIX fluorescence than the surrounding normal tissues. The contrast in fluorescence between pathological foci and healthy vulva were detectable as soon as one hour after ALAsens administration and reached the maximum value 4 h thereafter.

Three months after PDT, the complete antiviral effect was confirmed in 18 cases (67%) with pointed condylomata and 6 cases (46%) with pathology of cervix uteri by means of PCR diagnosis. No antiviral effect was observed in 9 cases (33%) with pointed condylomata and 7 cases (54%) with pathology of cervix uteri.

Within a follow-up period of up to three months, a complete regression of the lesions after PDT was observed in 16 cases (59%) with pointed condylomata and 5 cases (38%) with pathology of cervix uteri. Ten patients (37%) with pointed condylomata and 4 patients (31%) with pathology of cervix uteri had a remarkable regression (about 60–95%). In only one patient (4%) with pointed condylomata and four patients (31%) with pathology of cervix uteri was there no effect at all. Epithelization of the treated tissue was completed generally 2–4 weeks after the treatment.

Conclusion: *In vivo* fluorescence measurements revealed the fluorescence contrast between pathological lesions and the surrounding healthy mucosa of vulva and cervix uteri that confirmed the increased induction of PpIX in pathological tissue after oral application of ALAsens. *In vivo* local fluorescence spectroscopy allowed the kinetics of 5-ALA-induced PpIX fluorescence to be studied and to determine the optimal time for PDT treatment. The selective induction of PpIX in the epithelial layer of vulva and cervix uteri made it possible to preserve the structure of normal tissue and also to decrease the duration of post-treatment tissue healing without erosions and scars formation.

[20] Clinical experience of ALA-based laparoscopic photodynamic detection (LPDD) of peritoneal carcinoma in gynecological patients

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Objective: The aims of the study were to determine the diagnostic value of laparoscopic photodynamic detection (LPDD) in case of early recurrence of peritoneal carcinoma in gynecological patients and to evaluate the efficacy of LPDD for accurate staging in the case of an ovarian mass.

Patients and methods: Six hours before laparoscopic surgery, 5-aminolevulinic acid (5-ALA) was applied intraperitoneal via infusion at a concentration of 50 mg/kg body weight. The D-Light system (Karl Storz, Germany) served as the light source. Intra-peritoneal located red fluorescent lesions, which were suspected to be metastases, underwent a biopsy. As a control, several biopsy specimens were taken from the peritoneal cavity also in the white light mode. LPDD was used for primary staging in patients with suspicious ovarian carcinoma ($n = 2$) and for suspicions of ovarian cancer recurrence ($n = 7$).

Results: In all 9 patients both the application of photosensitizer and surgical procedures were performed without complication. In 3 of the 7 patients with suspicious cancer recurrence, carcinoma was confirmed histologically and cytologically. The white light imaging provided the visual detection of peritoneal cancer lesions. In these patients, metastases were visible as a strong red fluorescence. In one patient a few metastatic lesions were only visible in the fluorescent mode.

In 4 patients with second-look laparoscopy no signs of peritoneal carcinoma lesions could be observed either in white light or in the fluorescent light mode. The cytological examinations of the abdominal cavity were also negative.

72 biopsies were excised. All red fluorescent biopsy samples were histologically positive for ovarian carcinoma metastases except for 3 samples, where scar tissue and vegetations on "foreign body", i.e. surgical sutures after previous interventions were determined.

In one case laparoscopic bilateral adnexectomy was performed in an LPDD-negative woman with bilateral ovarian cysts. Cytological and histological examination of the adnexa and five random peritoneal biopsies were negative for cancer tissue.

In a second LPDD-negative patient with an ovarian tumor, the cyst wall was perforated during the laparoscopic ovarectomy due to adhesions, tumor size and thinness of the cyst wall. After evacuation of the fluid from the cyst, a strong red fluorescence of vegetations on the internal surface of the cyst wall was detected. Laparotomy and ovarectomy was performed under normal daylight with the operation lamps turned off. The intestine was carefully covered with sterile towels during the intervention to prevent possible phototoxic effects. The diagnosis of serosum papillar

middle differentiated ovarian carcinoma was proved in a section taken for immediate histological examination. Then the standard omentectomy was performed. The duration of laparotomy was 45 min. No complications were detected in the post-operative period. In the fully processed histological examination, five random peritoneal biopsies and omentum were negative for cancer cells; ovarian carcinoma was detected on the internal surface of the ovarian cyst. Ovarian cancer was staged T1cNxMoG2 due to the perforation of the cyst during the procedure.

Conclusion: LPDD may provide a higher sensitivity for finding peritoneal metastases of epithelial ovarian cancer compared to white light laparoscopy. However the impact of LPDD on survival has yet to be proved. The current study is the first report to evaluate the use of LPDD for primary accurate staging of ovarian cancer in humans. The possibility of a safe, open laparotomic intervention after LPDD was also shown.

[21] Photodynamic therapy at the treatment of virus-associated precancerous and non-invasive cervical cancer

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Objective: A study was performed to assess the efficacy of photodynamic therapy (PDT) in the treatment of virus-associated precancerous and non-invasive cervical cancer.

Patients and methods: At P.A. Hertsen Moscow Oncology Research Institute photodynamic therapy (PDT) of the uterine cervix was performed in two groups, in total 60 women with cervical intraepithelial neoplasia (CIN) aged 22–76 years; 60% of them were of reproductive age. Complex investigations were carried out using clinical, endoscopic, ultrasonic and morphological examinations. In a first group, which included three women with CIN II and 27 women with CIN III, PDT was carried out using the sensitizer Photosens (NIOPIK, Russia). In a second group, including 30 women with CIN III, PDT was carried out after surgical amputation of the cervix using the prosensitizer ALAsens (NIOPIK, Russia). Then all the women were tested for human papilloma virus (HPV) infection using polymerase-chain reaction (PCR). "High-risk" virus types (16, 18, 31, 33, 35, and 58) were found in 90% of the patients. Genotypes 16/18 were found in 95% of the cases.

Results: Complete regression of CIN II and CIN III was achieved in the first group in 100% of the cases. In the second group, complete regression of CIN III was achieved in 88.8% (significant regression: 7.4%, stabilization: 3.8%). The antiviral effect of PDT was registered in 94.3% of the cases. Two women with CIN II and 11 women with CIN III became pregnant after PDT.

Conclusion: PDT is the method of choice for an organ-sparing treatment, especially for women planning gravidity. PDT method is less radical as it preserves anatomical and functional integrity of the cervix. The interrelation between the achievement of complete regression of pathological changes of the uterine cervix and complete eradication of HPV is apparent.

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[22] Current application of photodynamic diagnosis in gynecology

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Fluorescence diagnosis constitutes a modern method for evaluating neoplastic and non-neoplastic lesions located on the surface of organ systems. In gynecology, accessible organs include the vulva, vagina, and the portio uteri. Furthermore, the uterine cavity can be accessed via hysteroscopy and the pelvis using laparoscopy or laparotomy. Protoporphyrin IX (PpIX) is a potent photosensitizer which can be accumulated intracellularly by the exogenic supply of 5-aminolevulinic acid (ALA) or modern ALA esters such as hexylaminolevulinate (HAL) or methylaminolevulinate (MAL). Thereby, dysplastic tissues present higher concentrations then normal tissues. The contrast that occurs is utilized diagnostically and therapeutically in different gynecological fields. In terms of the cervix uteri, fluorescence diagnosis can raise the sensitivity for the detection of neoplastic and preneoplastic changes. In ovarian cancer, different studies have provided evidence which indicate it could be used to identify peritoneal metastases and positive lymph nodes better. When performing diagnostic laparoscopy, given a suspicion of endometriosis, this method is especially useful in identifying non-pigmented lesions. Furthermore, histology studies have proved that there is no sustained tissue damage after PDD/PDT using ALA esters. Further studies are required in order to introduce fluorescence diagnosis into routine gynecological use.

Urology

[23] Focal therapy of malignant lesions in urology

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For decades, malignant tumors have been treated in most cases with the most radical approach. Even small renal tumors were treated almost exclusively by radical nephrectomy. The relatively rare cases of bilateral malignant tumors have forced new approaches, such as heminephrectomy, partial nephrectomy, and tumor excision, thus reducing relevant therapy related morbidity. Good oncologic outcomes have led to new routines and these surgical approaches have become standard practice for care of small renal masses at a favorable localization. With new ablative technologies, such as radiofrequency ablation, high intensity focused ultrasound, and cryosurgery becoming available, minimally invasive treatment for small tumors of the kidney has become possible. These focal therapies are currently under investigation.

The most frequently occuring malignancy in urology is prostate cancer. To date, in organ confined stages, radical prostatectomy and external beam radiation therapy are considered state-of-the-art, recently supplemented by brachytherapy. Treatment-related morbidities such as urinary incontinence and impotence which occur in a significant percentage of patients, even in centers with the highest standards, have led to a search for additional treatment options. Growing knowledge of tumor biology has given way to completely new thoughts on prostate cancer management, such as active surveillance, which can be adequate for individual patients. In addition, originating as second-line treatment options for prostate cancer relapse after first-line radiation therapy, thermal ablation techniques have also become potential treatment options for first-line therapy of locally confined prostate cancer. However, all initial trials employing these techniques were also designed as radical whole gland therapy. As a consequence, such treatments have the risk of complications, which are considered unacceptable for minimally invasive therapies. Half-gland or focal therapy, which is currently under investigation in several centers, might be the answer to these problems.

A review will be presented of the modalities of focal tumor therapy, ongoing studies, published literature, and the caveats and future aspects of focal tumor therapy.

[24] Adjuvant photodynamic therapy (PDT) in combination treatment of bladder cancer

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Background and objective: Bladder cancer is one of the most urgent topics in modern oncourology. Research for more efficient methods of tumor treatment and new ways of antitumor action with the aim to prevent recurrence and progression of the disease is an on-going process.

Photodynamic therapy (PDT) is a promising treatment option for malignant and benign neoplasms. It is based on the ability of photosensitizing products to selectively accumulate in tumor and dysplastic tissues. The irradiation of these tissues with a wavelength in the visible wavelength spectrum (400–760 nm) produces cytotoxic effects leading to necrosis, with further substitution by connective tissue.

The aim of the conducted study was the assessment of the efficacy of PDT using the photosensitizer Photosens as adjuvant therapy of non-muscle-invasive bladder cancer.

Patients and methods: Adjuvant treatment with PDT was performed on 44 patients with transitional-cell bladder cancer at stage T1N0M0G2 after transurethral bladder resection.

Twenty-four hours prior to the PDT session, an intravenous infusion was given with photosensitizer Photosens (0.3–0.8 mg/kg body weight). PDT was performed with the diode-laser equipment set LFT-630/675-01-BIOSPEC (Biospec Ltd., Russia) at a wavelength of 675 nm with a cylindrical fiber optic. Power density was 15 J/cm^2 in one session and the mean session time was 22 min. All patients underwent ultrasound, regular and fluorescent cystoscopy, and biopsy of postoperative scarring and of parts of the bladder mucosa suspicious for cancer.

Results: As yet, the follow-up period has not exceeded 25 months. Seven patients (15.9%) have had recurrences. The registered side effects and complications during the PDT session were not life-threatening. Ten patients (22%) had sunburn (edema and hyperemia of skin) due to misconduct of prescribed regime. During the PDT session, 12 patients (27%) had an annoying or painful sensation in the pelvic area, which passed by itself or required analgesics. There was no systemic or allergic reaction during intravenous infusion of Photosens.

Conclusion: Adjuvant PDT of bladder cancer can be used on an outpatient basis, or in a day hospital. There is a need for further study and development of PDT regimes. First results show a significant decrease in the bladder cancer recurrence rate after PDT. Further follow-ups will help in defining the efficacy of this method and produce practical recommendations for its usage.

[25] Adjuvant photodynamic therapy with ALAsens in combination with intravesical chemotherapy with Mitomicin C in patients with superficial bladder cancer

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Introduction and objectives: To develop a technique and to assess the results of a combination treatment for patients with non-muscle-invasive bladder cancer (NMIBC) after transurethral resection (TUR) using photodynamic therapy (PDT) in combination with intravesical chemotherapy with Mitomicin C.

Patients and methods: Twenty-five patients (17 male, 8 female) with intermediate risk of NMIBC were included in the study. The average patient's age was 60.0 ± 8.2 years. For PDT the photosensitizer 5-aminolevulinic acid hydrochloride (ALAsens; NIOPIK, Russia), and a diode laser with a wavelength of 630 nm (Biospec Ltd., Russia) was used. Silicon diffusers were used (Karl Storz, Germany) for introduction of laser radiation into the bladder through the cystoscope. In all patients 50 ml of 3% of ALAsens solution was intravesically instillated 1.5–2 h before the operation.

Firstly, TUR of the bladder was performed using fluorescence navigation with ALAsens. Then PDT was performed with irradiation of all the bladder walls with 25 J/cm^2. After PDT, intravesical instillation of 40 mg of Mitomicin C was performed with an exposure time of 1 h. A total of 6 courses of adjuvant treatment of PDT with ALAsens and instillation of Mitomicin C were performed, with an interval of 7 days between the courses. After that, all patients underwent postoperative examination every 3 months with ultrasound, cystoscopy and urine cytology.

In 14 patients the Mitomicin C concentrations were assessed in normal and tumor tissue using high-performance liquid chromatography (HPLC). HPLC was performed for all 14 patients after intravesical instillation of 40 mg of Mitomicin C with 1 h exposure. After that, biopsy of normal and tumor tissue was carried out to assess the Mitomicin C tissue concentrations with HPLC. Seven days after this procedure, a PDT with ALAsens was performed in all 14 patients with a radiation energy of 25 J/cm^2. After 1 h, Mitomicin C instillation was carried out. Biopsy of normal and tumor tissue was repeated to assess the Mitomicin C tissue concentration with HPLC.

Fifty patients with intermediate risk of NMIBC were included in a control group. Patients in all groups were compared by prognostic risk factors using European Organization for Research and Treatment of Cancer (EORTC) criteria.

Results: The median Mitomicin C concentration assessed using HPLC before PDT with ALAsens was 214.5 μg/g and 98.0 μg/g in tumor tissue. After PDT with ALAsens median Mitomicin C concentration was 52.5 μg/g in normal tissue and 130.5 μg/g in tumor tissue.

Median follow-up was 7.7 ± 3.6 months (range: 2–15 months) in the experimental group and 18.4 ± 14.7 months (range: 1–8.8 months) in the control one. No recurrence was observed in the experimental group during the follow-up. In the control group recurrence of bladder cancer was verified in 14 patients (28%). Median time to recurrence in control group was 7 months. Moderate cystitis was observed in 12 of 25 patients in the experimental group in the 3 months after the procedure. One patient (2%) had severe cystitis (grade 4) during 1-year period after the procedure. No complications were observed in the control group.

Conclusions: A new and effective method of complex therapy of NMIBC with a combination of TUR, PDT and Mitomicin C reduces the risk of recurrence for bladder cancer and has minimal toxicity.

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[26] Initial experience of photodynamic therapy in prostate cancer patients

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Introduction and objective: Photodynamic therapy (PDT) is one of the ablative minimal invasive methods of cancer treatment which might be useful for patients with small volume prostate adenocarcinoma as an alternative to radical local treatment or watchful waiting. PDT may be an alternative method for patients with local recurrence after radical prostatectomy, external beam radiation, or brachytherapy.

The aim of our study was to develop a method of PDT for prostate cancer (PC) patients and evaluate the side effects of this procedure.

Materials and methods: PDT was performed on 14 patients with verified PC. PDT was performed for 11 patients because of local recurrence after external beam radiation therapy (six patients) or radical prostatectomy (RPE) (five patients). Three patients had undergone PDT prior to radical prostatectomy. For five patients PDT was performed because of localized low volume prostate tumors. The median PSA level was 6.25 ng/ml (min 0.5; max 10.2). The Gleason score was 4–6 for seven patients, 7 for four, 8–10 for one and was not assessed due to small volume of tissue for two patients. The mean patient's age was 64.8 ± 7.2 years (min 56, max 78). The median prostate volume was 29 cm^3 (min 3, max 85). Two hours before PDT the photosensitizer Photosens (NIOPIK, Russia) was injected intravenously. The light from high-power diode laser system LPhT-675-01-BIOSPEC (Biospec Ltd., Russia) using a wavelength of 675 nm was delivered to the prostate using optical fibers within transparent needles. Transrectal ultrasound (TRUS)-guided installation of 4–7 needles (18 Gauge) was performed on 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 energy during PDT at one position was 200–500 J and the median irradiation time was 27 min.

Results: No complications were observed during the procedure. The urethral catheter was removed the day after PDT. In one patient a urinary irritation was observed which required prologue catheterization. No specific complications (allergic, dermatological and laboratory abnormalities) occurred. Erection in patients with preserved function was not significantly affected. The mean hospital stay time was 3 ± 1 days. There were no pre- and post-operative complications in patients who had undergone radical prostatectomy after PDT and a follow-up examination was made after 3–12 months. Six months after PDT a decreased PSA level of 3.1 ng/ml (min 0.1, max 6.8) was observed.

Conclusion: From the preliminary results obtained from a small number of patients, we can conclude that PDT is a non-invasive procedure with only minor adverse effects. PDT could be an alternative treatment method for patients who are not eligible for a radical prostatectomy or external beam radiation therapy. The clinical trials will provide additional information about the effectiveness of this method.

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[27] Photodynamic detection of the renal cell carcinoma during the kidney-preserving tumor resection

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Introduction and objectives: The aim of our study was to identify the distal margin of a renal cell carcinoma (RCC) with the help of photodynamic and spectroscopic detection after the systemic administration of 5-aminolevulinic acid (5-ALA) in humans during partial nephrectomy.

Materials and methods: In a pilot study, 14 patients with a renal mass <4 cm in diameter underwent open surgery partial nephrectomy. As a photosensitizer, 1.5 g of 5-ALA were administered orally 4 h prior to surgery. During the operation the resection site and the distal margin were exposed under light to the wavelength from red spectrum. Intensity of fluorescence of the tumor was evaluated by spectroscopy. All side effects were taken into account. The results of fluorescence diagnostics were compared to the histological findings.

Results: In the patients undergoing surgery of RCC we found that fluorescence of tumor and normal tissue clearly differed and was sufficiently intense to see the difference and to identify the distal margins of the tumors for nephron-sparing surgery. We did not observe any side effects of the photosensitizer 5-ALA with the dosages used and methods of administration. We consider that the sensitivity of this method is quite high, whereas the specificity is not high enough so there is a need for further studies to clear up the situation.

Conclusion: Photodynamic diagnostics with systemic administration of 5-ALA can be effective in visualization and assessment of surgical margins for the surgeons and that can make resection more radical. We expect that this method will lower the local recurrence rate and could increase disease-free survival rates in the first 1–2 years during follow-up. We assume that this diagnostic tool will be a reliable tool for the detection of renal tumor margins, so we will be able to assess the mode of resection of a suspected renal tumor mass during nephron-sparing surgery.

[28] Local fluorescence spectroscopy for urology

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Local fluorescence spectroscopy (LFS) is a non-invasive, sensitive method enabling the quantitative determination of the local tissue fluorescence using a fiber-optic probe scanned in contact with the tissue surface or mucosa of hollow organs. The method can be used for the detection of endogenous or exogenous fluorescence contrast between normal tissue and malignant lesions. For LFS examinations, an all-in-one remote probe fluorescence spectrometer Spectr-Cluster (Cluster Ltd., Russia) has been developed and applied with built-in excitation lasers at blue, green and red spectral wavelengths. The report presents the main results of *in vivo* LFS application in urology obtained during the last few years.

A complex of main spectral parameters of autofluorescence emission spectra recorded *in vivo* from healthy urothelium and foci of superficial bladder cancer under 442 and 532-nm laser excitation were studied. Specific parameters, in terms of intensity and shape of autofluorescence spectra have been revealed which could be used for reliable differentiation between healthy and neoplastic urothelium.

LFS at 442 and 532-nm excitation was also used *in vivo* to improve the predictive ability of photodynamic diagnosis (PDD) of superficial bladder cancer after intravesical instillation of ALAsens (5-ALA-based agent). Two approaches for LFS data interpretation were developed and tested, which allows a significant increase in the positive predictive value (PPV) of PDD. The results suggest that the combination of fluorescence imaging with *in vivo* LFS may minimize false positive fluorescence cases and reduce the required number of biopsies from 5-ALA-induced red-fluorescence zones.

Moreover, LFS at 638 nm excitation was applied (1) for *in vivo* fluorescence detection after application of a chlorine-based photosensitizer in normal urothelium and papillomas of the bladder and (2) for *ex vivo* studies on its accumulation, distribution and clearance in hyperplastic tissues of human prostate. The obtained results showed the possibility of PDT treatment with this photosensitizer and allowed the optimization of the incubation time interval after the intravenous injection.

[29] Innovative techniques in transurethral resection of urothelial bladder carcinoma: Combined photodynamic diagnostics and submucosal water-jet dissection

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Conventional wire loop transurethral resection (TURB) of bladder tumors is the therapeutic and diagnostic gold standard. The quality of performance of TURB is essential for the patient, as it determines the probability of recurrence and progression to higher tumor stages, and possibly tumor specific mortality. But even in high-volume centers specialized for urothelial carcinoma of bladder, high recurrence rates, occurring in up to 45% of the cases, can be seen within 6 months after TURB. This explains the great need for an improvement in conventional TURB. In this context, photodynamic diagnostics (PDD) is a very promising approach. Tumors are marked with a fluorescent dye, leading to an improved detectability and detection certainty of tumors, especially of carcinoma *in situ* (CIS). Randomized clinical trials have shown an improved recurrence-free survival in patients where PDD was used for tumor identification.

One reason for high recurrence rates following conventional TURB is that it contradicts a basic oncologic principle, namely to resect the tumor en-bloc by dissecting through normal tissue. Usually urothelial carcinomas are shredded during TURB and tumor cells are spilled out within the bladder. This issue could be overcome by submucosal dissection (SMD), a very promising resection technique that has been adopted from gastroenterology. The water-jet-dissector HybridKnife® (ERBE, Germany) consists of a microcapillary wire that is placed directly on to the bladder wall. With a high-pressure (30 bar) water jet of a fluid cushion is selectively created within the mucosa and upper muscular layers below the tumor, facilitating resection. We have performed the first clinical evaluation study in patients with urothelial carcinoma of the bladder. Thirty single tumors were resected. No perforation was observed. Time for application of the HybridKnife® seems to be comparable to common wire-loop TURB with a steep learning curve. We conclude that the application of the HybridKnife® in resection of urothelial carcinoma of the bladder is safe and reliable. This new method is an elegant en-bloc resection technique according to oncologic principles. It improves the transurethral resection of urothelial carcinoma of the bladder. Oncologic benefit still has to be proven in further studies addressing recurrence and progression rates.

Hyperthermia

[30] Combined treatment modalities with subtotal peritonectomy and HIPEC as the treatment of choice in primary peritoneal tumors

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Purpose: Irrespective of the increased aggressiveness of surgical treatment and implication of the new drugs in clinical practice, the long-term results of primary peritoneal tumors (peritoneal mesothelioma, and pseudomixoma peritonei) remain dismal. The standard treatment options with systemic chemotherapy cannot alter their dismal prognosis.

Patients and methods: Between 1.1.2006 and 31.03.2010, a total of 24 patients with primary peritoneal tumors of different origin were treated in the Russian National Cancer Center of RAMS and the Central Clinical Hospital of the Presidential Administration of the Russian Federation. The mean age of the patients was 47.8 ± 11.9 years (range: 20–69 years). The majority of the patients were women ($n = 20$; 83.3%). The main tumor entity was peritoneal pseudomixoma ($n = 17$). All patients were surgically treated and optimal cytoreduction was achieved for most of the patients (CC-0: 16 cases = 66.7%; CC-1: 3 cases = 12.5%; CC-2: 2 cases = 8.3%). The hyperthermic intraperitoneal chemoperfusion (HIPEC) was performed in all cases. HIPEC was performed for 90 min with an average temperature of 43.5–44.0 °C. There were no intra-operative complications.

Results: In most of the patients, combined surgical procedures had been carried out ($n = 21$; 87.5%), that is, mainly splenectomy ($n = 12$) and cholecystectomy ($n = 6$). Postoperative complications were observed in 11.7% of cases, whereas uneventful recovery occurred in 18 cases (78.3%). No postoperative mortality was observed.

The survival (Kaplan–Meier) was estimated separately: in the whole group the 1- and 2-year survival was 100%, with progression free 1- and 2-year survival of 86.0 ± 13.2%. In two cases with primary peritoneal mesothelioma, tumor relapse was diagnosed during the first year, and in one case a second laparotomy with tumor excision and second

HIPEC + early postoperative intraperitoneal chemotherapy (EPIC) was carried out and the patient lived for further 36 months without recurrence. For the whole group and in the subgroups, the median survival was not reached.

Conclusion: The HIPEC is a feasible procedure that does not increase the intra-operative and postoperative morbidity and mortality, and obviously increases the long-term results in cases of primary peritoneal tumors. In selected cases, HIPEC can be applied in cases with local relapse with good long-term results. Due to our data, HIPEC combined with adequate surgical treatment (subtotal peritonectomy and/or organ resection), is the treatment of choice in cases of primary peritoneal tumors.

[31] Hyperthermic intraperitoneal chemoperfusion (HIPEC) in the combined treatment of locally advanced and disseminated gastric cancer

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Purpose: In spite of the increased aggressiveness of surgical and combined treatment modalities intraperitoneal recurrences (IR) still remain the major problem after radical surgery in cases of locally advanced gastric cancer (GC). Moreover, the majority of patients with metastatic GC have peritoneal carcinomatosis. The standard treatment options with systemic chemotherapy cannot alter their dismal prognosis.

Patients and methods: Between 1.1.2002 and 31.03.2010, a total of 31 patients with locally advanced (pT3-4N+) and disseminated (P1-2) gastric cancer (curability B: $n = 11$; curability C: $n = 20$) and one patient with recurrent GC underwent surgical treatment combined with hyperthermic intraperitoneal chemoperfusion (HIPEC). Women were predominant ($n = 17$, 53.1%) and the mean age was 56.8 ± 1.7 years (range: 32–67 years). Total gastric wall infiltration was diagnosed in 13 cases (40.6%). In 17 cases, tumor infiltration of the cardia with esophageal involvement was diagnosed (53.1%). Infiltrative tumor growth types (Borrmann 3 and 4) were predominant ($n = 29$; 90.6%). In 23 cases (71.9%) a combined treatment modality (surgery + HIPEC) was applied, although in nine cases induction treatment was used. A resectional type of procedure was performed in 27 cases (curability B: $n = 12$ cases, 37.5%; curability C: $n = 15$, 46.9%). The majority of patients had signet ring cell ($n = 14$, 51.9%) or low differentiated adenocarcinoma ($n = 5$, 18.5%). In 13 cases (44.8%) R0 resection was feasible, whereas in 12 cases R1 and in 4 cases R2 resection was carried out. In 24 cases complete CC-0 cytoreduction was feasible (75.0%).

The main procedure was total gastrectomy (69.4%) combined with splenectomy (47.2%) or pancreato-splenectomy (22.2%).

The mean duration of the procedure was 346 ± 80 min and the HIPEC was performed for 90 min with an average temperature of 44.0 °C. There were no intra-operative complications.

Results: Postoperative mortality was observed in one case (3.1%). Uneventful recovery was observed in 23 cases (82.1%). A subphrenic abscess and intraparenchymal hemorrhage were diagnosed in one case each (3.1%).

For the whole group, mean and median survival were 23 ± 4 and 12 ± 5 months respectively, with a 5-year survival of 23%. In the stratification subgroup analysis, best results were observed in curability B group with a mean survival of 46 ± 6 months, and median survival was not reached. In curability C group mean and median survival were 10 ± 2 and 7 ± 3 months respectively. In the palliative subgroup best results were gained in patients with free cancer cells (Cy+) – mean survival was 24 ± 9 months with median survival of 12 ± 7 months.

Conclusion: The HIPEC is a feasible procedure that does not increase the intra-operative and postoperative morbidity and mortality, and can be combined with radical or palliative surgical procedures improving the long-term results. To draw a final conclusion, prospective randomized trials are needed in stratified groups of patients.

[32] Cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy – Experience of a "high-volume" center

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Peritoneal carcinomatosis is an expression of an advanced stage gastrointestinal cancer. Mostly, patients are treated with a systemic chemotherapy, but the overall long-term survival rates are not satisfactory. In fact there seems to be a paradigmal shift in the therapy of peritoneal carcinomatosis. The interdisciplinary treatment with cytoreductive surgery (CRS), hyperthermic intraperitoneal chemotherapy (HIPEC) and systemic chemotherapy is moving more and more into the focus of therapy, but is only suitable for a highly selected patient collective. After a complete macroscopic cytoreduction, these patients have a survival benefit. Depending on the tumor entity, a nearly 50% 5-year survival rate can be achieved.

Due to extensive surgery and the use of cytotoxic agents, CRS and HIPEC are associated with a significant number

of postoperative complications, although the postoperative complication rates are comparable to other major gastrointestinal surgery. In our patient collective of about 350 patients, we observed a mortality of 2%. Nevertheless, the chemotherapy-related complications and the possible side effects have to be taken into account in the context of preoperative patient selection and individual dosimetry for intraperitoneal chemotherapy.

Regarding the quality of life, the data show impairment 3 months postoperatively, with an improvement over 6–12 months at levels higher than the baseline.

Because of the complex treatment regime, including a long learning curve, patients with peritoneal carcinomatosis should only be evaluated and treated in a center with experience. If peritoneal carcinomatosis is diagnosed in an early stage, it is more likely that the described interdisciplinary treatment approach can be applied.

Tissue imaging

[33] Molecular fluorescence imaging: A new promising tool in surgical oncology

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Background and objective: Surgical excision of cancer is often confronted with difficulties in the identification of cancer spread and the accurate delineation of tumor margins. Currently, the assessment of tumor borders is afforded by post-operative pathology or, and less reliably intra-operative frozen sectioning. Fluorescence imaging is a natural modality for intra-operative use, since it relates directly to the surgeon's vision and offers highly attractive characteristics such as high-resolution, sensitivity and portability. By using targeted probes, it can also be highly tumorspecific and can lead to significant improvements in surgical procedures and outcome.

Investigations were made in order to improve the surgical procedure and outcome in the future by means of real-time molecular imaging feedback of tumor spread and margin delineation, using targeted near-infrared fluorescent probes with specificity to tumor biomarkers.

Materials and methods: Mice bearing xenograft human breast tumors were injected with an $\alpha_v\beta_3$-integrin receptor targeted fluorescent probe and were visualized *in vivo* using a novel, real-time, multi-spectral fluorescence imaging system. Confirmatory *ex-vivo* imaging, bioluminescence imaging and histopathology were used to validate the *in vivo* findings.

Results: Bioluminescence images were all in good correspondence with the fluorescence images with respect to anatomical localization. Fluorescence imaging detected all tumors and successfully guided total tumor excision by effectively detecting small tumor residuals, which occasionally were missed by the surgeon. Tumor tissue exhibited a target-to-background ratio (TBR) of ~4.0 which was significantly higher than compared to white-light (WL) images representing the visual contrast. Histopathology confirmed the capability of the method to identify tumor negative margins with high specificity and a better prediction rate compared to visual inspection.

Conclusion: Real-time multispectral fluorescence imaging using tumor specific molecular probes is a promising modality for tumor excision by offering real-time feedback to the surgeon in the operating theatre.

[34] Molecular imaging with optical tomographic methods

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Optical imaging is an emerging field in imaging sciences that encompasses a diverse range of methods and techniques utilized in biomedical research and clinical application. Fluorescence and bioluminescence methods have attained widespread use in the biomedical lab because light is an easily manipulated modality and the corresponding measurements or image formation can reveal highly diverse anatomical, physiological and molecular features of the structure studied.

Additionally, applications are found in the fields of biotechnology, genomics, proteomics, and systems biology while there is increasing use of fluorescence methods for *in vivo* imaging of diseases and in drug discovery.

Optical tomographic technologies have been developed to address the quantification and penetration limitations of planar imaging. Fluorescence molecular tomography (FMT) reconstructs the three-dimensional biodistribution of fluorescent contrast agents achieving submillimeter resolution in small animals utilizing multi-illumination approaches, efficient fluorescence image normalization schemes, and theoretical models of photon propagation.

This method can reveal cellular and sub-cellular information when imaging fluorochromes with specificity to certain tissue biomarkers or with fluorescent proteins. Similarly optoacoustic (or photoacoustic) tomography is an emerg-

ing imaging technology in which tissue is excited with short laser pulses and produces ultrasonic waves. The recorded acoustic signals are used tomographically to reconstruct the absorption map of tissue. Optoacoustic imaging combines the advantages of the high optical contrast with the high resolution of acoustic imaging, allowing the imaging of strong absorbers in tissue, mainly tissue structures which have a high blood concentration like blood vessels. Apart from the intrinsic tissue contrast, the biodistribution of probes that have a distinct spectral absorption profile (nanoparticles, fluorochromes, fluorescent proteins) can be resolved with multispectral optoacoustic tomography (MSOT) expanding the capability of optoacoustics to molecular imaging. The use of these optical imaging methods is demonstrated in animal models and their capacity, their limitations and their optimal use in biomedical research is discussed, with particular reference to cancer imaging applications in small animals.

[35] Optical tomography in treatment planning and treatment control in oncology

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Background and objective: The problem of adequate trade-off between radical tumor removal and maximum preservation of organs is very important for cancer management and patient's quality of life. Therefore, the detection of exact tumor borders is one of the critical questions. Results from using conventional methods are still unsatisfactory. OCT is one of the promising methods for solving this problem. We present examples of clinical application of optical coherence tomography (OCT) and experimental results of diffuse optical tomography (DOT).

Materials and methods: A conventional time-domain OCT-device (IAP RAS, Nizhny Novgorod, Russia) has been used. It utilizes near infrared light to enable real-time cross-sectional imaging with spatial resolution of 10–20 μm in a depth of approximately 2 mm. In our research we used flexible endoscopic forward-looking probes of 2.4 and 2.7 mm in diameter with linear transverse scanning that were introduced through biopsy channels of standard endoscopes. More than 850 patients with bladder and gastrointestinal tract cancer, and cervical pathology have been examined and a multicenter study has been carried out. Written informed consent was obtained from all patients.

DOT was tested for hypoxia identification of tumor models with different biological features and in the clinical examination of female patients with breast cancer. The study was performed using an experimental set-up with parallel plane geometry and a single source and detector pair (IAP RAS, Nizhny Novgorod, Russia). Three laser fibers coupled in a single bundle were used to illuminate the studied volume at 684, 794 and 850 nm. The frequency of amplitude modulation was 140 MHz. Independent scanning of the source and the detector in corresponding planes was performed by computer-controlled step motors; the scanning area was $15 \times 15\,cm^2$.

Results: According to our data, OCT can help to define the exact tumor margins in real-time and has the potential to improve biopsy precision, adequacy of tumor resection and to reduce the recurrence rate.

The DOT technique confirms the possibility of investigating the internal structure of deep tissues and of detecting neoplastic changes. The sensitivity of the created system allows tumors to be located at a depth of up to 8–10 cm. Illumination at multiple wavelengths provides determination of component distributions under appropriate image processing. Distribution of the oxygenated and deoxygenated forms of hemoglobin may give additional information which reflects the fact that tumor oxygenation that could be essential for prognosis and therapy selection.

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[36] Raman spectroscopy as a prospective tool in cancer research

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In the last few years Raman spectroscopy has emerged as a powerful tool for the analysis of isolated molecules, single cells or even tissue. By means of *in vivo* spectroscopy, information can be gained about the cell components and even living cells can be analyzed without destruction. With the aid of micro-Raman spectroscopy it is possible to resolve structures in the submicron range and to record Raman spectra within this spatial resolution.

Raman spectra of biological samples, like cells or tissues, are superpositions of the molecular information from all components within the laser focus. Therefore, special techniques are necessary to properly analyze the data, e.g. with statistical/chemometrical methods. Furthermore, since Raman images are hyperspectral images i.e. consist of complete Raman spectra at each pixel, data reduction by multivariate statistics is required to obtain data sets of manageable numbers of chemically significant descriptors for generating the image contrast.

In this contribution we describe some of our latest results concerning the application of micro-Raman spectroscopy in combination with innovative chemometric methods to characterize cancer cell lines and tissues. As even minor variations in cell chemistry during pathogenesis can be monitored by means of Raman spectroscopy, this information can be used to characterize and distinguish different cancer cell lines. In addition, knowledge about the mode of action of cytostatic drugs might lead to the development of novel therapeutic approaches, including the design of more effective and specific drugs.

[37] AOTF-based spectroscopic instruments for oncology

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Medical investigations and developments require new instruments for the elaboration of novel approaches and techniques for diagnostics and therapy. Basic requirements for this modern apparatus are sensitivity, stability and reliability, high-rate operation, and preferably, a compact and rugged design.

Spectrometers based on acousto-optical tunable filters (AOTF) generally satisfy all these conditions. They employ dynamic diffraction grating, which is induced in real-time with the use of ultrasonic waves, and this operation principle makes spectral selection of light possible by means of ultrasound frequency tuning. AOTFs and AOTF-spectrometers possess a unique set of specific physical properties (high optical throughput, rather high spectral resolution for small-size device, spectral agility, etc.) and technical characteristics (compactness, insensitivity to mechanical influence, i.e. shock and vibrations, real-time control). They are also capable of detecting spectral images of the object with use of matrix-array photodetector. This particular property enables application of all the scopes of spectral techniques developed for chemical and biomedical imaging.

A variety of AOTF-based devices, developed at the Scientific Technological Center of Unique Instrumentation of RAS, are presented and their medical applicability is described for the diagnosis of diseases. In particular, a photoluminescence spectrometer (0.55–0.9 μm) and a spectral imaging (500 × 300 pixels) device are reported for the visible–NIR range (0.6–1.2 μm). Both devices are compact, programmable, and provide double monochromatization for higher spectral discrimination. The imaging device ensures high-quality images. The only visually observable distortion is unidirectional extension up to 3–5%.

The most specialized device of this series is a spectral fluorimeter operating in time resolution mode (5 μs). It was demonstrated that a prototype device is capable of distinguishing tumor from normal muscle tissue by synchronous detection of photoluminescence caused by pulse-periodic laser (532 nm, 20 μJ/pulse, 1000 pulse/s) with an accumulation time of 1 s. The detectable threshold was estimated as 20 μg/kg of body weight with use of Pt-coproporphyrin III metal complex as a photoluminescence agent, which is accumulated preferably in malignant tissue. Such a dose is quite safe with regard to both toxicity and phototoxicity. Therefore, this device with a fiber-optic input can be used for early tumor diagnostics of endoscope-accessible forms of cancer.

Two extra prototype devices are discussed which are promising for biomedical applications. The first one is a differential-mode AOTF-spectrometer, which provides instrumental detection of differential characteristics of spectra. This device can be implemented for spectral characteristics detection against background illumination, which is the usual situation for medical measurements. The second device is an AOTF acting as fast optical shutter with a trigger-time down to 2 μs. It is a promising no-moving-parts unit which combines functions of optical filter and shutter.

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[38] Clinical experience with confocal laser scanning endomicroscopy

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The use of a confocal microscope enables non-superficial microscopic imaging of untreated tissue without previous fixation and preparation of tissue sections. This is enabled by passing focused laser-light of a defined wavelength through a confocal apparatus, and thereby reducing scattered light from above and below the focal plane. Dynamic images are then reconstructed by having all light-spots to be scanned in the horizontal und vertical plane.

Clinically, the method can be used during endoscopic examination to detect cancers or to differentiate suspicious

lesions. In this way, confocal laser scanning endomicroscopy would help to significantly reduce the number of biopsies. Moreover, it could potentially even replace standard tissue acquisition methods in areas where the performance of these methods is inadequate. The application of this technology would have the further advantage of allowing the visualization of dynamic processes on a microscopic level such as enabling the monitoring and determination of blood flow.

In summary, endomicroscopy could help to establish a better diagnosis. Future research will focus on the combining this method with other new imaging methods, the measurement of biological changes in patients *in vivo,* and finally on therapy monitoring, e.g. for anti-angiogenic therapy in cancer patients.

[39] Complex dermatoscopy diagnostics of melanoma

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Introduction: In spite of the fact that the incidence of melanoma only makes up 3–5% of all primary malignant tumors of skin, it is the main reason for the death of patients in oncodermatology. Over the last 4 years complex dermatoscopy examination has been intensively used for the detection and characterization of melanoma and other pigmented skin lesions in the clinical practice of Moscow Oncological Hospital No. 62.

Materials and methods: Complex dermatoscopy diagnostics include digital photo, zoom photo, standard- and microdermatoscopy, and fluorescent dermatoscopy. At the first stage, we took digital photos and performed computer mapping of the patient's skin. At the second stage, zoom photos with a 10-fold magnification were taken for each suspicious lesion. At the third stage, we performed a standard dermatoscopy with a 10-fold magnification, microdermoscopy with a 120-fold magnification and a fluorescent dermatoscopy with ALAsens (NIOPIK, Russia).

Results and conclusion: Applying the complex method of dermatoscopy diagnostics, we studied the reliability of characteristics describing malignant and benign pigmented skin lesions in 497 patients with 1735 pigmented skin lesions, 280 of them non-melanocytic and 1271 melanocytic (65 melanoma, 259 dysplastic nevi). The data of the complex dermatoscopical investigations were compared to the results of morphological investigations of surgery samples.

Sensitivity and specificity of dermatoscopy diagnostics of melanoma was 92% and 72% accordingly. Considering the high level of efficiency and the non-invasive character of the complex dermatoscopy diagnostics method for melanoma of the skin, it should be used as an examination method in groups at high risk of melanoma.

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Other

[40] Compact and high-efficiency solid-state lasers based on Tm-doped and Ho-doped crystals at an emission wavelength of 2 μm for medical applications

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Background and objective: Laser radiation in the 2-μm wavelength region is well-absorbed by water. Commercially available low-OH quartz optical fibers showed good transmittance thus enabling their medical application in endoscopic and open surgery. Medical laser systems in this wavelength range are flashlamp- or laser-pumped pulsed Ho:YAG lasers and continuous wave (cw) Thulium (Tm)-doped fiber lasers. In this study the laser–tissue effects on an *ex-vivo* tissue model were investigated using an innovative diode-pumped Tm:YLF laser and cw Ho:YAG laser.

Material and methods: The diode-pumped Tm:YLF laser (IAP RAS prototype) emits light at 1909 nm with an output power of up to 30 W. This air-cooled system showed an optical-to-optical efficiency of more than 41%. The operation mode can be technically switched from cw to pulsed. The Ho:YAG laser (IAP RAS prototype) is pumped by the radiation of a Tm:YLF laser and can be tuned by an intracavity etalon at wavelengths of 2091, 2097 and 2112 nm. The laser can operate in cw, pump-switched or Q-switch regimes. A cw power of up to 15 W can be used. The laser beam quality of both lasers is about M^2<1.3 and can be easily coupled in low-OH optical fibers.

Laser tissue interaction experiments were performed using pork kidney and liver tissue. In a reproducible set-up, the distance of the fiber tip to the tissue surface (d = 0–5 mm), the laser output power (P = 5, 10, 20 W) and the velocity of the linear fiber movement (v = 0–1 mm/s) could be varied. Macroscopic and histological evaluations were performed.

Results: The tissue effect showed precise and reproducible ablation. The cutting depth depended on the applied power and on the velocity. Interestingly, the histological findings, showed that the thickness of the coagulation zone in lateral

and axial direction was nearly constant and was 1–1.5 mm in each direction.

Conclusion: These laser sources showed promising capabilities for precise cutting due to the reproducibility of the coagulation boarders. The size of the coagulation region promises sufficient sealing, which should be proven in further experiments. The laser-induced combination of ablation and coagulation is especially useful for surgery procedures in order to protect certain tissue structures (e.g. nerves) from thermal exposure.