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Experimental ONCOLOGY

THE SECOND UKRAINIAN-SWEDISH WORKSHOP "TRANSLATIONAL ONCOLOGY: OLD AND NEW PARADIGMS"

The second Ukrainian-Swedish workshop "Translational Oncology: Old and New Paradigms" took place at the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology National Academy of Sciences of Ukraine (IEPOR NASU) on May 20–21, 2013. Karolinska Institutet (Stockholm, Sweden) was co-organizer of the event.

Academician Vasyl F. Chekhun, the director of R.E. Kavetsky IEPOR NASU, opened the conference with a welcoming speech. He greeted participants and stressed the importance of knowledge integration in field of cancer research. He also pointed out the necessity of experience exchange and collaboration between different institutes worldwide.

The goal of the Workshop was to discuss current views and newly emerging conceptual frameworks concerning mechanisms of cancer development. Considering the fact that cancer is major healthcare problem today, the pursuit of deep understanding how cancer actually works will lead to new effective approaches to treatment of proliferative disorders. Accordingly, all lectures and reports, presented by participants, included up-to-date issues and experimental data dealing with clinical and biological aspects of cancer research. The Workshop featured excellent lectures, delivered by invited speakers from Karolinska Institutet (Sweden) and speakers from IEPOR NASU, as well as informative reports on proceeding research.

The number of scientists, who took part in the event, reached more than hundred people this year. Apart of organizing institutes representatives of other institutions also attended the Workshop. Among them there were Institute of Cell Therapy, City Clinical Oncological Center, National Cancer Institute of the Ministry of Public Health of Ukraine, National University of Kyiv-Mohyla Academy, Center of Molecular and Cell Research, Taras Shevchenko National University of Kyiv, Educational and Scientific Center "Institute of Biology", State Institution "Institute of Pharmacology and Toxicology, National Research Center for Radiation Medicine, Shupik National Medical Academy of Post-Graduate Education, Reference-Centre for Molecular Diagnostic, Ovcharenko Institute of Biocolloidal Chemistry, Bogomolets National Medical University, Institute of Molecular Biology and Genetics (Kyiv, Ukraine), Danylo Galytskyj Lviv National Medical University, Ivano-Frankivsk National Medical University, Rivne Region Oncology Hospital, Bila Tserkva National Agrarian University; Bila Tserkva Regional Oncology Center; Belarusian Research Center for Pediatric Oncology, Hematology and Immunology (Minsk, Belarus), Cancer Research Institute of SB of the RAMS (Tomsk, Russia), Institute of Chemical Biology and Fundamental Medicine of SB of the RAS (Novosibirsk, Russia).

The program of the Workshop spanned plenary lectures and oral or poster reports. All talks evoked vivid interest among listeners and, thus, were thoroughly discussed. Some of these reports were included into current issue of *Experimental Oncology* journal for your appreciated consideration.

PART I. LECTURES

TUMOR PROGRESSION — TIME FOR A NEW PARADIGM?

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Around the millennium three decades of successful research into the biology of cancers were concluded in the most cited review on cancer ever "Hallmarks of Cancer" (Hanahan and Weinberg, Cell, 2000). It marked the end of the era when oncogenes and suppressor genes were discovered en masse, and when tumor progression at the level of cell and tissue pathology could be linked with molecular steps like that of the pioneering description by Bert Vogelstein of colon cancer progression ("The Vogelgram"). We then understood that cancer was indeed a disease of cells and depended on mutations of some ten genes. Tumor progression, when tumors "go from bad to worse", could be viewed as an evolutionary, Darwinistic process at the tumor cell population level, with sequences of mutation, improved cell survival, selection, another mutation etc. This seemed so transparent and logically sound that the scientific community at large believed it had understood the biology of cancer. Now we only needed to trace these mutated genes and eliminate their erroneous behaviour one way or another. It also gave a strong impetus to "personalized cancer medicine", now we only needed the genome of individual tumors for designing the cure. One impressive major extrapolation was that all the 200 different forms of cancer develop through a similar mechanism. With this modern paradigm of cancer biology (or the Hanahan — Weinberg — Vogelstein/HWV-paradigm) as a basis, ten years later it was clear that the success rate in exploiting its implications for cancer treatment was quite low.

During the same time the understanding of cell and tissue biology took quantum leaps in new directions, which clearly exposes the HWV-paradigm as totally insufficient and oversimplified, although not directly wrong. The conclusions are two: we do not really un-

derstand cancer biology, and cancer is a very complex disease reflected by the enormous complexity of its "host" — the cell. Major findings have been: epigenetic deregulation is as important as mutations, cancer tissues are highly heterogenous with cell hierarchies (including stem-cell like cells) and a participating stroma, microRNAs represent a large new set of regulatory genes, low level chronic inflammation is a key component in early carcinogenesis, there are not only some ten mutations in cancer cells but rather several thousands, and cancer cells usually switch metabolism from oxidative glycolysis to fermentation (the Warburg effect).

How can we comprehend all these significant findings in our understanding of cancer biology? Firstly we should be humble. Secondly we should explore new tools and technologies in interdisciplinary initiatives to adopt an approach that accommodates complex or complicated features of biological systems like cancer. This will be a long and uncertain way. Meanwhile it must be perfectly motivated to continue to develop treatments based on trial-and-error without sufficient information about the enemy.

EXPLOITING THE P53 PATHWAY TO ACHIEVE CURE IN CHRONIC MYELOGENOUS LEUKEMIA

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In 2008 we reported the discovery and antitumor activity of a small molecule named tenovin-6. In addition, we showed that this compound activates the tumour suppressor p53 as well as inhibits the protein deacetylase activity of sirtuin SirT1. In 2012, a series of remarkable studies were published showing that tenovin-6 increases apoptosis in Chronic Myelogenous Leukemia (CML) stem cells and reduces their growth *in vitro* and *in vivo* in combination with imatinib. These exciting preclinical results have encouraged us to synthesize new tenovin-6 analogues and investigate their activity in cells and biochemical assays. Here I will present new findings obtained with our current collection of tenovin analogues and suggest which of these features may influence therapeutic activity. I will also discuss our progress in the identification and mechanism of new compounds for testing in leukemia preclinical models and patient samples.

INDIVIDUALIZATION OF CANCER TREATMENT: SUCCESSES AND CHALLENGES

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Biomedical research delivered a new look on the medicine. New developments of biochemistry, molecular biology, engineering, biomedical research deliver new insights in the cancer mechanisms, new treatment methods and new drugs.

Medicine success needs new techniques.

New techniques are coming from biochemistry, chemistry, physics, molecular and cell biology.

More than 100 anti-cancer drugs are used in the clinic today, and new drugs are under development for use tomorrow. The challenge is to match a patient with the right drugs. The clinicians face this challenge with practically every patient, because of the limitations of diagnostics. Information obtained with current diagnostics is still far from predicting securely how the tumor and patient would respond to a treatment.

The recent developments of cancer research have opened for the qualitative improvement of diagnostics. We have developed Functional Molecular Diagnostics (FMDx) which is for the clinical use. FMDx tests responsiveness of individual patient's tumors to different drugs by testing responsiveness of the living tumor samples in organ culture (Organ Culture FMDx), testing targets and modulators of the drugs' action (Functional Biochemical Assays), and by unbiased testing of the tumor's proteome profile (Proteomics FMDx).

FMDx is a set of assays performed on living and processed tumor cells following removal from patients upon surgery or biopsy. These assays measure in a real time how the patients' tumor may respond to different drugs before the patient is offered treatment, and whether the tumor is of an aggressive type. The successfulness of FMDx is based on novel proprietary technologies.

FMDx assays are of 3 types — organ culture-based, functional biochemical assays and proteomics-based. The assays may be used all together or separately, depending on the clinical requirements. All 3 types of assays analyze a tumor with different methods, but for the common goal of finding the best treatment. This increases the confidence of the recommendations for treatment. FMDx has been used for diagnostics of cancer patients, and in my talk, Functional Molecular Diagnostics applications will be discussed.

HIGH-RISK HPV L1 CAPSID PROTEIN AS A MARKER OF CERVICAL INTRAEPITHELIAL NEOPLASIA IN HIGH-RISK HPV-POSITIVE WOMEN WITH MINOR CYTOLOGICAL ABNORMALITIES

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Human papillomavirus (HPV) L1 capsid protein is only produced during a productive HPV infection at the end of the natural viral life cycle and is a major target of the immune response in women with HPV-related squamous intraepithelial lesions.

We evaluated the usefulness of L1 detection by immunocytochemistry in high-risk (HR) HPV-positive women with minor cytological abnormalities detected at organised population-based cervical cancer

screening in Sweden, and assessed the relationship with histological diagnoses. Cytological slides were immunocytochemically stained using a HPV L1-specific monoclonal antibody for all known HPV types. HPV DNA analysis was performed by Linear Array. Out of 13 L1-positive women infected with HPV16, only two (15.0%) progressed to cervical intraepithelial neoplasia grade 2 or worse (CIN2+); compared to four L1-positive women infected with other HR-HPV types. Among L1-positive women with CIN2+, 35.7% harboured both HR and low-risk HPV types, 25.0% harboured HR-HPV types only, and 13.3% were infected with HPV16. Loss of L1 expression could be a prognostic marker for the development of preinvasive cervical lesions. We show that different HPV types may initiate a parallel oncogenic process, but only loss of L1 expression predicts the development of CIN2+, suggesting that HPV typing in combination with L1 detection could be used for more focused investigations of women with minor cytological abnormalities.

CARCINOMA OF THE ENDOMETRIUM: PREDICTION MARKERS OF TUMOUR PROGRESSION, AGGRESSIVENESS AND POTENTIAL TARGETS FOR THERAPY IN A MITOCHONDRIAL PERSPECTIVE

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Endometrial cancer (EC) is the most common gynaecological cancer in the Western world, and its incidence continues to increase. At present, molecular markers are not commonly used to detect EC, nor are there molecular markers that can distinguish between the more and less aggressive forms of EC. Treatment is currently based on histological results from a single pre-surgical endometrial biopsy, which is often quite different from the biopsy obtained during surgery. There is a clear need for early molecular markers that can be used to determine EC diagnosis, as well as tumour progression and aggressiveness, in order to improve the prognosis of EC and to reduce recurrence. Further investigation of tumour gene mutations is also needed to find new targeted treatments for EC.

We summarized the known molecular alterations that occur in EC, with a focus on mitochondrial alterations, and the potential to use these alterations to develop targeted treatments in the future. Studies concerning mitochondrial dysfunction in carcinogenesis reported that the LRPPRC protein is highly expressed in early endometrial carcinogenesis, and may be considered an early marker of EC. Moreover, four proteins were reported to be molecular markers of aggressiveness in patients with EC: 1) the ratio of progesterone receptor (PR) A/PR B less than 1 was associated with a shorter disease-free period and shorter survival in patients with endometrioid EC; 2) glucose transporter proteins (GLUT 1 and GLUT 3) were correlated with increased malignancy, invasiveness and poor prognosis; and 3) the TKTL1 protein was overexpressed in low-grade EC.

Currently, there are no tumour markers that can resolve diagnostic problems in EC. The present review indicates that molecular markers and tumour characteristics greatly vary. Consequently, a combination of molecular markers would be necessary to give information on early diagnosis, tumour progression and metastatic capability.

EBV-ENCODED EBNA-5 BINDS TO P18ARF AND PREVENTS P53-INDUCED GROWTH ARREST AND APOPTOSIS

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The EBV transformed LCLs maintain wild type p53 during serial culturing. We addressed the question why the p53 downstream growth arrest and apoptotic pathways are non-functional in p53 expressing LCLs, unless activated by genotoxic agents, by examining p53 containing protein complexes in LCLs.

We have shown that trimolecular complexes were formed where MDM2 served as a bridge between EBNA-5 and p53. All three proteins colocalized in the nucleus of lymphoblastoid cells with a high p53 expression.

To explore the functional consequences of the MDM2–EBNA-5 binding, p53 polyubiquitination and degradation assays were performed *in vitro*. GST-EBNA-5 inhibited MDM2-dependent polyubiquitination (but not monoubiquitination) of p53, similarly to GST-p14ARF. The p53 degradation on commercially purified 26S proteasome subunits was inhibited by EBNA-5 as well.

These findings indicate that the high p53 levels in LCL are due to EBNA-5 dependent inhibition of p53 degradation. Chromatin immunoprecipitation showed that p53 could bind to the p21 promoter in mitogen stimulated but not the EBV activated B-cells. Taking together, these findings are consistent with the interpretation that the trimolecular EBNA-5-MDM-2-p53 complex inhibits the binding of p53 to DNA.

We have cloned full length of human p14ARF protein (p18ARF) from the EST clone. We have shown by GST pull-down and transfections experiments that p18ARF binds to MDM2, MDMX, and EBNA-5. We have found that an enhanced expression of p18ARF in cells bearing wt p53 resulted in the cell death, similarly to mouse p19ARF. Simultaneous expression of p18ARF and EBNA-5 rescued these cells. Biological significance of the binding between MDMX, p18ARF and EBNA-5 is currently under investigation.

TRANSCRIPTIONAL NETWORK IN B CELLS: IRFS IN THE DEVELOPMENT OF B CELLS AND LYMPHOMAGENESIS

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The family of interferon regulatory factors (IRF) is comprised of 9 members: IRF1, IRF2, IRF3, IRF4/PIP/LSIRF/ICSAT/MUM1, IRF5, IRF6, IRF7, IRF8/ICSBP and IRF9/ISGF3γ. All members of this family of transcription factors contain the homological DNA-binding domain, which is responsible for interaction with DNA binding motifs ICS, ISRE, IRFE, EICE. IRF4 and IRF8 proteins share the most homology among other IRFs, and their expression is narrowed to the cells of immune system. For transcriptional regulation of target genes IRF4 and IRF8 form complexes with each other or with other transcription factors and adaptor proteins (Ets family — PU.1, SpiB, TEL; E47, NFAT, FOXP3, STAT proteins, MyD88, IBP, TRAF6). IRF1, IRF2, IRF4 and IRF8 play the central role in the regulation of immune cells differentiation and maturation. IRF4 and IRF8 are key regulators of multiple steps of B cell differentiation, starting from stage of pre-B cells. IRFs 4 and 8 together with other transcription factors (BCL6, BLIMP1, XBP1, Pax5) control GC formation, CSR, memory versus plasma cell differentiation. IRF4 and IRF8 were shown to be deregulated in B cell malignancies. IRF8 was mainly shown to function as tumor suppressor protein, but IRF4 was found to be overexpressed in some tumors (multiple myeloma, Hodgkin's lymphoma, some diffuse large B cell lymphoma cases, etc), and at the same time could have tumor suppressor functions in B-acute lymphoblastic leukemia.

Still there is not so much data on regulation of IRFs expression by different signals and signaling pathways in normal and malignant B cells. B cell receptor and CD40 are the most potent up-regulators of IRF4 expression. However, nothing is known about receptor-mediated downregulation of this transcription factor. We have shown that CD150 receptor expression decreased the levels of IRF4 mRNA and protein in DT40 $CD150^+$ transfectants — the stage of pre-B cells. Prolonged stimulation of activated human tonsillar B cells via CD150 resulted in upregulation of IRF4 mRNA. Signals via CD150 and BCR induced the upregulation of IRF4 mRNA expression by Burkitt's lymphoma cells BJAB. Overall, CD150 and BCR had different impact on IRF4 expression by malignant and normal human B cells. The identification of surface receptors and signaling pathways involved in the regulation of IRFs expression could help to develop new strategies for tumor cells elimination.

IMMUNOGENIC CANCER CELL DEATH: HOW IT CAN BE EXPLOITED

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When considering normal, non-transformed cells, it is commonly assumed that the immunological consequences of cell death follow a classical dichotomy of immunogenic necrosis versus non-immunogenic (or even tolerogenic) apoptosis. However, recent studies have revealed that apoptotic tumor cells induced by ionizing irradiation, anthracyclines and some other cytotoxic agents are able to induce a potent immune response *in vivo*. This led to the hypothesis that cell stress or death may result in the release of endogenous danger signals that act as adjuvants to stimulate an immune response. Some characteristics of the plasma membrane, acquired at preapoptotic stage, can cause immune effectors to recognize and attack preapoptotic tumor cells. Depending on the signal-transduction pathway, tumor cells responding to chemotherapy or radiotherapy can express "danger" and "eat me" signals on the cell surface (such as NKG2D ligands, heat-shock proteins and calreticulin) or can secrete/release immunostimulatory factors (such as cytokines and high-mobility group box 1) to stimulate innate immune effectors. These endogenous danger signals (or alarmins) promote activation of innate immune cells and recruitment and activation of antigen-presenting cells engaged in host defense through pattern recognition receptors such as the TLRs, many of which have a key role in the detection of pathogens. In addition, these danger associated molecules act broadly with many other immunostimulatory molecules (endogenous and exogenous including pathogen associated molecular patterns or PAMP) to amplify their activity in a synergistic manner. In our investigations we used cytotoxic drug NSC-631570 in combination with PAMP in the treatment of melanoma B16 bearing mice. Our results suggest that bacterial adjuvants synergized with chemotherapy to inhibit tumor growth and significantly augment immune responses. Current evidence suggests that once in the extracellular milieu, alarmins can bind to molecules such PAMP and, depending on its interactions with DNA, can form complexes of varying structure and with a varying the number of components. Here we analyse the current knowledge and our data to discuss the hypothesis that the formation of extracellular complexes is an important mechanism for generating pro-inflammatory signals during cell death and reverting some of the established immunosupressive barriers present within the tumor microenvironment, ideally recovering the role of the tumor as an effective immunogenic hub.

HUMAN BETA-DEFENSINS IN REGULATION OF BIOLOGIC PATTERNS OF CANCER CELLS

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Human beta-defensins (hBDs) belong to a family of small cationic peptide antibiotics produced by human epithelial cells and are characterized by wide spectrum of biologic activities. These molecules exert direct antimicrobial action and immunomodulatory activity, and are shown to be involved in many human pathologies including cancer. Beta-defensins are involved also in wound healing processes and may affect proliferation of epithelial cells. To understand the mechanisms of biologic activities of beta-defensins, we have produced two recombinant defensins — hBD-2 and hBD-4 expressed in bacterial cells as GST-hBD-2 or -4 fusion proteins. Analysis of *in vitro* activity of rec-hBD-2 toward different human cancer cell lines (A341, A549, 3 thyroid cancer cells, 2 melanoma cell lines) has demonstrated that this defensin is capable to affect cell proliferation and viability in a concentration-dependent manner and in nanomolar range may significantly suppress cell growth via cell cycle arrest in G1/S checkpoint, dephosphorylation of pRB and significant suppression of cyclin D1 expression. The action of hBD-2 is not cell type specific, at nanomolar concentrations hBD-2 significantly suppresses colony forming activity of human cancer cells and their migration ability in scratch assay. *In vitro* hBD-2 may enter tumor cells, could be detected in cell cytoplasm and also in cell nuclei. Similarly, this defensin has been detected in cell nuclei in human lung tumor samples, moreover, in such cases hBD-2 expression levels positively correlated with human lung adenocarcinoma differentiation grade, and negatively correlated with PCNA expression. Hypothetically, concentration-dependent effects of hBD-2 could be explained by its capability to oligomerization. The study of recombinant hBD-4 (presented in details in current issue of Exp Oncol) has demonstrated a concentration-dependent bimodal effect of this defensin on biologic patterns of human cancer cells: in low nanomolar concentrations rec-hBD-4 significantly stimulates cancer cell proliferation and viability, promotes cell cycle progression through G2/M checkpoint, greatly enhances colony-forming activity and migration ability of the cells, but at higher concentrations the effects are of opposite character — significant suppression of cell proliferation and viability, cell cycle arrest in G1/S checkpoint, significant inhibition of cell migration and colony forming activity. It's of interest to analyze further with the use of *in vitro* system what mechanism is exploited by beta-defensins for cancer cell growth suppression — senescence, MET, other, whether it is reversible or irreversible, and to find applications of this knowledge in the field of clinical oncology.

PART II. ORAL AND POSTER PRESENTATIONS

NEW MECHANISMS OF CLINICAL EFFICACY OF LOW-DOSE METRONOMIC CHEMOTHERAPY TREATMENT OF PATIENTS WITH METASTATIC COLORECTAL CANCER

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***Background and Aim*:** This work describes the experience of treatment of metastatic colorectal cancer (mCRC) patients with liver metastases (stages $T_{1-4}N_0$–$2M_1$) by low-dose chemotherapy (LDCT) in metronomic regimen including interferon-alpha (IFN), and demonstrates growth behavior and immunophenotypical changes in CRC cells after influence of LDCT *in vitro*.

***Patients and Methods*:** Treatment of 254 patients was carried out using different schemes, which included cyclophosphamide, cisplatin (CP) and irinotecan (IT) with IFN, efficiency of therapy was monitored by clinical methods and computer tomography. Cell culture and immunocytochemical methods were used for *in vitro* investigation. Statistical analysis using the Student's t-test and Kaplan — Meier survival curves.

***Results*:** Metronomic mode of LDCT in patients with mCRC demonstrated high performance compared with conventional treatment using only high dose of IT. Among the most effective schemes metronomic mode of application of LDCP and IT combination was shown: duration of partial clinical effect was nearly 3 fold higher, duration of the stabilization process and the overall survival rate increased respectively by 81% and 34.5% in comparison with a high dose IT therapy. Using of IFN in all the metronomic CT schemes significantly increased their effectiveness. We have found new targets in the CRC cells for the effects of LDCT *in vitro*. Cells of human colorectal adenocarcinoma of COLO 205 line were cultivated *in vitro* with LD (10–20 times lower IC50) of IT, CP, IFN and their combination for 30 days. The ability of cells after prolonged exposure by LDCT to the agar colony formation decreased by 3–5 times, the maximal effect was observed with the combination of IT + CP + IFN (from 9.25% to 0,25%). A significant decrease of number of cells with mesenchymal and malignant stem cell characteristics was found: number of N-cadherin-, SLUG- and CD44-positive cells decreased more than 10 fold for various combinations of drugs. Expression of ERCC1 marker in COLO 205 cells completely disappeared in all combinations with IFN, but increased somewhat under the influence

of IT + CP. At the same time sensitivity of this cells to drugs in IC50 doses didn't change. ***Conclusions*:** Low-dose metronomic chemotherapy of patients with mCRC is a new and perspective approach that provides suppression of the disease progression. LDCT drugs inhibit signs of epithelial-mesenchymal transition and malignant phenotype in CRC cells *in vitro*.

THE COMET ASSAY: APPLICATIONS TO STUDY CANCER RISK INFLUENCED BY EXTERNAL EXPOSURE

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Introduction*:** The comet assay is a reliable method for measuring DNA damage and repair in human cells such as lymphocytes, epithelial cells, and tumor cells of different origin and has applications in genotoxicity studies, bio-monitoring and cancer research. The potential use of assay as a tool for estimation an individual cancer risk in patients with high predisposition to cancer and clinical management of cancer is widely discussed. ***Aim: To study DNA damage and repair capacity in epithelial cells and peripheral blood lymphocytes (PBL) after exposure to genotoxic agents in healthy and cancer patients with family history of cancer. ***Methods*:** PBL from 45 endometrial cancer (EC) patients were exposed to bleomycin for 30 min. DNA repair capacity was assessed after 15 min incubation in RPMI 1640 without bleomycin. Primary and immortalized by HPV16 *E6/E7* oncogenes of ovarian surface epithelial (OSE) cell cultures obtained from 5 women at high risk (HR) of ovarian cancer were exposed to mitomycin C (MMC) at concentration 50 nmol/L. PBL from 10 healthy volunteers and 5 primary OSE cell cultures from women without family history of cancer were evaluated for DNA damage as a control. DNA damage was assessed by comet assay as a mean % of DNA in the tail and the comet tail moment (TM). ***Results***: The level of basal DNA damage in PBL of EC patients was significantly higher (TM 2.1±0.2) compared to healthy volunteers (TM 0.2±0.1). Among EC patients the sensitivity of PBL to genotoxic exposure to bleomycin was higher in patients with family history of cancer (TM 104.9±1.2) then in patients with sporadic cancer (TM 97.3±0.6). It has been found that efficiency of DNA repair in PBL of EC patients depended on family history of cancer. After 15 min repair PBL restored the damaged DNA to the level of TM 55.0±2.4 in EC patients with family history of cancer and up to 22.7±1.1 in patients with sporadic EC. Basal damage of DNA was significantly higher for the primary HR OSE cells than in control OSE cells (TM 15.1±0.1 and 1.0±0.2, respectively). Cell incubation with MMC resulted in progressive increasing of the level of DNA damage of HR OSE cells (TM 25.3±0.2) whereas normal OSE cell line was resistant to genetoxic exposure (TM 1.1±0.5). After immortalization of OSE cells by HPV *E6/E7* oncogenes the intensity of DNA damage significantly increased independently of family history of cancer. HR OSE cells immortalized with HPV *E6/E7* oncogenes demonstrated increased sensitivity to genotoxic exposure compared to normal immortalized OSE cells (TM 88.5±0.1 and 16.0±0.1, respectively). ***Conclusion***: Enhanced DNA damage of PBL and OSE cells in healthy and cancer patients with family history of cancer under genotoxic and viral oncogenes exposure could expand our understanding of endometrial and ovarian cancer etiology and biology. Comet assay is a useful tool for assessment of individual sensitivity to genotoxic and viral exposure to evaluate the risk of malignancy in patients with predisposition to cancer.

ANTITUMOR EFFECT OF OXY CORRELATES WITH ITS ABILITY TO DECREASE THE LEVEL OF NITROSYL-HEM COMPLEXES IN LEWIS LUNG CARCINOMA CELLS

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Introduction*:** It is known that the tumor growth is accompanied by changes of redox carriers in the mitochondrial electron transport chain. These changes may result in decrease of energy metabolism. The functional state of the mitochondrial electron transport chain also defines the intensity of production and accumulation of the oxygen and nitrogen reactive species (ROS and NOS), which affect different biological processes and can promote cell damage. It has already been observed that non-cytotoxic doses of oxyresveratrol (OXY) showed significant antineoplastic and anti-metastatic effects and prolonged the life of animals with tumors. ***Aim*:** To analyze the influence of OXY on the mitochondrial iron-sulfur centers and nitrosyl-hem complexes of tumor cells during Lewis lung carcinoma growth. ***Materials and Methods: EPR method was used to analyze the OXY influence on the content of the mitochondrial iron-sulfur centers (g=1.94) and nitrosyl-hem complexes (g=2.007) in tumor tissues. The total dose of OXY was 0.2 mg/g of animal weight. The agent was administered *per os* from the 2nd day after cancer cell inoculation, 5 times/week, during 3 weeks. Tumor volume as well as characteristics of tumor mitochondrial electron transport chain was registered on the 10th, 14th, 16th, 20th, 25th day of the tumor growth. ***Results*:** It was shown that OXY affected the formation of nitrosyl-hem complexes in mitochondrial electron transport chain. In OXY free mice the level of nitrosyl-iron complexes was significantly increased during tumor growth, while the level of iron-sulfuric centers was progressively decreased. Treatment of LLC-bearing mice with OXY resulted in a considerable LLC growth inhibition and caused the reduction of NO-hem complexes. Its level at the end of the treatment (20th, day of tumor growth) was by 64% ($p < 0.05$) lower than that of control mice. In 5 days after therapy completion the level of NO-hem complexes in OXY treated mice was not changed compared to untreated animals. It was shown that OXY

didn't influence the content of iron-sulfuric centers in LLC. ***Conclusions*:** LLC growth is accompanied by decrease of iron-sulfur centers' content and increase of nitrosyl-hem complexes in mitochondria of cancer cells. Decrease of the level of nitrosyl-hem complexes in cancer cells under OXY treatment correlates with an ability of OXY to inhibit LLC growth.

COMBINED APPLICATION OF AUTOLOGOUS AND XENOGENEIC HEAT SHOCK PROTEINS IN VACCINOTHERAPY OF MALIGNANT TUMORS

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***Introduction*:** The use of immunotropic substances of natural and synthetic origin, including heat shock proteins (HSP) is one of the successful strategies to improve the efficiency of existing tumor vaccines. ***Aim*:** Combined application of heat shock proteins derived from the chicken xenogeneic embryonictissue and from autologous tumor cells for creating effective antitumor vaccines. ***Methods*:** Protein extracts from embryonic and tumor tissue were obtained by protein ammonium sulphate salting-out. Column chromatography was used for HSP separations with molecular weight of 70 kDa. HSP were analyzed by western blot with anti-HSP-70 monoclonal antibody (Enzo, USA). Based on HSP fraction, a number of vaccines were constructed and their efficacy was analyzed *in vivo* in Balb/c and C57Bl/6 mice with transplantable Ehrlich carcinoma and Lewis lung carcinoma (3LL). All animals were divided on 6 groups consisting of 10 animals each. Antitumor effect of vaccines was evaluated by the dynamics of tumor growth, survival rate and antimetastatic effect. ***Results*:** Immunoblot test demonstrated antigenic homology of HSP-70 derived from embryonic chicken tissues and Ehrlich carcinoma. It was found that level of HSP-70 was 1.68 times higher in chicken embryonic tissues compared to Ehrlich carcinoma. Vaccines enriched with HSPS 70 were made from tumor samples and chicken embryonic tissues and their antitumor efficacy, manifested in inhibition of tumor growth (IG 24.35%) and the increase in life expectancy of experimental animals, has been shown. The purification of HSP-peptide complexes in tumor extracts and embryonic tissues was made and on their basis a number of vaccines has been developed, antitumor activity of which has been tested in the system *in vivo* on animals with transplantable 3LL. The effective vaccines stimulated cytotoxic activity of lymphocytes and macrophages (straight by 25% and antibody-dependent by 27%), increased serum cytotoxic activity and decreased the content of circulating immune complexes. Anticancer activity of vaccines was mainly reflected in the inhibition of metastasis. ***Conclusions*:** Combined use of xenogeneic and autologous HSP-peptide complexes for design of vaccines significantly increases their antitumor activity and immunomodulating effect compared with the vaccine prepared by the traditional technology.

MISMATCH REPAIR PROTEINS MSH2, MLH1 EXPRESSION AND DNA DAMAGE IN TUMOR CELLS OF ENDOMETRIAL CANCER PATIENTS WITH FAMILY HISTORY OF CANCER

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***Introduction*:** DNA repair deficiency may cause genetic instability that results in high sensitivity of cells to exogenous and endogenous DNA damaging agents. Mismatch repair has important role in the maintenance of genome integrity in tissues with high proliferative activity such as endometrium. Genetic and epigenetic defects of mismatch repair genes *MSH2* and *MLH1* induce accumulation of mutations leading to malignant transformation. Patients with family history of cancer may be characterized by inherited DNA repair deficiency therefore further studies are needed to assess DNA repair activity in these patients. ***Aim*:** To evaluate the level of DNA damage and mismatch repair activity in tumor cells of endometrial cancer patients considering family history of cancer. ***Methods*:** 20 endometrial cancer (EC) patients with the mean age 57.2 years were included in the study. Assessment of DNA damages in endometrial tumor cells was performed by DNA comet assay. The level of DNA damage was quantified as the mean percentage of DNA in the tail and the comet tail moment (TM). Mismatch repair activity was assessed by immunohistochemistry using primary monoclonal antibodies MSH2 (clone 25D12) and MLH1 (clone G168–15) ("Diagnostic BioSystems"). The mean percantage of immunopositive cells was calculated (LI, %). ***Results*:** 30% of EC patients had family history of gastrointestinal tract and female reproductive system cancers. Assessment of DNA damage in endometrial tumor cells revealed that TM equaled to 32.2±1.2 and the % DNA in comet tail — 51.7± 1.3. It was found that level of DNA damage in tumor cells of EC patients with family history of cancer was almost two times higher (TM 50.4±2.9; % DNA in comet tail 75.72±1.96) compared to sporadic EC (TM 27.3±1.2; % DNA in comet tail 45.2±1.5). Immunohistochemical study didn't reveal significant difference of MSH2 expression in EC of patients with and without family history of cancer (LI 40.0±3.4% and 40.5±7.7%, respectively). Expression of MLH1 was higher in sporadic EC (LI 45.8±2.3%) compared to EC of patients with family history of cancer (LI 37.8±4.9%). ***Conclusions*:** Endometrial cancer cells of patients with family history of cancer are characterized by increased level of DNA damage that may be caused by low level of MLH1 protein expression indicating abnormalities of mismatch repair capacity.

THE INFLUENCE OF THE PELVIC PLEXUS PRESERVATION UNDER RADICAL HYSTERECTOMY IN PATIENTS WITH INFILTRATIVE CERVICAL CANCER ON THE CONTRACTILE FUNCTION OF THE URINARY BLADDER

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Aim*:** To justify the need to preserve pelvic plexus under radical hysterectomy (RH) in patients with infiltrative cervical carcinoma (CC). ***Patients and Methods: 30 patients with infiltrative CC (mean age 32.7±4.9 years) which underwent RH were enrolled into study: in 15 patients (group I) RH was performed with preservation of the pelvic plexus and in 15 patients (group II) RH was performed by standard method without preservation of the pelvic nerve plexus. The study protocol was approved by Ethical committee permission of National Cancer Institute (Kyiv, Ukraine). Cytomanometric assessment of urinary bladder's contractile function after RH with preserving of the pelvic plexus in infiltrative CC patients was performed by using urodynamic stand URO-PRO according to the standard procedure. ***Results***: According to cytomanometric data contractile function of urinary bladder was fully restored in 2–3 days after RH in 80% of group I patients and only in 20% of group II patients. In patients of group II the function of the lower urinary tract restored within 7 days after RH, in 20% of these patients persistent paresis of the urinary bladder detrusor was observed, which partially restored from 7 to 21 days. It indicates strong neurological disorders of contractile function of urinary bladder under surgical trauma of pelvic plexus during standard RH. ***Conclusion*:** Recovery of the contractile function of urinary bladder in 24 hours after RH justifies necessity of the pelvic plexus preservation during RH in patients with infiltrative CC.

ALTERATIONS IN FUNCTIONAL PROPERTIES OF BONE MARROW STEM CELLS IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH DIFFERENT RESPONSE TO TYROSINE KINASE INHIBITORS TREATMENT

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Introduction: It has been revealed the relative quiescence and resultant therapeutic resistance of primitive chronic myeloid leukemia (CML) progenitor cells providing a basis for identifying the hematopoietic developmental stage of chronic phase initiating events, such as altered stem cell differentiation and survival. This survival advantage is largely mediated by leukemic stem cells (LSCs) microenvironment. ***Aim***: The aim of this study was to determine the functional characteristics of hemopoietic and mesenchymal stem cells from CML patients with different response to the tyrosine kinase inhibitor (TKi) Imatinib (Novartis). ***Patients and Methods***: All 32 patients who were treated with TKi between 0 and 41 month have undergone cytogenetic analysis for the Philadelphia chromosome as well as *in vitro* assays: CFU-A in the semisolid agar and suspension cultures. According to the TKi therapy response patients were divided into groups: with optimal response (no Ph^+ cells), suboptimal response (Ph^+ > 0%) and the group of patients who received hydroxyurea before TKi treatment. ***Results***: The functional activity of bone marrow progenitor cells of patients with an optimal response to therapy was significantly lower (p <0.05) compared to patients with resistance to the therapy (the average CFU-GM numbers 29.3 and 79.3, respectively). Leukemic progenitor cells from CML patients were able to survive in stroma-free suspension culture without any additional growth factors supplementation during 21 day (median survival of 10 days). Such autonomous growth, not typical for normal cells, could be explained by autocrine mechanisms in primitive leukemia cells. Mesenchymal stem cells (MSCs) from CML patients with suboptimal response to TKi therapy showed 6 fold increases in proliferation rate compared to MSCs from bone marrow of patients with optimal response, suggesting that stem cell microenvironment is also involved in transformation process. ***Conclusions***: Our data indicate the presence of individual response to the TKi therapy in CML's patients that is probably associated with specific changes in the population of stem and progenitor cells due to alterations of their functional activity. Further researches of leukemic stem cells and their niche would allow better understanding of mechanisms of drug resistance and disease progression.

THE FREQUENCY OF HORMONE-RELATED TUMORS IN BREAST CANCER PATIENTS AFTER SURGICAL TREATMENT

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***Introduction*:** The frequency of hormone-related tumors is about 20%, among which 10.8% belong to breast cancer (BC). Women with BC have increased risk of developing a new primary cancer in the opposite breast and second tumors of different localization that can be linked to genetics and hormonal risk factors. However, there is no data about the frequency, character and preventive measures of origin of the second hormone-related tumors in BC patients. ***Aim*:** To study the frequency of malignant tumors in women who were treated for BC. ***Methods*:** We have analyzed clinical data of 51 BC patients with stage I–IV. The patients' age varied from 26 to 76 years (the mean age 54.3). Immunohistochemical staining of BC samples was performed using the primary monoclonal antibodies against progesterone (PR) (clon 1A6, "Dako", Denmark), estrogene (ER) (clon 1D5, "Dako", Denmark) receptors and HER2/neu (cerv2 oncoprotein, "Dako", Denmark).

28 (54.9%) BC patients received neoadjuvant treatment and all patients received complex treatment including surgery. The study was approved by the local Institutional Ethic Committee. ***Results*:** BC of stage I was diagnosed in 8 (15.7%), stage II — 33 (64.8%), stage III/IV — 10 (18.6%) patients. In BC patients second oncological pathology has been developed: in 19 (37.3%) patients — endometrial cancer, in 10 (19.6%) patients — ovarian cancer, in 7 (13.7%) — cervical cancer, in 2 (3.9%) — cancer of vulva, in 9 (17.6%) — colorectal cancer, in 1 (1.9%) — stomach cancer, in 1 (1.9%) — thyroid gland cancer and in 1 (1.9%) — kidney cancer. Family history of cancer with prevalence of BC was observed in 32 (62.7%) patients. Immunohistochemical phenotype ER^+HER2/neu^+ was detected in 45 (88.2%), PR^+ER^+HER2/neu^{++} — in 10 (19.6%) and PR^+ ER^+ $HER2/neu^{+++}$ — in 3 (5.9%) BC samples. ***Conclusions*:** Oncogynaecological tumors occupy the first place (74.5%) among hormone-related tumors in BC patients. The further study of risk factors for developing second malignant tumors in BC patients after treatment is needed.

EVALUATION OF INTRAPERITONEAL HYPERTHERMIC CHEMOPERFUSION EFFICIENCY FOR CHEMORESISTANT OVARIAN CANCER

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***Introduction*:** Intraoperative hyperthermic intraperitoneal chemoperfusion (HIPEC) is a new method of combined treatment of chemoresistant ovarian cancer (OC) with peritoneal carcinomatosis, which efficiency was not fully determined. ***Aim*:** To improve the efficiency of treatment of patients with chemoresistant recurrent ovarian cancer after cytoreductive operation with HIPEC. ***Patients and Methods*:** We conducted a full clinical examination and treatment of 181 patients with recurrent OC, including 72 patients with chemoresistant recurrent OC. Depending on the treatment of OC, patients were divided into three groups. The first group — 72 patients who received systemic chemotherapy: 1A group — 17 patients with chemoresistant recurrent OC, 1B group — 55 patients with chemosensitive recurrent OC. The second group — 61 patients who underwent cytoreductive operation and systemic chemotherapy: 2A group — 29 patients with chemoresistant recurrent OC, 2B group — 32 patients with chemosensitive recurrent OC. The third group — 48 patients who underwent cytoreductive operation, HIPEC and systemic chemotherapy: 3A group — 26 patients with chemoresistant recurrent OC, 3B group — 22 patients with chemosensitive recurrent OC. ***Results*:** Median of survival for patients, who received combined treatment including cytoreductive operation and HIPEC with systemic chemotherapy, was 27 months and median of survival for patients with chemoresistant recurrent OC, who received treatment including cytoreductive operation with systemic chemotherapy without HIPEC, was 18 months, median of survival for patients, who received treatment with systemic chemotherapy, was 10 months. Procedure of HIPEC didn't increase the frequency of surgical complications and postoperative lethality in OC patients. Surgical complications after cytoreductive operation and HIPEC was observed in 10 (21%) patients, after cytoreductive operation without HIPEC — in 14 (22.9%) patients. Postoperative lethality after the procedure of HIPEC was observed in 2 (4.2%) patients, and in 3 (4.9%) patients after the procedure of HIPEC. ***Conclusions*:** Application of HIPEC in combined treatment of patients with chemoresistant recurrent OC evidently increases survival of patients.

PROGNOSTIC VALUE OF SCCA TUMOR MARKER IN PATIENTS WITH CERVICAL CANCER

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Squamous cell carcinoma antigen (SCCA) level in blood serum is proven to be valuable for diagnosis and monitoring of patients with cervical carcinoma. Since cervical carcinoma as a rule originates from squamous epithelium, SCCA estimation in blood serum is significant for the study of the disease development***. The aim*** of the study was to monitor SCCA level in patients with cervical carcinoma with primary and metastatic disease for determination of recurrence probability value. ***Patients and Methods***: Changes in SCCA level in blood serum and clinical data were analyzed in 224 patients with cervical carcinoma of I-IV stage. Primary cancer was diagnosed in 171 patients; recurrence was confirmed in 53 patients. SCCA concentration in blood serum was determined by chemiluminescent immunoassay at micro particles (HIAM). Statistical analysis was performed with the use of Statistica 6.0 software. The study protocol was approved by Ethical Committee permission of National Cancer Institute (Kyiv, Ukraine). ***Results***: It was shown that SCCA values different reliably in groups of patients with different development of the disease ($p<0.05$). SCCA level in patients with Ca *in situ* and IA stage was equal to 0.6 ± 0.1 and 0.9 ± 0.1 ng/mL, respectively. At the higher stage of the disease, the higher SCCA level in blood serum was detected (I 5.9 ± 1.4 ng/mL in IIIB stage). It was confirmed that SCCA level in patients with primary cervical cancer with metastases in regional lymph nodes was higher compared to SCCA level in patients without metastases (5.6 ±1.4 ng/mL vs 2.3 ± 0.1 ng/mL, $p < 0.05$). SCCA level in blood serum increased up to 86.4% of patients with the recurrence and equaled 19.4 ± 3.7 ng/mL (from 0.5 to 70.0 ng/mL). SCCA value in blood serum increased up to 60.4% of patients with cervical carcinoma of IB-III stage. Test-sensitivity increased from 7.8% (I stage) to 77.8% (III stage). ***Conclusion***: SCCA level in blood serum can be considered as a sensitive method in prognostic study and determination of disease stage in patients with cervical carcinoma.

OVARIAN TISSUE CRYOCONSERVATION IN PATIENTS WITH GYNECOLOGICAL CANCER

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Introduction: During recent years the increase in incidence rate of gynecological malignancies in reproductive age patients, who had no time to implement reproductive function, has been registered. In majority of cases special treatment results in partial or full deprivation of fertility as a result of the high ovarian sensitivity to chemotherapy and radiation therapy. Cryopreservation and autotransplantation of ovarian tissue appear to become the method of fertility preservation. ***Aim***: To evaluate the ovarian reserve and develop policy of treatment in patients with gynecological malignancies with the purpose of cryoconservation of ovarian tissue for further realization of reproductive function. ***Methods***: 10 patients of reproductive age (< 35 years) have been treated in National Cancer Institute from 2010 to 2012 year with diagnosis IB–IIB stage cervical cancer. Panhysterectomy type 3 has been executed in 9 patients, radical abdominal trachelectomy — in 1 patient. Ovarian tissue obtained after laparotomy was placed in aseptic terms in container with special medium with temperature +20–25 °C and after that it was transported to Institute of Cell Therapy for following investigation, cryopreservation and storage. Currently all the patients are placed under dynamic surveillance. There is no evidence of the disease. The study protocol was approved by the Ethical Committee permission of national Cancer institute (Kyiv, Ukraine). ***Results***: As a result, cryoconservation of ovarian tissue can be fulfilled with the aim to realize the delayed reproductive function. ***Conclusions***: This method does not require the delay of the treatment, ovarian stimulation and can be accepted for the patients with gynecological malignancies.

MODERN CHEMOIMMUNOTHERAPY APPROACH BASED ON DENDRITIC CELLS AND LOW-DOSE CISPLATIN IN EXPERIMENT

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Introduction: Nowadays, combined therapy based on dendritic cells (DC) and low-dose chemotherapy is intensively investigated worldwide. There are no commonly accepted regimens of combined therapy and development of efficient chemoimmunotherapy schemes remains an actual problem. ***Aim***: To develop scheme of combined chemoimmunotherapy based on DC and low-dose cisplatin. ***Materials and Methods***: 120 CBA mice have been involved in the experiment. Sarcoma-37 was injected intramuscularly at lethal dose ($2x10^6$ cells per animal). Cisplatin was injected intraperitoneally 5 times in metronomic regimen according to the two schemes: 0.2 or 2 mg/kg on the 7th day after tumor transplantation with interval of 1 day and 3 days, respectively. DC vaccines were administered intravenously 3 times on the 4 day after the chemotherapy. All experiments were approved by Ethical Committee permission of National Cancer Institute (Kyiv, Ukraine). ***Results***: We have found that both chemoimmunotherapy schemes had significant antitumor and immunomodulation effect. The most pronounced effect was observed with DC-vaccine and cisplatin at concentration of 2 mg/kg in combination. Combination of DC vaccine and low-dose cisplatin had a synergistic effect on the reduction of primary tumor. Significantly decrease of primary tumor volume in animals was received in combined therapy compared to the control ($p = 0.001$) and DC-vaccine ($p = 0.007$) groups have been found. We showed that administration of combined therapy improves survival by 20% in comparison with the control group. A significant increase of cytotoxic activity of splenocytes in response to allogeneic stimulation to 42.33 ± 4.8% in the combined therapy group compared to 29.67 ± 4.1% in the control group had been observed, $p < 0.05$. We also found that phagocytic activity of splenocytes in this group increased up to 52.33 ± 4.67% compared to 37 ± 5.29% in the control group, $p < 0.05$. The coefficient of reactive oxygen species induction was statistically significant and increased to 1.61 ± 0.23 in comparison with 1.05 ± 0.13 in the control group. ***Conclusions***: Low-dose chemotherapeutics enhance the antitumor effect of DC-based immunotherapy. These investigations form the basis to a new multimodality treatment of cancer patients.

THE ROLE OF CD150 SURFACE RECEPTOR IN REGULATION OF TOPOLOGY AND EXPRESSION OF P50 AND P65 NF-KB IN NORMAL B CELLS

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Introduction: CD150 receptor is widely expressed in hematopoietic cell lineage and is involved in lymphocyte activation, differentiation and apoptosis via regulation of signal transduction pathways. Moreover, CD150 is expressed on malignant cells at Hodgkin's lymphoma and diffuse large B cell lymphoma that are characterized by enhanced level of NF-kB activation. CD150-mediated signal transduction pathways are not fully understood. Particularly, it is not clear whether CD150 is involved in regulation of NF-kB transcription factors that play a key role in immune system. ***Aim*** of our study was to find the potential role of CD150 in regulation of NF-kB transcription factors function in normal human B-cells. ***Methods***: Dense human tonsillar B cells were obtained in Percoll gradient, were stimulated *via* CD150 cell surface receptor, followed by immunofluorescence staining with 3D deconvolution and Western blot analysis. ***Results***: In dense B cells that comprise

IgD^+ naive cells p65 was predominantly localized in cytoplasm and p50 — in the nucleus, while several p65/p50 heterodimers were observed in nucleus, but not in cytoplasm. After 90 min of CD150 ligation p50 was also detected in cytoplasm. Stimulation of dense B cells via CD150 for 16 h resulted in complete translocation of p50 and p65 from nucleus to the cytoplasm. Further cell stimulation via CD150 up to 24 h resulted in returning only p50 back to the nucleus, although, a high level of p50 was still observed in cytoplasm where it was co-localized with p65. Western blot analysis has shown that after 24 hours of CD150 crosslinking on dense B cells, the expression level of p50 did not change, however, p65 level was slightly enhanced. The expression level of IkBα was significantly increased after 24 hour of CD150 ligation that points on the role of IkBα in p65 and p50 subunits re-exportation from the nucleus and sequestering in the cytoplasm. ***Conclusions*:** CD150 is involved in regulation of gene expression in B cells via intracellular relocalization of both p50 and p65 and minor upregulation of p65 expression level. Thus CD150 may also contribute to the regulation of NF-kB1 activation in CD150 expressing malignant B lymphocytes.

MOLECULAR-GENETIC MARKERS FOR DIAGNOSIS AND PROGNOSIS IN PEDIATRIC SOLID MALIGNANCIES

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Introduction: Molecular-genetic markers are very important tools for diagnosis and prognosis of solid tumors in children. ***Aim***: To study chromosomal translocations in patients with pediatric solid malignancies. ***Patients and Methods***: Study has been performed on 279 tumor specimens, which were collected at diagnosis, and 90 specimens of bone marrow (BM) puncture biopsy from 200 patients with neuroblastoma (NB), 21 — Ewing sarcoma family tumors (ESFT), 29 — alveolar rhabdomyosarcoma (ARMS), 29 — embryonal RMS (ERMS). The study protocol was approved by Ethical Committee permission of National Cancer Institute (Kyiv, Ukraine). Chromosomal translocations were detected by fluorescence in situ hybridization (FISH) or real-time PCR on fresh or paraffin-embedded tissue samples. ***Results*:** Chromosomal translocation t(2;13)(q35;q14) exhibiting chimeric PAX3-FKHR and PAX7-FKHR gene products are the hallmarks of the ARMS. PAX-FKHR fusions were detected in 58 RMS samples: 10 (17,3%) had PAX3-FKHR and had 24 (41,4%) PAX7-FKHR fusions, 24 (41,3%) cases were fusion-negative. PAX3-FKHR or PAX7-FKHR fusions were found in all ARMS. PA3/7-FKHR was detected in 5 of 29 patients with eRMS; it indicates the type of mixed type RMS and requires a change of tactics on the protocol for the treatment of ARMS. In addition, PAX3-FKHR fusion products were detected in biopsy tissue and BM from 3 patients. ESFT is associated with chromosomal translocation t(11;22)(q24;q12). Out of the 21 samples with translocations detected, 16 (76.3%) had EWS-FLI1 type 1, 1 (4.7%) had EWS-FLI1 type 2, 2 (9.5%) had EWS-ERG, 2 (9.5%) had EWS-ETV4 translocation. Fusion transcript EWS-FLI1 type 1 was found in BM of 6 patients. The most significant abnormality in NB is amplification of proto-oncogene *MYC*; its locus lies on the short arm of chromosome 1. In 44 (22%) cases of primary NBs amplification of *MYCN* gene is present. *MYCN* gene is a factor of unfavourable prognosis and influences the choice of treatment schedules. One sample with *MYCN* amp pos and 1p36 del pos, and 3 samples in *MYCN* amp neg and 1p36 del pos were detected. Loss of heterozygosity (LOH) at 1p36 was independently associated with a worse outcome in NB patients. NB expresses the tyrosine hydroxylase (TH) mRNA, the first enzyme in the catecholamine pathway. TH expression was found in BM of 74 (37%) patients with NB. Molecular-genetic testing is more accurate than standard morphological methods; it enables detection of the presence of tumor cells in BM at diagnosis and during treatment, allows optimization of the treatment strategy. ***Conclusion***: Molecular-genetic analysis helps to identify specific genes in children with solid malignancies in order to specify the diagnosis, prognosis; it also influences the selection of at-risk patients and treatment monitoring.

NUCLEIC ACID MARKERS IN BLOOD PLASMA OF PATIENTS WITH COLORECTAL CANCER

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Introduction: Colorectal cancer (CRC) is the third commonly diagnosed cancer that causes 400 000 deaths worldwide. The most sensitive modern diagnostic tool of CRC is colonoscopy, which is a painful procedure and can not be recommended for patients with altered topography of colon. ***Aim*:** Development of less invasive tools for CRC screening based on detection of nucleic acids in blood plasma. ***Methods*:** Concentration of cell-free circulating DNA in blood plasma was analyzed by qPCR. Methylation status of *LRRC3B*, *APC*, *FHIT* and *HIC1* genes in cell-free circulating DNA of samples was determined by using methyl-specific PCR (MSP) with subsequent melting curve analysis. ***Results*:** It was shown that mean-value of cell-free blood plasma DNA is statistically higher in CRC patients than in healthy donors ($p < 0.01$). Thus the mean-value of concentration of cell-free circulating DNA in blood plasma of CRC patients was 17.57 ± 3.43 ng/mL and 7.07 ± 0.84 ng/mL — in healthy donors. We have revealed hypermethylation of *APC*, *FHIT* and *LRRC3B* genes in 45% (10/22), 73% (16/22) and 68% (15/22) of tumor samples, respectively. Altogether hypermethylation of at least one of the selected genes was detected in 95% (21/22) of samples. Using MSP with subsequent melting curve analysis we have detected methylated fragments of *APC*, *FHIT*

and *LRRC3B* genes in blood plasma of 29% (6/21), 19% (4/21) and 14% (3/21) of CRC patients, respectively. We have identified hypermethylation at least in one of the selected genes in 48% (10/21) of blood plasma samples of CRC patients. Additionally we have registered high frequency of *HIC1* hypermethylation in blood plasma of CRC patients (8/10). We have suggested that two stage verification might be applied for CRC screening, which includes measurement of cell-free circulating DNA concentration with following detection of methylated fragments of *APC*, *FHIT* and *LRRC3B* genes in blood plasma of CRC patients. Overlapping of the above mentioned approaches allowed to increase sensitivity of studied panel up to 71% (15/21) in CRC detection. ***Conclusion*:** We have developed approach for screening of CRC which is based on determination of cell-free DNA and methylated fragment of well-known tumor related genes *APC*, *FHIT*, *LRRC3B* in blood plasma. The sensitivity of CRC detection might be increased by using of additional perspective genes like *HIC1*.

COMPLEX INFORMATION SYSTEM WITH WEB-INTERFACE CONCERNING BREAST CANCER

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Introduction: Breast cancer (BC) is the most frequent cancer among women with an estimated 17232 new cancer cases diagnosed in 2010 in Ukraine (19.9% of all cancers among women). As a result BC ranks as the first cause of death from cancer among women. BC is an actual social economical problem in Ukraine due to high rates of mortality and disability of women of active working age and requires significant funding. Economic situation in Ukraine gives priority to the prevention of BC, its most important component — increasing awareness of the public. Today a significant amount of information about BC has been accumulated and solution of the problem can be accomplished through the use of up-to-date tools of information, such as electronic information technologies that offer quick access to large amounts of information. The primary tool of obtaining this information is a global Internet network, but a significant amount of different information makes the problem of choosing the right materials more complicated. So there is a need for systematization and generalization of data to create a complex information system concerning BC. ***Aim***: To develop the Ukrainian language information system about prevention, diagnosis and treatment of BC aimed on informing and self-education of Ukrainian population as well as specialists in the medical field. ***Results***: Technical specification for creation of a web-portal (conceptual, logical and physical design), software development, portal and template design, is developed. The site is filled up with thematic material content according to the designed structure. Created web-resource contains information concerning BC, which uses brunched structure of the site and satisfies the information needs of the target audience — specialists in the field of medicine and ordinary citizens. Section of the site target audience, which defines the general population of Ukraine, is devoted to various aspects of BC in women such as questions disclosing BC risk factors, symptoms and diagnosis, primary prevention, clinical classification of tumors and treatment. Module that contains data appointed to provide information to medical specialists reflects the modern aspects of the pathogenesis of hormonal and molecular-genetic features of BC as a result of new post-genomic research. ***Conclusion***: Complex Ukrainian language information system concerning nature, early symptoms, prevention, diagnosis and treatment of BC as well as providing medical specialists with the latest structured scientific data about molecular biology of BC, has been created.

SIGNIFICANCE OF COX-2 AND VEGF EXPRESSION IN TUMOR FOR ESTIMATION OF TREATMENT RESULTS IN CERVICAL CANCER STAGE IIB

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Introduction: One of the important tasks of modern oncogynecology is the estimation of biological characteristics of tumors. Among the biological markers studied in relation to cervical cancer, cyclooxygenase-2 (COX-2) and vascular endothelium growth factor (VEGF) play an important role in the processes of the tumor invasion and neoanginogenesis. ***Aim***: To determine the COX-2 and VEGF expression in tumors and their connection with clinico-morphological indicators of the efficiency of treatment in patients with cervical cancer. ***Patients and Methods***: Operation materials of 48 patients with cervical cancer stage IIB after chemotherapy was used. The immunohistochemical analysis of COX-2 and VEGF expression was performed using primary monoclonal antibodies (MCAB) of the "Diagnostic BioSystems" company, USA (clone 4H12) and "DakoCytomation", Denmark (clone VG1) on the paraffin sections of the tumors. ***Results***: A positive immunohistochemical reaction of specific MCAB to COX-2 was determined in 44 (92%) tumors, and to VEGF — in 42 (88%). It was shown that tumors with partial regression (PR), stabilization of the tumor process degree II of pathomorphosis after the treatment and the disease recurrence are characterized by high levels of the COX-2 and VEGF expression. Tumors with PR and with degree III of pathomorphosis after the treatment and disease recurrence are characterized by high levels of the COX-2 and VEGF expression

but of low intensity. In patients with complete tumor regression after the treatment and without cervical cancer recurrence for more than 5 years no COX -2 and VEGF expressions were found. ***Conclusion***: The results indicate that determining of the COX-2 and VEGF expression in tumor cells after treatment of patients could be considered as additional indexes for estimation of treatment efficiency and the prognosis of the cervical cancer course.

A COMPARISON OF MULTIPLEX SHORT TANDEM REPEAT PCR AND REAL-TIME PCR INSERTION/DELETION POLYMORPHISMS METHODS FOR QUANTIFICATION OF CHIMERISM AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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***Introduction*:** Quantitative monitoring of chimerism after allogeneic hematopoietic stem cell transplantation (HSCT) by molecular methods has become an indispensable diagnostic tool in detection of engraftment/graft failure, predicting rejection and disease relapse. Despite the great utility of chimerism analysis there is no unique standard method for its quantification. ***Aim*:** The objective of the present investigation was to compare the sensitivity (detection limit) and the quantification accuracy of two perspective methods: multiplex short tandem repeat polymerase chain reaction (STR-PCR) and real-time PCR insertion/deletion polymorphisms (InDel-PCR) for the quantification of chimerism after stem cell transplantation. ***Methods*:** We performed a perspective study analyzing the chimerism status in 46 patients by STR-PCR using AmpFlSTR SGM Plus PCR Amplification Kit (ABI, UK) with capillary electrophoresis and by InDel-PCR with primers and probes to 20 allele-specific markers and reference gene albumin. ***Results*:** Recipient-donor discrimination was possible with STR-PCR in all patient-donor pairs (100%), whereas informative alleles for recipient were found in 94% pairs with InDel-PCR. The sensitivity (detection limit) of STR-PCR was 1–5% donor DNA with variation among STR markers. The sensitivity of InDel-PCR was more than 0.01% donor cells. The accuracy of quantification was higher for InDel-PCR than for InDel-PCR, when level of donor chimerism was <5% or >95% as it had higher sensitivity. The accuracy of quantification was higher for STR-PCR than for InDel-PCR, when level of chimerism was >10%, because there was 0.5 threshold cycle error for InDel-PCR and this corresponded to a variation in DNA of 50%. The results obtained by two methods showed a good agreement and correlated well (r=0.98; p<0.0001). ***Conclusions*:** These methods can be successfully used to determine chimerism after allogeneic HSCT. Considering the higher sensitivity and quantification accuracy of InDel-PCR it should be chosen if donor chimerism level is less than 5–10% or more 90–95% (i.e. after myeloablative conditioning) and in other cases STR-PCR should be chosen.

MATRIX METALLOPROTEINASES 2 AND 9 ACTIVITY AND TUMOR-ASSOCIATED MACROPHAGES IN HUMAN GASTRIC CANCER: CORRELATION WITH METASTASIS

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Introduction*:** Matrix metalloproteinases-2 and 9 (MMP-2 and -9) participate in the degradation of extracellular matrix (ECM) in tumor that results in the invasion, neoangiogenesis and metastasis. It is also known that tumor-associated macrophages (TAM) are important source of MMPs. ***Aim*:** To study the interrelationship between TAM number (CD68-positive cells) and concentration of MMP-2 and -9 active forms in tumor tissue as well as their link with clinicopathologic characteristics, metastasis into lymph node (LN) and survival of patients with gastric cancer (GC). ***Material and Methods: 112 resected specimens of primary gastric cancer were used. CD68 (tumor-associated macrophages) expression was evaluated using immunohistochemistry, and MMP-2 and MMP-9 activity, zymography. Statistical analysis was conducted using Spearmans's test, Kaplan — Meier survival analysis, log-rank test and Cox proportional hazards model. All patients were thoroughly informed about the study, which was approved by the local Ethics Committee. ***Results*:** It was shown that TAM number in GC categorized as N_{1-2} and M_1 was significantly higher than that in GC categorized as N_0 and M_0 ($p < 0.05$). Correlation of concentration of MMPs active forms in GC categorized as N_0 and N_{1-2} was not statistically significant. At the same time MMP-2 activity was higher by 1.5-fold in tumors categorized as N_1 in comparison with those in tumors categorized as N_0 ($p < 0.05$). Moreover, the inverse correlation between tumor concentration of MMP-2 active form and M category was shown: concentration of MMP-2 active form in tumor was higher by 2-fold in patients without distant metastasis ($p < 0.05$). The positive correlation between TAM number and tumor concentration of active form of both MMP-2 and MMP-9 was observed (*rho* = 0.4 and *rho* = 0.51, respectively; $p < 0.05$). Overall survival (OS) of patients with concentration of active form of MMP-2 (< 2 μg/g) was significantly better than that in patients with higher activity of MMP-2 in tumor (p=0.004). It was also shown that risk of unfavorable outcome increased by more than a factor of 3.5 (hazard ratio 3.8, (95% CI 0.78–8.2; $p < 0.05$) in patients with high concentration of active MMP-2 in tumor. OS of patients with concentration of active form of MMP-9 (< 4.5 μg/g) was also

significantly better than that in patients with higher activity of MMP-9 in tumor (p=0.015). Hazard ratio for patients with MMP-9 activity of tumor > 4.5 μg/g (HR 4.7, 95% CI 0.83–7.7; $p<0.05$) was significantly higher than that for patients with MMP-9 activity < 4.5 μg/g. The survival rate of patients with high number of TAM in tumor tissue (number of CD68-positive cells > 23%) was significantly lower than that of those with a low TAM number ($p = 0.003$). Risk of unfavorable outcome in patients with TAM number higher than the median value (> 23%) increased by more than a factor of 2 (HR=2.4; 95% CI 0.86–7.25; $p < 0.05$). ***Conclusion*:** It was suggested that TAM number and concentration of active form of MMP-2 and MMP-9 in gastric cancer may be used to the disease prognosis.

GENE EXPRESSION OF ACTIVATING TRANSCRIPTION FACTOR 5 AS A PREDICTOR OF SURVIVAL IN GERMINAL CELL DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: Prognostic models based on currently used methods of prediction of the outcome in diffuse large B-cell lymphoma (DLBCL) do not identify the molecular basis of clinical heterogeneity. ***Aim***: To identify new independent predictor of survival in germinal B-cell like subtype (GCB) of DLBCL. ***Patients and Methods***: Analysis was performed on RAW data from 203 DLBCL samples from a previously studied cohort of patients treated with CHOP (Series 11318) using Partek Genomics Suite software (methods of regression analysis and Cox-model for prognostic significance of studied parameters). Study was conducted according to bioethical standards of National Cancer Institute (Kyiv, Ukraine). Only GCB samples were choosing for the next analysis. According to the IPI score 71 GCB samples were divided into two groups: with "high" IPI (3–4 risk factors, 14 samples) and "low" IPI (0–2 risk factors, 50 samples). Detection of differentially expressed genes was performed (IPI high *vs* low IPI). Gene list was created (fold changes < -1.3, > 1.3, p-value with FDR < 0.05). 37 differentially expressed genes were detected. Highest fold-change was in gene activating transcription factor 5 (ATF5). The medium expression of this gene in GCB subtype of DLBCL was 5.42 and the expression was higher in high IPI samples *vs* low IPI (7.5 vs. 4.9 respectively). GCB samples were divided into two groups depending on APF5 expression. In Kaplan — Meier survival analysis the probability of survival was better for patients with low expression of ATF5 compared to high expression. ***Results***: Higher expression of ATF5 was observed in patients from high risk group (IPI score 3–4) (p-value with FDR 0.05) and the probability of survival was less for this group of patients treated with CHOP, especially after one year of follow up. ***Conclusions***: ATF5 could be a useful prognostic factor for DLBCL, but its significance need to be verified in further studies.

ABERRANT EXPRESSION OF *IKZF1* GENE IN BLAST CELLS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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***Introduction*:** Modern molecular-genetic methods of acute lymphoblastic leukemia (ALL) research revealed alterations of a number of novel genes involved in pathogenesis. The most frequent are transcription factors of lymphoid differentiation, such as *pax5*, *EBF-1*, *IKZF1* (*Ikaros*), *IKZF2* (*Helios*), *IKZF3* (*Aiolos*). *Ikaros*, coded by *IKZF1* gene, is a key factor of early lymphocyte development. Knock-out of *IKZF1* leads to high frequency of lymphoid malignances and autoimmune disorders. The Ikaros protein contains 4 DNA-binding zinc fingers forming DNA-binding domain near the N-terminus. The *IKZF1* gene contains 8 exons and is transcribed as at least 16 isoforms due to alternative splicing. Long isoforms (Ik1–3) have at least three zinc fingers, which are able to bind DNA and are considered to be functional. Short isoforms (Ik6,8,9,0) lack two or more zinc-finger domains and impair the function of Ikaros proteins in a dominant-negative manner (DN-Ik). ***Aim*:** Estimation of *Ikaros* isoforms expression by RQ-PCR in bone marrow (BM) blast cells of primary and relapsed childhood ALL patients in comparison with mononuclear cells of healthy donors' BM. ***Methods*:** We developed panel of primers and probes for independent quantitative assessment of all isoforms expression. Deletion of exon 3–6 was examined at the genomic level by PCR. ***Results*:** 132 BM samples from 104 ALL patients were analyzed, including 43 relapses and 27 paired cases. 9 BM from healthy donors composed a control group. Profile of *Ikaros* expression was similar in controls and most patients. Levels of *Ikx*, *Ik2*, *Ik4* and *Ik8* isoforms were significantly lower in patients. In 18 (27.3%), 3 (4.5%) and 1 (1.5%) out of 66 cases aberrant overexpression of *Ik6*, *Ik9* and *Ik0*, respectively, was found. In 13 of 18 (72%) cases *DN-Ik* overexpression was detected in relapsed and primary patients who finally relapsed. Deletion in *IKZF1* gene was found in 11 of 103 (10.7%) cases. In 24 cases both deletion and *Ik-DN* expression was absent, and in 4 both features were present. In 9 cases *Ik6* was overexpressed in the absence of deletion. Pair cases at diagnosis and relapse displayed the same Ikaros status. ***Conclusions*:** Expression of *DN-Ik* is a relatively frequent event for ALL. It may be detected by RQ-PCR analysis of cDNA apart from genomic analysis and possibly associated with poor prognosis. *Ik6* expression may be caused not only by the exon 3–6 deletion in *IKZF1* gene. *Ik6* overexpression is hardly the factor of relapse, but rather it is an initial event at the time of *de novo* diagnosis.

CREATION OF NEW COMBINATIONS OF ANTITUMOR REMEDIES (EXPERIMENTAL STUDY)

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Introduction: Development of a new combination of antitumor remedies replacing one or two components in well-known combinations by preparations with more selective antitumor activity is one of approaches to improve effectiveness of cancer pharmacotherapy. ***Aim*:** To create new combinations of antitumor drugs on a basis of initial combinations from standard treatment protocols for cancer replacing cyclophosphamide, fluorouracil and bonefos by chlofiden, fludinat and mebifon, which were developed in SI "IPT NAMSU". ***Materials and Methods*:** Toxicological and histological methods and method of tumor allografts were used. Melanoma B16 and Lewis lung carcinoma (3LL) were used as tumor models. Drugs were injected intraperitonially 24 hours after tumor implantation. Drug dosage for mice based on single dose for human was calculated using rapid extrapolation method. ***Results*:** The substitution of phosphorylated chlorethylamine chlofiden and fludinat for traditional combinations of cyclophosphamide and fluorouracil restored high antitumor activity of initial combinations but reduced their toxicity (chlofiden does not induce nephro- and hepatotoxicity, unlike cyclophosphamide; toxicity of fludinat is 3.3 fold lower than that of fluorouracyl). Thus, new remedies have more selective antitumor action. In melanoma B16, combination of doxorubicine, 3.0 mg/kg, cisplatine, 3.0 mg/kg and cyclophosphamide, 30.0 mg/kg, caused 80.5% of tumor growth inhibition, while replacement of cyclophosphamide by chlofiden, 30.0 mg/kg, in this combination enhanced tumor inhibition up to 85.9%. Doxorubicine, 3.0 mg/kg, cyclophosphamide, 30.0 mg/kg and fluorouracil, 37.0 mg/kg caused 90.2% of 3LL growth inhibition, meanwhile the same combination with fludinat, 37.0 mg/kg, instead of fluorouracil showed 85.9% of 3LL growth inhibition. Toxicity of mebifon and bonefos is similar, but mebifon showed higher antitumor activity than bonefos (72.0% and 37.4% of B16 growth inhibition respectively). Combination of dacarbazine, 60.1 mg/kg, cisplatin, 1.5 mg/kg, vinblastine, 0.2 mg/kg and bonefos, 14.0 mg/kg inhibited growth of melanoma B16 by 81.7%, the substitution of mebifon, 14.0 mg/kg, for bonefos resulted in 91.6% of B16 growth inhibition (10% higher than initial combination). ***Conclusions*:** On the basis of combinations from standard cancer treatment protocols of NCI of Ukraine, there were developed combinations of anticancer remedies with replacement of cyclophosphamide, fluorouracil and bonefos by chlofiden, fludinat and mebifon. Combinations of chlofiden and fludinat had the same antitumor activity and were less toxic than standard combinations. The replacement of bonefos by mebifon enhanced antitumor activity of initial combination.

THE ROLE OF PRO- AND ANTIOXIDANT PROCESSES IN DNA DAMAGE ORIGINATING FROM COMBINED EFFECTS OF ENVIRONMENTAL FACTORS AND TUMOR GROWTH

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Introduction: Influence of environmental factors plays a crucial role in the formation and development of cancer. The peculiarity of combined action of adverse factors on the human organism is the ability of each factor to modify the overall pathogenic effect. Nitrogen oxides (NOx) and low doses of ionizing radiation (LDIR) are genotoxic factors. Their interaction can lead to increased levels of DNA damage, changes in cell response to stressors, alterations in microenvironment of cells in tissues, the development of nitrosative and oxidative stress, resulting in increase of carcinogenic risk. At present, genotoxic effect of combined influence of environmental factors on organism upon tumor growth is studied insufficiently. In this regard, investigation of the antioxidant system status and the development of genetic instability upon the tumor growth and combined action of exogenous NOx and LDIR is important. ***Aim*:** To investigate changes in DNA damage and dynamics of the activity of superoxide dismutase (SOD) and catalase (CAT) in the process of tumor growth in the conditions of the individual and combined effect of exogenous NO and LDIR. ***Methods*:** The formation of DNA strand breaks was determined using horizontal gel electrophoresis of isolated rat's peripheral blood lymphocytes (PBL). The object of study were PBL of rats exposed to NO inhalation (150 mg/m^3 of air) for 30 days and LDIR (10-fold by 0.1 Gy) in the conditions of individual and combined treatment. Changes in CAT and SOD activity in rat peripheral blood were investigated in parallel. Guerin carcinoma (GC) was used as a tumor model. Study was approved by Ethical Committee permission of IEPOR NASU (Kyiv, Ukraine). ***Results*:** GC growth was accompanied by decrease of CAT activity in the blood of rats after 12 days of tumor growth. CAT activity increased both upon single LDIR and joint influence of NO+LDIR and reached its maximum upon combined action. The activity of SOD in blood increased in the conditions of the action of LDIR and NO+LDIR, and also upon the GC development. GC inoculation after treatment with NO+LDIR significantly increased the SOD activity on the 12 day followed by normalization on the 18 day of tumor growth. However, the increased activity of antioxidant enzymes was insufficient in the conditions of oxidative and nitrosative stress. Individual action of NO and LDIR resulted in DNA damage gradual increase during tumor growth. Maximal genotoxic effect was observed in the case of NO and LDIR combination (4-fold excess of the control value). ***Conclusions:*** Preliminary impact of NO and/or LDIR resulted in persistant

additive genotoxic effect accompanied by the increase of genetic damage on background of GC growth after the direct action of these factors. Tumor development and combined effect of environmental factors of different nature were accompanied by complex interactions between the main enzymes of antioxidant protection — CAT and SOD. An imbalance of free radical processes under oxidative and nitrosative stresses lead to the development of genetic instability, which in turn is a factor of increased carcinogenic risk and implemented as accelerated tumor growth

THE CLINICAL EFFICACY OF DRUGS OF "ERBISOL" CLASS IN THE TREATMENT OF COLORECTAL CANCER PATIENTS WITH LIVER METASTASES

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Introduction: In recent years the incidence of colorectal cancer (CRC) is significantly increased in all countries, including Ukraine. The problem is CRC metastasis in the liver at the time of diagnosis in 25–30% of patients. ***Aim***: To improve the efficiency of treatment of CRC patients with liver metastases using drugs of "ERBISOL" class (Kyiv, Ukraine). ***Patients and Methods***: The study included 111 CRC patients with liver metastases at the disease stage T1–4N0–2M1. All patients underwent surgical treatment. The patients were divided into 2 groups: group 1 (control, 54 patients) who underwent treatment by Roswell-Park scheme; group 2 (57 patients), which, on chemotherapy Roswell-Park scheme held 18 cycles of treatment with Erbisol Ultrapharm.. The study protocol was approved by Ethical committee. ***Results***: According to preliminary research, Erbisol Ultrapharm is a metabolic antimutagen, its use helped to reduce the number of multiaberrant and aneuploid cells, also Erbisol Ultrapharm exhibits properties of natural cytostatic agent that inhibits the abnormal cell growth. The analysis of the clinical course of the disease showed that in patients of group 2 general condition has been improved without local and general side events, and allergic reactions. In group 2, stabilization of the disease occurred in almost half of cases, compared with control patients where the progression of the disease was recorded in two thirds of patients. Additional biotherapy with Erbisol showed improvement in clinical response and duration of stable disease (13.5 months ± 1.0 in the main group, 7.4 ± 1.1 months in the control group, $p < 0.05$); and the median duration of effect (13.3 ± 1.0 months in the main group, 7.0 ± 1.1 months in the control group, $p < 0.05$). The trend for improved clinical effect was observed in patient survival rate. ***Conclusion***: The use of drugs of "Erbisol" class in the combined treatment of CRC patients is a new and promising approach improving treatment effectiveness. Combined therapy of CRC patients with liver metastases using 5-FU and drugs of "Erbisol" class prolongs duration of disease stabilization and increases patient survival ($p < 0.05$).

MOLECULAR GENETIC STUDIES IN PATIENTS WITH CANCER AND BENIGN TUMOR OF THE FEMALE REPRODUCTIVE SYSTEM FROM THE FAMILIES WITH HISTORY OF CANCER

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***Introduction*:** Screening of single nucleotide polymorphisms (SNP) allows determining the genetic predisposition to various multifactorial diseases and may help to predict individual sensitivity to pharmacological agents. ***Aim*:** To conduct clinical-genealogic, molecular-genetic studies in patients with ovarian cancer (OC) and / or breast cancer (BC), benign tumor of the female reproductive system from the families with cancer history. ***Patients and Methods*:** The clinical-genealogic study of 45 patients aged 25–72 years was carried out. 19 (42.2%) patients had cancer of the female reproductive organs (OC, BC, stage I-II), 26 (57.8%) — benign tumor of the uterus, ovary and breast. The genetic analysis was used to identify *BRCA1* (*185delAG* and *5382insC*) and *BRCA2* (*6174delT*) genes mutations and *ESR1* (*T-397C*, *A-351G*), *Cyp2D6*4* (*G1846A*) genes polymorphism in peripheral blood DNA of the patients. ***Results*:** Among 19 OC and BC patients with familial cancer *5382insC* mutation in BRCA1 gene was detected in 2 (10.5%) patients. Other mutations in *BRCA1/2* genes were not found. The *-397C* allele (*CC* and *CT* genotypes) of *ESR1* gene was detected with the same frequency in patients with cancer and benign tumor, respectively, 50.0 and 45.7% ($p > 0.05$). The frequency of *-351G* allele (*GG* and *AG* genotypes) of *ESR1* gene was significantly higher in cancer patients compared to patients with benign tumor, respectively, 71.4 and 25.7% ($p < 0.05$). However, the frequency of *1846A* allele (*GA* and *AA* genotypes) of *Cyp2D6*4* gene was significantly higher in women with benign tumor than in cancer patients, respectively, 17.1% and 0.05% ($p < 0.05$). Among cancer patients we found more individuals (13–68.4%) with combination of two polymorphisms in investigated genes in comparison with benign tumor patients (12–46.2%).

***Conclusion*:** Analysis of *BRCA1/2* genes mutations and polymorphic variants of *ESR1* gene among healthy patients with familial cancer can be useful tool for genetic risk estimation of cancer development.

ABERRANTLY METHYLATED CIRCULATING DNA AS A TOOL FOR DIAGNOSTICS AND EVALUATION OF THERAPEUTIC EFFECT IN LUNG CANCER

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***Introduction*:** Cancer cell-specific aberrantly methylated DNA was found in blood plasma from cancer patients, indicating that cell-free DNA circulating in blood (cirDNA) is a convenient source of tumor-associated DNA markers for the minimally invasive diagnostic tests. ***Aim*:** Estimation of the diagnostic and prognostic significance of methylation changes in RARB2, RASSF1A tumor suppressor genes detected in the cirDNA from lung cancer patients before and after combined treatment. ***Methods*:** Blood samples were taken from 33 healthy subjects (HS) and 60 patients with non-small cell lung cancer (NSCLC) before treatment, after neoadjuvant chemotherapy, surgery and during post-treatment follow-up. CirDNA was extracted from blood plasma and cell-surface-bound cirDNA (csb-cirDNA) fraction which was obtained by successive treatment of blood cells with PBS/EDTA and trypsin solutions. Concentration of methylated and unmethylated RASSF1A and RARB2 tumor suppressor genes circulating in blood was quantified by methylation-specific PCR and methylation index (IM) was calculated as IM = 100 x [copy number of methylated /(copy number of methylated + unmethylated gene)] for plasma cirDNA and csb-cirDNA. ***Results*:** Methylation index for RASSF1A, RARB2 genes was elevated 2–3 fold in plasma cirDNA and csb-cirDNA from lung cancer patients versus HS (Mann — Whitney U-test, $p<0.05$). Exceeding of at least one of RASSF1A or RARB2 IM values over the selected cut-offs discriminates NSCLC patients from healthy subjects with 90% sensitivity and 82% specificity when plasma cirDNA and csb-cirDNA were analyzed. The mean RARB2 IM in csb-cirDNA was higher for stage III patients compared with stage I–II patients ($p < 0.05$). In post-surgery period most patients had more pronounced decrease of RASSF1A, RARB2 IM in both plasma cirDNA and csb-cirDNA compared with IM values detected before treatment and after chemotherapy. The increased level of methylation markers was found in blood of patients with recurrent NSCLC after treatment. ***Conclusions*:** Detection of RARB2, RASSF1A methylation changes in csb-cirDNA and plasma cirDNA of NSCLC patients' blood appeared to be more informative in comparison with analysis of plasma cirDNA only. The obtained data provides evidence that RARB2, RASSF1A IM analysis in the cirDNA is valuable for lung cancer diagnostics, evaluation of cancer treatment efficiency and post-treatment monitoring.

PECULIARITIES OF MODIFYING EFFECTS OF CHEMICAL AGENTS ON THE FORMATION OF RADIATION-INDUCED DAMAGE IN GENETIC APPARATUS OF HUMAN CELLS

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Introduction*:** Accumulation of chromosomal aberrations in cells is potentially oncogenic; thus, ionizing radiation (IR), which induces such changes, is a carcinogenic factor. Combination of chemical agents, IR with different mechanisms of action on human genome still has to be elucidated. ***Aim*:** To determine and provide a comparative analysis of combined action of irradiation, co-mutagens, and mutagens using cell culture of human peripheral blood lymphocytes (LPB). ***Materials and Methods*:** Culture of LPB, metaphase chromosomes cytogenetic analysis after ionizing radiation in the range of 0.3–2.0 Gy; co-mutagen verapamil (V) treatment in different concentrations (1.5–4.0 μg/ml); mutagen — nitrozotiol (GSNO) treatment as a transport form of nitrogen oxide (0.25–1.0 mM/ml) (experiments *in vitro*, two cell generations after irradiation). ***Results*:** A phenomenon of co-mutagenesis, which stands for combined action of radiation exposure (0.3–2.0 Gy) and V treatment (1.5–4.0 μg/ml of blood), on the level of chromosomal aberrations in PBL has been established. It has been shown that V at concentration of 4.0 μg per ml of blood increased the damaging effect of low doses of radiation up to 1.5-fold. Frequency of chromosomal aberrations (radiation markers) became higher with the increase of concentration of co-mutagen V (1.5–4.0 μg/ml). Combined treatment of PBL with IR and mutagen GSNO (two cell generations after irradiation) resulted in synergistic effect which enhanced the additive effect due to chromosomal aberrations, which are known to be the genetic markers of chemical agents' action. Proliferative potential of PBL decreased more than 60% under IR and GSNO treatment. Level of aberrations of the chromatid type rose with increasing concentrations of GSNO (0.5 mM to 1.0 mM) by 3-fold. Combination of low doses of radiation and V inhibited cell proliferative potential, but increasing of exposure dose did not alter this effect. ***Conclusions: Increase in concentration of co-mutagens in combination with ionized radiation enhances chromosomal aberrations in PBL. Moreover, proliferative potential of PBL decreases under IR and GSNO treatment. Low doses

of radiation and V inhibit cell proliferative potential; nevertheless, it remained unchanged when the doses of radiation were increased.

COMPARATIVE ANALYSIS OF POLYMERASE CHAIN REACTION AND CONVENTIONAL CYTOLOGICAL EXAMINATION FOR PERITONEAL RECURRENCE RISK ASSESSMENT IN PATIENTS UNDERGOING SURGERY FOR COLORECTAL CANCER

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Introduction*:** Peritoneal recurrence is a major cause of surgical treatment failure in patients with colorectal cancer (CRC). Diffuse metachronous peritoneal carcinomatosis is associated with poor prognosis and median survival of 5–6 months. There is a chance for prolongation of survival in patients with limited peritoneal carcinomatosis who are eligible for aggressive combined treatment. Evaluation of peritoneal relapse risk at a time of surgery for primary colorectal tumor is essential for early diagnosis and treatment of metastasis. The presence of free cancerous cells (FCC) within the peritoneal cavity is believed to be a powerful predictor of peritoneal carcinomatosis. A method of real-time polymerase chain reaction (PCR) was proposed recently to detect FCC in peritoneal washes. ***Aim*:** To evaluate the feasibility of real-time PCR for detection of FCC within the peritoneal cavity of patients with CRC who underwent curative surgery and to compare the results with those of conventional cytology. Patients and Methods*:** Twenty-two patients were enrolled in this study with ethical considerations. Patients were divided into three groups. The main group consisted of 16 patients radically operated for CRC. Positive control group included three patients with macroscopic peritoneal carcinomatosis diagnosed at a time of surgery for CRC. Negative control group consisted of three patients with benign colorectal tumors. A laparotomy and subsequent peritoneal lavage were performed in all 22 patients. Peritoneal wash samples were analyzed microscopically by Giemsa staining and by PCR analysis with primers for carcinoembryonic antigen (CEA) gene as a molecular target of cancerous cells. Real-time thermal cycler "Bio-Rad iCycler iQ5" (USA), PCR reagent kits "Fermentas" (Lithuania) and oligonucleotide primers "Metabion" (Germany) were used. Cases with CEA signal were defined as PCR-positive. ***Results*:** PCR reaction was positive in three (19%) of 16 patients in the main group, while cytology was negative in all samples. No positive results of PCR and cytology in negative control group was detected, i.e. both methods yielded no false-positive results. Cytological examination was positive only in one (33%) of three patients of positive control group showing lack of sensitivity, whereas all (100%) of them were PCR-positive. ***Conclusions*:** CEA identification by PCR appears to be reliable method of FCC detection. It can be useful for identifying patients at high risk of peritoneal relapses of colon cancer following primary tumor resection. PCR is a more sensitive method for FCC detection in peritoneal washes compared to conventional cytology.

THE PREDICTIVE MODEL OF CHEMOTHERAPY RESPONSE IN MULTIPLE MYELOMA PATIENTS

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***Introduction*:** Multiple myeloma (MM) is plasma cell neoplasm with different sensitivity to drug treatment. The drug resistance worsens the individual prognosis for MM patients. ***Aim*:** To determine the predictive markers of refractory forms in MM patients. ***Methods*:** 130 patients with first time diagnosed MM from different regions of Ukraine were examined during the period of 2007–2011. 26 (20%) of 130 patients underwent treatment according to the scheme MP, 55 (42.31%) — according to the scheme M2, 49 (37.69%) — according to the scheme VAD. Patients were divided into two groups: the 1st group — 52 patients, who had no response to treatment, the 2nd group — 78 patients with response to treatment. Patients of both groups were scrutinized closely with a help of 68 clinical-laboratory indexes and molecular-genetic tests, and *GSTT1, GSTM1* genes deletion polymorphism, *GSTP1* gene A*313G* polymorphism, *MDR1* gene *C3435T* polymorphism were characterized. Binary logistic regression with consequent including of predictors (program SPSS 16.0) was implemented to create a predictive model. ***Results*:** The patients of the 1st group had significantly lower frequency of *GSTM1* gene deletion polymorphism as compared to patients of the 2nd group (32.20% and 62.82%, respectively; $\chi^2 = 11.33$, OR = 0.29 (0.14–0.60), $p < 0.001$). The *GSTM1* gene allele polymorphism correlated with elevated risk of development of refractory forms in MM patients (OR = 3.48 (1.66–7.29)). There was no difference in the frequency of others genes polymorphism. We have found that the higher prognostic value had *GSTM1* gene polymorphism (69.90%), while calcium and α_2-globulin level in blood serum before treatment had lower prognostic value (62.30%). The better prognostic value was obtained using statistical model which includes *GSTM1* gene polymorphism, α_2-globulin and calcium levels in blood serum (73.60%). ***Conclusion*:** Implementation of predictive model which considers *GSTM1* polymorphism, α_2-globulin

and calcium levels in blood serum from the beginning of treatment increases the accuracy of prognosis of response to treatment for each individual patient.

EXPRESSION OF CD44 AND E-CADHERIN IN THE SEROUS OVARIAN CANCER AND ITS CLINICAL SIGNIFICANCE

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Introduction*:** Molecules of cell adhesion (CD44 and E-cagherin) are significant for tumor growth and are connected to changes of cell proliferation and migration. Recently, it was hypothesized that cancer stem cells (CSCs) express CD44 which regulates cancer cell survival, metastatic potential, resistance to conventional radio-chemotherapy, disease relapse and prognosis. ***Aim: To evaluate the interrelations of CD44 and E-cadherin expression with clinical and cytomorphological features of serous ovarian cancer and its clinical significance. ***Patients and Methods*:** Immunohistochemical and morphological of CD44 and E-cadherin expression analysis was applied to pathological material obtained after surgery of 72 patients with ovarian cancer of I-III stage (FIGO classification) before neoadjuvant chemotherapy. Monoclonal antibodies against CD44 (clone DF 1485) and E-cagherin (clone NCH-38) were used. Results of immunohistochemical analysis were evaluated by semi-quantitative assessment, the percentage of CD44 (+) and presence/absence of E-cadherin (+) cells were estimated. Survival of patients was analyzed by Kaplan — Mayer method. ***Results*:** Variability in the number, distribution, and location of CD44 (+) and E-cadherin (+) tumor cells was observed in ovarian cancer patients. Expression of CD44 was defined in solid structures, and also in clusters of tumor cells (budding) located in stroma, in cells of tumor papilles, in adenomatous structures, and in gland lumen which in total provides the evidence of cellular discohesion and active invasion. The expression of CD44 (+) (over 10%) was observed in 58.3% patients. The negative correlation between CD44 (+) and E-cadherin (-) expression was determined ($r = -0.38$ $p < 0.05$). Survival analysis of ovarian cancer patients showed that 5-year survival of patients with tumor phenotype CD44 (+), E-cadherin (-) was lower compared to survival of patients with tumor phenotype CD44 (-), E-cadherin (+). ***Conclusion***: In serous ovarian cancer variability of CD44 and E-cadherin expression was determined. CD44 expression in zones of cellular discohesion and active invasion of tumor cells was shown. We demonstrated the correlation between the percentage of CD44 (+) cells and survival of ovarian cancer patients. The results from this study suggest that quantification of CD44 (+) and E-cadherin (-) expression and their localization in ovarian cancer can be helpful as predictors of tumor progression.

THE ROLE OF SOME CYTOKINES AS A LOCAL MICROENVIRONMENT IN BREAST CANCER PROGRESSION

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Introduction*:** Breast cancer (BC) is a leading cause of cancer death among women. In most cases, death results from the dissemination of cancer cells and the development of distant metastases. Approximately 65–75% of patients with advanced breast cancer will develop bone metastases, which result in bone destruction. The presence of disseminated tumor cells (DTC) in bone marrow (BM) of BC patients has been proven to have clinical relevance. Despite the clinical significance of DTCs in the BM, the biological relevance remains controversial. Microenvironment plays an important role in tumor progression both within primary tumor and distant metastases in secondary sites. BM is a depot of various growth factors. It is not excluded that increased level of some cytokines may serve as the additional factor of prediction progression of tumor growth, because certain cytokines create the microenvironmental conditions for the establishment of distant metastases from a primary BC. Therefore, a search of new comprehensive prognostic algorithm, based on simultaneous detection of DTC in BM and cytokine profile in BC patients, should promote the development of personalized therapeutic approaches. ***Aim: The aim of this study was to detect DTC in BM and determine an impact of cytokine status of peripheral blood (PB) and BM of primary BC patients to cancer prognosis. ***Methods:*** Immunocytochemistry, bioassay tests, ELISA, cultivation tests and statistical methods. ***Results*:** In our study DTC in samples of BM was revealed in 50% breast cancer patients of progression group. The level of TNF, CSF-1, IL-6, TGF-β1, VEGF and IFN was analysed. It was found that most significant additional markers of tumor progression with detection of DTC in BM are the levels of TNF in BM and PB, of CSF-1 and IL-6 in PB and of endogenous IFN in BM and PB of BC patients. In patients of disease progression group in comparison with patients in remission group the levels of TNF in BM were increased by 44% ($p < 0.01$), of CSF-1 and IL-6 in PB — by more than on 40–60% ($p < 0.05$). ***Conclusion:*** Comprehensive detection of DTC in BM and identification of the levels of TNF, IFN, CSF-1, VEGF, IL-6 and TGF- β1 in PB and BM of BC patients could be one of the ways to predict metastatic process and correct antitumor individualized therapy.

CONNECTIONS OF SERUM AND TUMOR FERRITIN LEVELS WITH SENSITIVITY TO NEOADJUVANT CHEMOTHERAPY IN BREAST CANCER PATIENTS

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Introduction: Design of new strategies for breast cancer (BC) treatment is still one of the actual problems in oncology. The main phase of complex therapy of locally disseminated BC stage II–III is neoadjuvant chemotherapy. Nowadays different prognostic values are being used in clinical practice, but objective criteria of BC sensitivity to neoadjuvant chemotherapy, which could give data about answer to chemotherapy on organism and tumor levels, are still unclear. ***Aim*:** To study connections between ferritin levels in serum and tumor tissue with sensitivity to neoadjuvant chemotherapy in BC patients. ***Patients and Methods*:** We studied 168 women with BC stage IIA–IIIB. Age of patients ranged from 28 to 69 years. Serum ferritin was measured by solid-phase immunoenzyme assay after histological verification of diagnosis before the first cycle of neoadjuvant chemotherapy. Patients received FAC, AC chemotherapy (2–6 cycles) every 21 days. Neoadjuvant chemotherapy efficacy was evaluated every 2 cycles by mammography according to RECIST criteria. Ferritin expression studies were performed on operation material of BC patients by standard immunohistochemical assay with specific monoclonal antibodies. Statistical analysis was performed using STATISTICA 6.0 software. ***Results*:** Patients were divided into 2 groups according to the degree of the clinical effect after neoadjuvant chemotherapy. The first group consisted of 79 BC patients with positive reaction on chemotherapy: complete tumor regression was observed in 8, partial — in 71 patients from this group. The second group consisted of 89 women with tumors resistant to chemotherapy. Among them 61 patients showed stabilization of tumor growth and 28 — tumor progression after neoadjuvant chemotherapy. The highest levels of serum and tumor ferritin were registered in group of patients with tumors resistant to neoadjuvant chemotherapy. In particular at the beginning of the therapy before the first cycle of neoadjuvant treatment average ferritin concentration in serum of these patients was 226.2 ± 5.7 ng/ml (213.4 ± 4.8 ng/ml — in patients with stabilization and 254.2 ±6.2 ng/ml — in patients with progression of tumor growth). At the end of treatment patients with resistant tumors showed significant ($p < 0.05$) elevation of serum ferritin levels (to 294.7 ± 7.3 ng/ml in patients with stabilization and to 351.9 ± 5.5 ng/ml — in patients with tumor progression). Most part (87%) of tumors from the resistant group of patients showed elevated levels of ferritin expression in operation material. The highest levels of expression of this protein (more then 92% of positive cells) was observed in tumors with progression after neoadjuvant treatment. Patients with tumors sensitive to neoadjuvant therapy had slightly elevated average concentration of serum ferritin before the first cycle of neoadjuvant chemotherapy (93.1 ± 2.8 ng/ml: 56.8 ± 4.0 ng/ml in patients with full and 97.2 ± 3.1 ng/ml in patients with partial tumor regression). After treatment patients with sensitive tumors showed significantly ($p < 0.05$) lower serum ferritin levels (4.3 and 3.2 times for patients with full and partial tumor regression, respectively). Average and low levels of ferritin expression were observed only in 18% of tumors with partial regression, although tumors with full regression showed absence of this protein expression. Correlation analysis showed reverse correlation between levels of serum and tumor ferritin and sensitivity of BC patients to FAC and AC neoadjuvant chemotherapy ($r = -0.39$; $p < 0.05$). ***Conclusions*:** So, we found relations between levels of ferritinin in serum and tumor tissue with sensitivity to neoadjuvant chemotherapy of BC patients. High serum and tumor ferritin levels point on resistance of BC patients to neoadjuvant chemotherapy. The acquired data showed that ferritin level should be used as objective criteria for determination of BC sensitivity to neoadjuvant chemotherapy on both organism and tumor levels.

GOLD NANOPRISMS AS POTENTIAL DELIVERY AGENTS FOR PHOTODYNAMIC THERAPY PHOTOSENSITIZERS

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Introduction: It is well known, that properties of nanomaterial strongly depend on particle shape and size. Gold nanospheres have already become an object for targeted drug delivery research. However, recently more attention is drawn to nanoparticles with different geometrical parameters, particularly nanoprisms. Such nanocarriers can be successfully applied as delivery agents for photodynamic therapy (PDT). One of the most questionable aspects of this approach is nanoparticle interaction with different cell types. ***Aim*:** To assess the gold nanoprism toxicity and ability to accumulate in malignant and normal cells *in vitro* and to evaluate the effectiveness of PDT *in vivo* using Fotolon-nanoprism composite as a photosensitizer. ***Methods*:** Human glioblastoma cell line A172 (kindly provided by Dr. S.P. Sydorenko), human Burkitt's lymphoma cell line Namalwa, peritoneal mouse macrophages, normal human monocytes and lymphocytes were used for studies *in vitro*. Mice C57Bl/6 with transplanted Lewis lung carcinoma were used in PDT experiments. Nanoprisms with average diameter of 88.3 nm (determined by DLS analyzer «Analysette 12 DynaSizer", «Fritsch", Germany) were stabilized with chlorella polysaccharides. Chlorine e6-

based drug Fotolon (RUE Belmedpreparaty, Belarus) was used as a photosensitizer. Accumulation of nanoprisms in target cells was studied using dark-field microscopy. Cell viability in toxicity tests was assessed by trypan blue dye exclusion method. *In vivo* effectiveness of gold nanoprisms was studied by assessment of tumor growth inhibition. ***Results*:** Cultured cells were incubated with nanoprisms at concentrations of 10 or 100 μg/ml for 1 hour in accumulation studies and 1 or 24 hours in toxicity studies. Dark-field microscopy studies of Namalwa cells showed that the nanoparticles accumulate mostly on the cell surface. Apparently, they do not penetrate cell membrane. Normal human lymphocytes do not accumulate nanoprisms even at concentration 100 μg/ml. On the contrary, treatment of peritoneal mouse macrophages and human blood monocytes, as well as human glioblastoma cells A172 with gold nanoprisms leads to nanoprism accumulation in cytoplasm. Gold nanoprisms were not toxic to normal human lymphocytes in both concentrations tested (10 and 100 μg/ml). As to Namalwa cell line, viability of the cells, treated with nanoprisms, decreased slightly (by 6–8%) only after their incubation with maximal nanoparticle concentration. Evaluation of Fotolon-nanoprism composite activity in PDT tests *in vivo* showed inhibition of Lewis lung carcinoma growth to 70% versus 32% in the group of animals treated with free Fotolon. ***Conclusions*:** 1. Gold nanoprisms are relatively nontoxic to cells. 2. Nanoprisms aggregated on the surface of transformed lymphocytes and did not aggregate on the surface of normal cells. 3. The nanoparticles were absorbed mostly by phagocytic cells. 4. Antitumor photodynamic activity of Fotolon-nanoprism composite exceeded such of the free Fotolon more than twice.

CHARACTERISTIC OF HLA-GENOTYPE IN CHILDREN WITH ALL AND AML

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***Introduction*:** The immunogenetic study has disclosed the correlation between HLA alleles and diseases. Investigated alleles could be used as genetic markers in the diagnosis of malignant diseases. However, the results of an immunogenetic research obtained in any population can not be automatically transferred to other ethnic groups. The HLA-distribution specificity analysis in acute leukemia patients is of primary importance. This information can help practitioners to reveal individuals with a high probability of malignant diseases. ***Aim*:** The HLA-genotype analysis in children suffering from acute leukemia and in children with non-malignant diseases. Marker specificy detection of high and low risk of leukemia progression. ***Methods*:** 25 children with acute lymphoblastic leukemia (ALL), 16 children with acute myeloblastic leukemia (AML) and 16 children with non-malignant diseases were included in the study. HLA-genotyping of leucocytes was performed by PCR-SSO (sequence specific oligonucleotide) and PCR-SSP (sequence specific primers) with reagent kits (Invitrogen, USA). The significance of discrepancy between groups was assessed by using chi-squared test. ***Results*:** The distribution pattern of HLA-alleles in two subgroups of patients with acute leukemia has been analyzed. The most frequent alleles in ALL patients were A*02 (15 patients, 34%), A*24 (9 patients, 18%), B*35 (9 patients, 18%), B*07 (6 patients, 12%), C*07 (9 patients, 20%), C*04 (6 patients, 12%), DRB1*01 (9 patients, 18%), DRB1*04 (8 patients, 16%), DQB1*05 (11 patients, 50%), DQB1*03 (15 patients, 36%). The most frequent alleles in AML patients were A*02 (8 patients, 34%), A*24 (4 patients, 16%), B*35 (5 patients, 16%), B*51 (3 patients, 13%), C*07 (6 patients, 22%), C*06 (6 patients, 22%), DRB1*07 (6 patients, 22%), DRB1*11 (5 patients, 19%), DQB1*03 (7 patients, 30%), DQB1*02 (7 patients, 27%). In patients with non-malignant diseases HLA-alleles distribution was the following: A*02 (8 patients, 31%), A*03 (5 patients, 19%), B*44 (8 patients, 28%), B*08 (4 patients, 13%), C*07 (10 patients, 31%), C*04 (7 patients, 25%), DRB1*03 (5 patients, 19%), DRB1*15 (6 patients, 19%), DQB1*02 (9 patients, 31%), DQB1*06 (9 patients, 28%). We displayed that differences between groups of ALL patients and patients with non-malignant diseases on HLA-DRB1*03 are statistically significant ($p = 0.04$). ***Conclusion*:** Analysis of HLA-DRB*03 locus has revealed the significant difference in ALL patients when compared with the group of children with non-malignant diseases. Obtained results are of certain interest and require further investigation.

PHOTODYNAMIC THERAPY OF TUMORS WITH CONTROLLED DELIVERY OF PHOTOSENSITIZER HEMATOPORPHYRIN

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***Introduction*:** Photodynamic therapy (PDT) is a tumor treatment modality, which is grounded on application of a photosensitizing drug (PS) and light of appropriate wavelength which activates PS leading to tumor cell destruction. To enhance PDT antitumor effect, a number of approaches are now under investigation, including elaboration of targeting techniques, such as use of metal or polymeric nanoparticles, and development of novel regimens of light and PS delivery. One of the promising approaches, which we exploited in this study, may be called metronomic or chronic PDT. It is based on continuous low level photoirradiation of a tumor and delivery of PS over extended period of time — from several hours to a few days. ***Aim*:** To establish a chronic regimen of PDT, providing controlled release and prolonged accumulation

of PS in tumors by employment of natural polymeric materials with simultaneous low-intensity light illumination, and to evaluate PDT effects on murine Lewis lung carcinoma (LLC) tumor growth and metastatic dissemination. ***Methods*:** C57Bl6 female mice (6 per qroup) with intracutaneous transplanted LLC were used. A special photodiode panel was developed for continuous low-intensity blue-light irradiation of tumor-bearing animals. Photosensitizer hematoporphyrin was loaded into 4% hyaluronic acid hydrogel which was inoculated intratumorally twice: on the 1st and 3rd days of light treatment. On the whole, mice were illuminated for 6 days. Before the start of experiments, kinetics of hematoporphyrin release from hyaluronic acid gel, introduced to mice, was determined by recording its fluorescence in tumors for 5 days using USB 4000 fiber optic spectrometer ("Ocean Optics", USA). All procedures involving animals were performed in accordance with the Institutional Animal Care and Use Committee. ***Results*:** Hyaluronic acid hydrogel during three days provided controlled release and higher accumulation (up to 7 times) of hematoporphyrin compared to free hematoporphyrin. This enabled us to use the gel-carrier for controlled release of hematoporphyrin in PDT experiments. For accomplishment of this chronic PDT, six-day illumination of LLC-bearing mice with hematoporhyrin-containing hydrogel was carried out. Remarkable inhibition up to 80% of tumor growth was observed in a group of treated animals compared to untreated ones. Moreover, the number of lung metastases in treated animals was 24-fold lower than in control group, indicating clear antimetastatic effect of chronic PDT. ***Conclusion:*** A feasibility of controlled continuous release of hematoporphyrin photosensitizer from hyaluronic acid hydrogel was demonstrated. Six-day blue-light low-level illumination of tumors concomitant to controlled hematoporphyrin release resulted in remarkable antitumor and antimetastatic effect, indicating significance of this approach to PDT of tumors.

THE PROGNOSTIC VALUE OF LEUKEMIC STEM CELLS PERCENTAGE IN B-LINEAGE CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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One generally accepted theory is that leukemia is maintained by leukemia stem cells (LSCs), which play the central role in drug resistance and metastasis. It would be of great interest to study the quantity of LSCs during follow-up and their prognostic impact on different types of leukemia. Moreover, developing LSCs-targeted therapy may provide, in combination with standard therapy, a way to eradicate the leukemic process. ***The aim*** of our study was to evaluate LSCs percentage and their prognostic meaning for childhood B-precursor acute lymphoblastic leukemia. ***Patients and Methods***: We investigated cord blood samples from 87 children with B-ALL. The percentage of LSCs with phenotype CD34+CD38-CD19+ (**Wilson K., 2010**) in the blast population was determined at diagnosis. Minimal residual disease (MRD) was assessed by detection of aberrant immunophenotype using flow cytometry (FC 500, "Beckman-Coulter"). Comparisons of patient's characteristics were performed using the Mann — Whitney U-test. Data were presented as median and percentiles (25–75) and accepted as reliable at $p < 0.05$. ***Results***: We observed that initial increased level of CD34+CD38-CD19+ cells correlates with a poor response to induction chemotherapy. The median content of CD34+CD38-CD19+ was 0.13% (0.01–3.7) for MRD-negative (<0.01% of blasts) and 2.1% (0.2–5.7) for MRD-positive (> 0.01% of blasts) patients at 15th day of induction chemotherapy ($p < 0.05$). We compared the proportion of the LSCs within the blast population in both diagnostic groups at day 36 and found that MRD-positive patients have higher content of LSC at diagnosis than MRD-negative: 3.3% (1.4–5.7) and 0.6% (0.06–3.7) respectively ($p < 0.05$). ***Conclusion***: Thus, increased LSCs percentage correlates significantly with a lack of complete response in patients with childhood B-ALL. CD34+CD38-CD19+ compartment at diagnosis was higher for MRD-positive patients at 15 and 36 days of chemotherapy. The prognostic impact of the MRD stem cell quantity might improve the already strong impact of total MRD quantity on outcome. Identification of new therapeutic targets based on LSCs biological features is the aim of the future studies.

ROLE OF ENDOMETRIAL HYPERPLASIA IN PROGNOSIS OF SURVIVAL OF PATIENTS WITH OVARIAN CANCER

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***Introduction*:** In ovarian cancer (OC) patients endometrial pathology is diagnosed, that is explained by the common risk factors and pathogenesis of the diseases. ***The aim*:** To study the relationship between general and recurrence-free survival rate in OC patients and presence/absence of associated endometrial hyperplasia (EH). ***Patients and Methods*:** Retrospective analysis of history of diseases in 303 OC patients with stages I-IV who underwent standard treatment according to the radical program in National Cancer Institute within 2001–2008 years. The study protocol was approved by Ethical Committee permission of National Cancer Institute (Kyiv, Ukraine). ***Results*:** Group of OC patients with EH composed 59.1% (among those — reproductive age patients made up 46.9%, menopausal period patients — 53.1%), without EH — 40.9% (among those — reproductive age patients made up 30.5%, menopausal period patients — 69.5%). The morphology of ovarian tumor of patients with associated EH were allocated as follows: papillary adenocarcinoma — 69.8%,

stromal tumors — 11.2%, mucinous cancer — 6.1%, germinogenic tumors — 3.3%, non-differentiated adenocarcinoma — 1.7%, endometrioid cancer — 1.1%. Correlation relationship of general and recurrence-free survival rates was defined in the investigated patients due to presence or absence of associated EH. It was shown that the median of recurrence-free survival of OC patients with EH made up 60 months, while in absence of EH — 24 months ($p < 0.05$). The five-year survival rate in OC patients with EH composed 53%, in absence of EH — 32% ($p < 0.05$). It is worth to note that early recurrences of the disease (*prolongatio morbi*) was observed in 24% of OC patients in presence of EH and in 76% patients with its absence ($p < 0.05$). ***Conclusions***: Thus, the presence of benign endometrial changes is a favorable prognostic sign that is associated with OC prognosis. The obtained data have practical significance as predictive factors for estimation of OC prognosis.

THE PHOSPHO-C-JUN CONTENT IN GASTRIC MUCOSA CELLS UNDER EXPERIMENTAL GASTRIC CARCINOGENESIS

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Introduction: The protein c-Jun belongs to the subgroup of DNA binding transcription factors that form AP-1 dimers. c-Jun is unique in its ability to positively regulate cell proliferation through the repression of tumor suppressor gene expression and function, and induction of cyclin D1 transcription. c-Jun is phosphorylated on Ser63 and Ser73 following activation of signalling cascades, which made c-Jun maximally effective in stimulating transcription. However, the exact role of c-Jun in gastric oncogenesis is unknown. ***Aim***: To determine the phosphorylated form of c-Jun content in the lysate of rat gastric mucosa cells in chemically induced gastric cancer development. ***Methods***: The content of phospho-c-Jun was measured by Sandwich ELISA method using the assay kit PathScan Phospho-c-Jun (Ser63) (Cell Signaling Technology, USA) and represented in conventional unit of absorbancy on mg of the protein. The protein concentration was registered by Bradford's method. Gastric carcinogenesis was initiated by 10-week replacement of drinking water by 0.01% solution of carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), at the same time the rats were redefined on diet containing 5% NaCl. The samplings of material were performed at the end of 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th}, 18^{th} and 24^{th} week. The study protocol was approved by Ethical Committee permission of Educational and Scientific Center "Institute of Biology" (Kyiv, Ukraine). ***Results***: At the end of the 4th week of MNNG and NaCl consumption the control reference of phospho-c-Jun content was observed. The gastric mucosa cells were characterized by increased content of phospho-c-Jun at 4 and 6.3 fold over the control at the end of 6^{th} and 8^{th} weeks, respectively, of the of MNNG and NaCl treatment. Also it was established that the MNNG and NaCl treatment for 10 weeks causes 1.9-fold increase in phospho-c-Jun content compared to the control. At terminal stages (12, 18 and 24 weeks) of the gastric carcinogenesis study there was a stable increase of phospho-c-Jun content on the average at 3.6-fold in comparison with reference values. ***Conclusions***: The increased level of the phosphorylated form of c-Jun from 6^{th} to 24^{th} week of gastric cancer development was probably caused by activation of MAP-kinase cascade and inactivation of phosphatases that would lead to the intensification of target genes transcription responsible for cell proliferation.

EPIDEMIOLOGICAL AND EXPERIMENTAL APPROACHES FOR RISK ASSESSMENT OF LOW INTENSITY RADIOFREQUENCY RADIATION ON THE PUBLIC HEALTH EFFECTS

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Introduction: Global development of wireless technologies, including cellular telephony and Wi-Fi, leads to a tremendous growth of radiofrequency electromagnetic radiation (RF-EMR) level in human environment. Today some studies revealed both epidemiological and experimental evidence on potential carcinogenicity of long-term exposure to the low intensity RF-EMR from the modern wireless devices. ***Aim***: To demonstrate the effectiveness of epidemiological approach (a public survey) as well as the model biological experiments in RF-EMR risk assessment. ***Materials and Methods***: 1) We interviewed students of Kyiv region on a specificity of cellular phones use and self-assessment of their health, including a headache, an earache, and a physical discomfort manifestation. 2) We used a model of Japanese quail embryos *in ovo* for the assessment of metabolic changes in living cells under the low intensity RF-EMR exposure. ***Results***: The survey of Ukrainian students (17–21 years old) revealed that about a half of them used cellular phones for talking more than 1 h per day, and about 25% of them — more than 2 h per day. And 42% of students surveyed had a headache and/or an earache during the long-term cellular-phone conversations. A strong correlation between headache prevalence and the duration of everyday cell-phone conversations was revealed. We could not exclude at least partial effect of RF-EMR from the cellular

phones in the phenomena revealed. On the other hand, the exposure of quail embryos *in ovo* to extremely low intensity RF-EMR of GSM 900 MHz standard during at least 158 h led to significantly increased levels of superoxide and nitrogen oxide, TBA-reactive substances and 8-oxo-dG in embryo cells. This was accompanied by a depression of antioxidant enzymes activity in the cells. Previously the distinctive mutagenic effects of this regimen of RF-EMR exposure on the quail embryos were demonstrated by alkaline comet assay. ***Conclusions*:** Both epidemiological and experimental approaches used in this study were effective in the risk assessment of the low intensity RF-EMR. Our findings indicate the necessity of at least wide implementation of the precautionary principle as for uncontrolled exposure of the public to RF-EMR from the modern wireless devices.

DECREASE OF POSTNEUROLYTIC PAIN IN PATIENTS WITH ADVANCED PANCREATIC CANCER AFTER PARAVERTEBRAL NEUROLYTIC BLOCK

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Introduction: More than 60% of advanced pancreatic cancer (APC) patients suffer from resistant pain syndrome whether they were previously resected or not. One of effective and minimal invasive pain control techniques is paravertebral neurolytic block (PNB). The most frequent adverse effect of PNB is postneurolytic pain (PP) persisting up to 7 days and more after the procedure. ***Aim:*** To decrease PP in the patients after PNB and to study the effectiveness of PNB in combination with prolonged ropivacaine epidural infusion (PREI) in APC patients. ***Patients and Methods:*** Group I consisted of 21 sequential patients matched to inclusion criteria and treated by PNB in our clinic before we have started PREI application in postneurolytic period. Group II consisted of 21 sequential patients matched to inclusion criteria and treated with PNB and PREI. Neural roots were identified at Th5–Th10 level by rhythmic eliciting paresthesia and/or pain radiating to corresponding body segment according to frequency of Stimuplex® DIG electrostimulation after puncture. 0.2% ropivacaine was used for PREI. Numeric Pain Rating Scale (NPRS) from 0 to 10 and morphine equivalent dose (MED) for tramadol daily intake were used for evaluation of pain. The study protocol was approved by Ethical Committee permission of Bogomolets National Medical University (Kyiv, Ukraine). ***Results:*** Pain assessment in postneurolytic period is represented in Table. Average duration of PREI application was 6.4 ± 1.3 days. 14 patients from group I and 11 patients from group II completely refused opioids. No severe side effects of PREI as pronounced arterial hypotension or paresis of lower extremities were recorded.

Table. Pain assessment in patients with advanced pancreatic cancer.

Patients	Initial level		Day 3rd after PNB		Day 7th after PNB	
	NPRS	MED	NPRS	MED	NPRS	MED
Group I	7.11±0.23	38.20±0.90	8.44±1.21	54.60±5.30	4.85±0.86	15.70±2.40
Group II	6.98±0.29	39.10±1.20	3.73±0.45	12.40±0.80	3.96±0.58	14.90±1.90
p value	>0.05	>0.05	<0.05	<0.05	>0.05	>0.05

***Conclusion*:** PREI application significantly reduces PP in early postneurolytic period. PNB in combination with PREI is effective and safe technique to reduce pain and improve quality of life in APC patients.

FEATURES OF TREATMENT OF THE BREAST CANCER PATIENTS WITH DISSEMINATED TUMOR CELLS AND OVERPRODUCTION OF TUMOR NECROSIS FACTOR IN BONE MARROW

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Introduction: One of the main problems of oncology is the low efficiency of treatment against disseminated forms of neoplastic process — recurrence and metastasis, which is the main cause of high mortality among cancer patients. The important characteristic of disseminated cancer is the presence of tumor cells in the bone marrow (BM), as in many cases the use of high-dose chemotherapy by accepted protocols is inefficient. ***The goal*** was to prevent cancer progression and bone metastasis in breast cancer patients with disseminated tumor cells (DTC) in BM and with elevated levels of TNF in BM using modified schemes of polychemotherapy with bisphosphonates. ***Patients and Methods:*** This research was carried out during the therapy of 119 patients with breast cancer in a disease stage T1–4N1–2M0. DTCs and TNF were analyzed in BM of all patients before therapy. The patients with DTCs in BM were divided into 2 groups. Group 1 (n = 20) underwent preoperative therapy according to AC (doxorubicin + cyclophosphamide) scheme. Group 2 (n = 27) received preoperative therapy in compliance with AC — Paclitaxel scheme and + Zoledronic acid (Zometa) in the postoperative period. Patients with high level (more than 150 pg/ml, n = 53) TNF in BM were divided into the same two therapeutic groups (n = 27 and n = 26). ***Results:*** It was found that DTC had been present in 47 patients (39.4%). Proportion of patients who were in 36 months remission was about the same in both groups — with DTC in BM or without it (59.5% and 60.2% respectively). The efficacy of treatment schemes AC → P + Zometa schemes vs. 48.9% in AC group. A similar pattern is evident while comparing the same therapeutic patterns for patients with high level of TNF in BM. Disease

progression was observed in 25.9% of patients with AC → P + Zometa schemes vs. 73.1% in AC group. ***Conclusion****:* Presence of DTC and TNF in the bone marrow indicates a high risk of disease progression for patients with breast cancer. Preoperative polychemotherapy according to the scheme of AC → P followed by Zometa in the postoperative period for patients with DTC in BM and high TNF levels in BM is more effective in comparison with AC scheme.

EFFECT OF A FENUGREEK ON LIFETIME OF EXPERIMENTAL RODENTS WITH TRANSPLANTED TUMORS

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***Introduction*:** Recent studies show that *Trigonella foenum graecum* (*Leguminosae* family), commonly known as fenugreek, has growth-inhibiting activity against some kinds of experimental tumors. ***Aim*:** To study the effect of fenugreek on lifespan of tumor bearing animals. ***Materials and Methods*:** Experiments were carried out on the adult C57Bl/6 female mice with subcutaneously grafted Ca755 mammary carcinoma, and adult C57Bl/6 male and female mice transplanted with Lewis lung carcinoma (3LL). The animals in the experimental group were administered with the mix of fenugreek powder (250 mg/kg of body weight) and standard mash from the day of tumor grafting up to the end of experiments. All experiments were carried out according to the rules of local Ethic Committee and were approved by the Ethic Board of IEPOR NASU. ***Results*:** The fenugreek consumption increased the lifetime of Ca755 carcinoma mice by 19%, and 3LL-bearing male and female mice by 9% and 26%, respectively. Moreover, it was shown that fenugreek consumption has resulted in the inhibition of Ca755 adenocarcinoma growth by 48%. Anti-metastatic effect of mentioned agent was also observed: an average volume of the metastases per animal was decreased by 86% and 18% in the female and male mice, respectively. ***Conclusions*:** The results have shown that fenugreek can be considered a potential anticancer agent with more significant effect in the female animals.