Eighty years of fighting against cancer in Serbia

This year on December 10th, *Serbian society for the fight against cancer* has celebrated the great jubilee - 80 years of its foundation. The celebration took place in *Crystal Room* of Belgrade's *Hyatt* hotel, gathering many distinguished guests from the country and abroad. This remarkable event has been held under the auspices of the President of Republic of Serbia, Mr. Boris Tadić.

The whole ceremony was presided by Prof. Dr. Slobodan Čikarić, actual president of Society, who greeted guests and wished them a warm welcome. Than followed the speech of Serbian health minister, Prof. Dr. Tomica Milosavljević who pointed out the importance of preventive measures and public education along with timely diagnosis and multidisciplinary treatment in global fight against cancer in Serbia. After that many eminent speakers paid their respects to great efforts and achievements of Serbian Society in its permanent struggle to overcome the cancer disease.

Serbian Society for fight against cancer has its origins in *Yugoslav Society for study and treatment of cancer*, which was founded in 1927, being the fourth such association in the world. Due to the visions of founding fathers, great scientists and doctors, Dr. Joannović , Dr. Šahović, and Dr. Antić, this society was conceived as a response to increasing sociomedical problems related to malignant diseases in former Kingdom of Yugoslavia .

The legacy of Yugoslav Society was great – first cancer statistic reports, press and radio coverage of oncological issues, modern approaches in diagnosis such as ex tempore biopsy and multidisciplinary in treatment. The crown was the construction of new building, Prince Paul's Institute for research and cancer treatment in 1939 – the present Institute for oncology and radiology of Serbia in Belgrade.

After the pause of two decades the work of Society was resumed in 1966, under the name of *Serbian Society for the fight against cancer*. Most important role in restoring activities has had Dr. Blagoje Nešković, prominent doctor and politician. Today, Society is non-government, non-profitable humanitarian organization with 1450 members, 11 subsidiaries in central Serbia and 12 in Vojvodina. Society is also a full UICC member. Main goals are public education regarding prevention and cancer treatment, support and informing of patients and their families, support of professional education and research activities in oncology and improvement of law regulations concerning malignant diseases. Also, Society has published twenty booklets on various topics in oncology, and has been issuing a journal ***„Rak - bolje sprečiti nego lečiti"*** on regular quarterly basis. The journal is distributed all over the country, free of charge.

Having in mind, the ever increasing number of cancer patients in Serbia, Serbian Society for the fight against cancer has to continue its battle along the other relevant institutions to beat this plague of our time. We hope it will win!!

Miroslav Kreačić

Ovarian cancer vaccine

Starting with first decade of this century modern biological and immunological science point to important role of an immune system and effect of that system on malignant cells. Developing an immunology many of studies have shown effects of immune system on malignant cells and expressed antigens.

Today around the globe many studies investigate effect of some therapeutic vaccines in some types of malignant cells.

Recent study by Kunle Odunsi et al., Roswell Park Cancer Institute, Buffalo, New York, USA, showed an effects of vaccine based on NY-ESO-1, a „cancer testis" antigen in preventing the recurrence of ovarian cancer. NY-ESO-1 is a „**cancer**-testis" antigen expressed in epithelial **ovarian cancer** (EOC) and is among the most immunogenic tumor antigens defined to date.

Author's previous study point to the role of presence of intraepithelial $CD8^+$-infiltrating T lymphocytes in tumors that was associated with improved survival of patients with the disease. The NY-ESO-1 peptide epitope, $ESO_{157–170}$, is recognized by HLA-DP4-restricted $CD4^+$ T cells and HLA-A2- and A24-restricted $CD8^+$ T cells. To test whether providing cognate helper $CD4^+$ T cells would enhance the antitumor immune response, Odunsi et al., conducted a phase I clinical trial of immunization with $ESO_{157–170}$ mixed with incomplete Freund's adjuvant (Montanide ISA51) in 18 HLA-$DP4^+$ EOC patients with minimal disease burden. NY-ESO-1-specific Ab responses and/or specific HLA-A2-restricted $CD8^+$ and HLA-DP4-restricted $CD4^+$ T cell responses were induced by a course of at least five vaccinations at three weekly intervals in a high proportion of patients.

Eighteen EOC patients (HLADPB1*0401 or *0402) with NY-ESO-1-expressing tumors who had completed adjuvant chemotherapy for primary or recurrent disease were entered into the trial.

Vaccine-induced $CD8^+$ and $CD4^+$ T cell clones were shown to recognize NY-ESO-1-expressing tumor targets. T cell receptor analysis indicated that tumor-recognizing $CD4^+$ T cell clones were structurally distinct from non-tumor-recognizing clones. Long-lived and functional **vaccine**-elicited $CD8^+$ and $CD4^+$ T cells were detectable in some patients up to 12 months after immunization.

No major (more than grade II) treatment-related toxicity was observed in any patient. Transient injection site pain was seen in all patients, and systemic hypersensitivity reactions were not observed.

Authors point that these results confirm the paradigm that the provision of cognate $CD4^+$ T cell help is important for **cancer vaccine** design and provides the rationale for a phase II study design using $ESO_{157–170}$ epitope or the full-length NY-ESO-1 protein for immunotherapy in patients with EOC.

Aljoša Mandić

Reference

1 Odunsi K, Qian F, Matsuzaki J, Mhawech-Fauceglia P, Andrews C, Hoffman EW, et al. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. *PNAS*. 2007;104(31):12837-42.

Pharmacogenomics of gemcitabine: Is it an important issue for future determination of chemotherapy?

Ueno et al.(1) from Japan point to the effects of genetic polymorphism of an enzyme involved in gemcitabine metabolism that can cause interindividual variations in the pharmacokinetics and toxicity of this agent

Gemcitabine is a deoxycytidine analogue that has a broad spectrum of antitumour activity in many solid tumours including pancreatic cancer.

The impact of genetic polymorphisms as well as tumor-specific expression of mRNA/proteins on gemcitabine efficacy and toxicity has been described. According to collected data authors point that tumour-specific expression of equilibrative nucleoside transporters (ENT1), subunit of ribonucleotide reductase - M1 (RRM1), excision repair cross-complementing group 1 (ERCC1), and some DNA repair genetic polymorphisms could be promising indicators of prognosis in

patients receiving gemcitabine chemotherapy. However, prospective pharmacogenetic-based clinical studies will be necessary to clarify the usefulness of these biomarkers in patients receiving gemcitabine-based chemotherapy.

With regard to adverse reactions caused by gemcitabine, the expression level or genetic polymorphism of cytidine deaminase (CDA) seems to be a good predictor. Single-nucleotide polymorphisms (SNPs) in the cytidine deaminase gene (CDA 208A4G) or CDA expression level may be candidate biomarkers for individualised gemcitabine-based chemotherapy. In this way it could be possible to avoid severe toxicity at least in Japanese and some African populations in which considerable numbers of homozygote carriers exist similarly as is the case of UGT1A1*28 for irinotecan and TPMT genotypes for thiopurine drugs.

Aljoša Mandić

Reference

1. Ueno H, Kiyosawa K, Kaniwa N. Pharmacogenomics of gemcitabine: can genetic studies lead to tailor-made therapy? *Br J Cancer.* 2007;97:145–51.

Adjuvant treatment of colon and rectal cancer: What is the optimal regimen?

Colorectal carcinoma is one of the most frequent malignant neoplasms. Worldwide 1,000.000 new cases and 500,000 deaths of colon and rectal Cancer every year are reported. Adjuvant treatment is established for stage III and high risk stage II colorectal cancer. Nowadays, latest generation of drugs as monoclonal antibodies - vascular endothelial growth factor (VEGFR) and epidermal growth factor receptor (EGFR) takes part in adjuvant treatment trials. The U.S. Gastrointestinal Intergroup (GI Intergroup), including the National Cancer Institute of Canada, has created a portfolio of clinical trials for patients with stage II and III colon and rectal cancer, integrating therapy for metastatic disease with either irinotecan or oxaliplatin plus bevacizumab, has resulted in significant improvement in response and disease-free and overall survival. Cetuximab and irinotecan have produced intriguing response and progression-free survival data from randomized phase II trials. Although patients with stage II and III rectal cancer are uniformly included in individual clinical trials, the GI Intergroup conducts separate trials patients with stage II and III colon cancer, with the exception of the National Surgical Adjuvant Breast and Bowel Project (NSABP), which continues to merge both stages in their statistical designs. The U.S. chemotherapy platform for adjuvant therapy clinical trials is based on the positive adjuvant data from NSABP C-07 {FLOX with bolus 5-Fluorouracil (5-Fu)} and the MOSAIC trial (FOLFOX with infusional 5-Fu). Three irinotecan-based adjuvant trials (one U.S. and two European) did not reach designated statistical end points. In addition, the GI Intergroup has consistently integrated molecular biological and other laboratory projects as important components of past and current trials. NSABP has recently completed accrual of patients to C-08, which is evaluating FOLFOX with or without bevacizumab in stage II/III colon cancer. E5202, the largest U.S. stage II colon cancer trial determines patients risk by the initial evaluation of tumor 18q loss of heterozygosity and microsatellity status. Low-risk patients are observed, whereas high-risk patients are randomized to FOLFOX with or without bevacizumab. N0147 evaluates FOLFOX with or without cetuximab in patients with stage III disease. Two large rectal cancer trials have begun to accrue patients. NSABP R-04 compares neoadjuvant radiation with either continuous infusion 5-Fu with or without oxaliplatin. E5204 is the adjuvant comparison of FOLFOX with or without bevacizumab and is also available to NSABP R-04 patients.

Nevertheless, prospective randomized studies need to be performed to confirm the superiority of these adjuvant regimens.

Ivan Nikolić

Reference

1 Benson AB, III. New approaches to Assessing and Treating Early-Stage Colon and Rectal Cancers: Cooperative Group Strategies for Assessing Optimal Approaches in Early-Stage Disease. *Clin Cancer Res.* 2007;13(22Pt2):6913s-20s.

Pax-5 gene loss makes possible conversion B cells into T lymphocyte and macrophages

Cobaleda C, Jochum W, Busslinger M (2007) reported that B lymphocytes may convert their differentiation into T lymphocytes and macrophages after deletion of transcription gene (Pax-5). However, it is not known whether the gene loss can converse pro-B cells into T cells by direct transdifferentiation or the differentiation of pro-B cells into uncommitted progenitors that then proceed along a conventional pathway to the T-cell lineage (Figure 1).

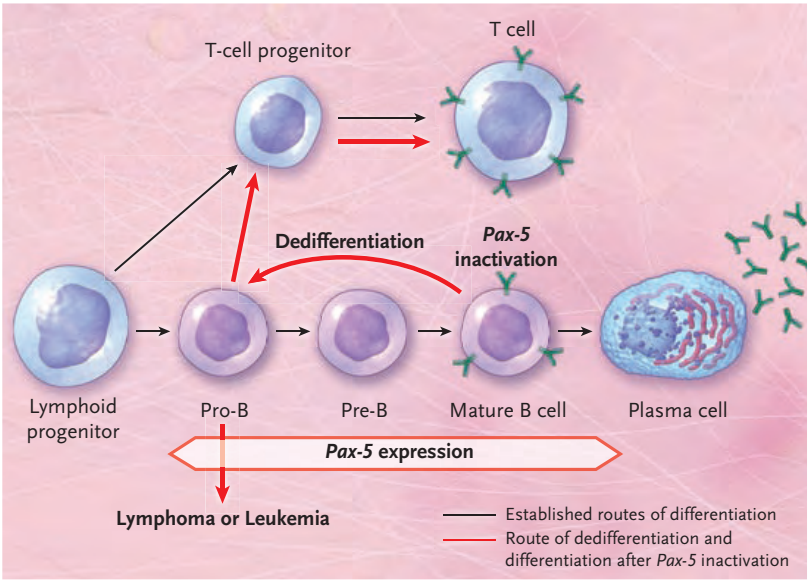

Figure 1. Converting B cells to T cells in mice.
B cells and T cells differentiate from a common lymphoid progenitor in the bone marrow. Committed B cells express *Pax-5* and pass through progenitor (pro-B), precursor (pre-B), and marute stages. *Pax-5* expression is down-regulated as B cells undergo their final differentiation into antibodysecreting plasma cells. Mice that do not have Pax-5 generate pro-B cells that are not committed to the B-cell lineage and have the abikity to differentiate into multiple cell types, including T cells.[3] A recent study by Cobaleda et al.[1] showed that inactivation of *Pax-5* in mature B cells result in dedifferentiation to the pro-B-cell stage and the generation of T cells. The loss of *Pax-5* also results in lymphoma in mice[1] and is know to contribute to the developnemt of acute lymphoblastic leukemia in humans.[5]

The results of B cell differentiation point to potential development of aggressive lymphomas and acute lymphoblastic leukemia. Due to re-programming of B cells into other cell lines a great caution should be paid when stem cells are used in therapeutic purpose.

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1 Cobaleda C, Jochum W, Busslinger M. Conversion of mature B lymphocyte into T-cells by differentiation to uncommitted progenetors. *Nature*. 2007; 409:473-7.
2 Nutt SL. B-cell identity – commitments is not forever. *N Engl J Med*. 2008;358:82-3.

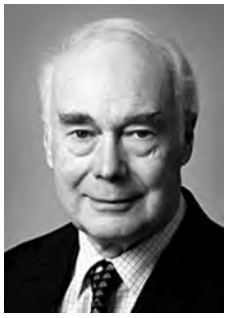
Vladimir Baltić

The Nobel Prize in Physiology or Medicine 2007

Mario R. Capecchi, Martin J. Evans and Oliver Smithies: „Principles for introducing specific gene modifications in mice by the use of embryonic stem cells“

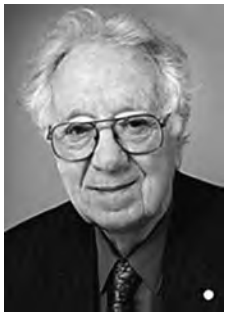
Mario R. Capecchi,

born 1937 in Italy, US citizen, PhD in Biophysics 1967, Harvard University, Cambridge, MA, USA. Howard Hughes Medical Institute Investigator and Distinguished Professor of Human Genetics and Biology at the University of Utah, Salt Lake City, UT, USA.



Martin J. Evans,

born 1941 in Great Britain, British citizen, PhD in Anatomy and Embryology 1969, University College, London, UK. Director of the School of Biosciences and Professor of Mammalian Genetics, Cardiff University, UK.



Oliver Smithies,

born 1925 in Great Britain, US citizen, PhD in Biochemistry 1951, Oxford University, UK. Excellence Professor of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, NC, USA.

This year's Nobel Laureates have made a series of ground-breaking discoveries concerning embryonic stem cells and DNA recombination in mammals. Their discoveries led to the creation of an immensely powerful technology referred to as *gene targeting in mice*. It is now being applied to virtually all areas of biomedicine – from basic research to the development of new therapies. Gene targeting is often used to inactivate single genes. Such gene „knockout“ experiments have elucidated the roles of numerous genes in embryonic development, adult physiology, aging and disease. To date, more than ten thousand mouse genes (approximately half of the genes in the mammalian genome) have been knocked out. Ongoing international efforts will make „knockout mice“ for all genes available within the near future. With gene targeting it is now possible to produce almost any type of DNA modification in the mouse genome, allowing scientists to establish the roles of individual genes in health and disease. Gene targeting has already produced more than five hundred different mouse models of human disorders, including cardiovascular and neuro-degenerative diseases, diabetes and cancer.

Modification of genes by homologous recombination

Information about the development and function of our bodies throughout life is carried within the DNA. Our DNA is packaged in chromosomes, which occur in pairs – one inherited from the father and one from the mother. Exchange of DNA sequences within such chromosome pairs increases genetic variation in the population and occurs by a process called *homologous recombination.* This process is conserved throughout evolution and was demonstrated in bacteria more than 50 years ago by the 1958 Nobel Laureate Joshua Lederberg. Mario Capecchi and Oliver Smithies both had the vision that homologous recombination could be used to specifically modify genes in mammalian cells and they worked consistently towards this goal. Capecchi demonstrated that homologous recombination could take place between introduced DNA and the chromosomes in mammalian cells. He showed that defective genes could be repaired by homologous recombination with the incoming DNA. Smithies initially tried to repair mutated genes in human cells. He thought that certain inherited blood diseases could be treated by correcting the disease-causing mutations in bone marrow stem cells. In these attempts Smithies discovered that endogenous genes could be targeted irrespective of their activity. This suggested that all genes may be accessible to modification by homologous recombination.

Embryonic stem cells – vehicles to the mouse germ line

The cell types initially studied by Capecchi and Smithies could not be used to create gene-targeted animals. This required another type of cell, one which could give rise to germ cells. Only then could the DNA modifications be inherited. Martin Evans had worked with mouse embryonal carcinoma (EC) cells, which although they came from tumors could give rise to almost any cell type. He had the vision to use EC cells as vehicles to introduce genetic material into the mouse germ line. His attempts were initially unsuccessful because EC cells carried abnormal chromosomes and could not therefore contribute to germ cell formation. Looking for alternatives Evans discovered that chromosomally normal cell cultures could be established directly from early mouse embryos. These cells are now referred to as *embryonic stem (ES) cells*. The next step was to show that ES cells could contribute to the germ line (see Figure). Embryos from one mouse strain were injected with ES cells from another mouse strain. These *mosaic* embryos (i.e. composed of cells from both strains) were then carried to term by surrogate mothers. The mosaic offspring was subsequently mated, and the presence of ES cell-derived genes detected in the pups. These genes would now be inherited according to Mendel's laws. Evans now began to modify the ES cells genetically and for this purpose chose retroviruses, which integrate their genes into the chromosomes. He demonstrated transfer of such retroviral DNA from ES cells, through mosaic mice, into the mouse germ line. Evans had used the ES cells to generate mice that carried new genetic material.

Two ideas come together – homologous recombination in ES cells

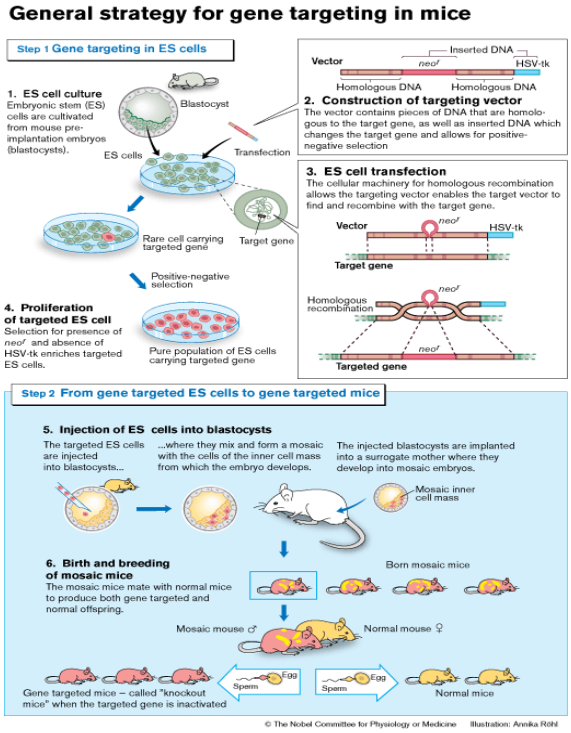
By 1986 all the pieces were at hand to begin generating the first gene targeted ES cells. Capecchi and Smithies had demonstrated that genes could be targeted by homologous recombination in cultured cells, and Evans had contributed the necessary vehicle to the mouse germ line – the ES-cells. The next step was to combine the two. For their initial experiments both Smithies and Capecchi chose a gene (hprt) that was easily identified. This gene is involved in a rare inherited human disease (Lesch-Nyhan syndrome). Capecchi refined the strategies for targeting genes and developed a new method (positive-negative selection, see Figure) that could be generally applied.

Birth of the knockout mouse – the beginning of a new era in genetics

The first reports in which homologous recombination in ES cells was used to generate gene-targeted mice were published in 1989. Since then, the number of reported knockout mouse strains has risen exponentially. Gene targeting has developed into a highly versatile technology. It is now possible to introduce mutations that can be activated at specific time points, or in specific cells or organs, both during development and in the adult animal.

Gene targeting is used to study health and disease

Almost every aspect of mammalian physiology can be studied by gene targeting. We have consequently witnessed an explosion of research activities applying the technology. Gene targeting has now been used by so many research groups and in so many contexts that it is impossible to make a brief summary of the results. Some of the later contributions of this year's Nobel Laureates are presented below. Gene targeting has helped us understand the roles of many hundreds of genes in mammalian fetal development. Capecchi's research has uncovered the roles of genes involved in mammalian organ development and in the establishment of the body plan. His work has shed light on the causes of several human inborn malformations. Evans applied gene targeting to develop mouse models for human diseases. He developed several models for the inherited human disease cystic fibrosis and has used these models to study disease mechanisms and to test the effects of gene therapy. Smithies also used gene targeting to develop mouse



models for inherited diseases such as cystic fibrosis and the blood disease thalassemia. He has also developed numerous mouse models for common human diseases such as hypertension and atherosclerosis. In summary, gene targeting in mice has pervaded all fields of biomedicine. Its impact on the understanding of gene function and its benefits to mankind will continue to increase over many years to come.

Taken from: Karolinska university press release.
Adapted by: Lazar Popović

44th Annual Meeting of Oncology Section – Serbian Medical Association
21st Annual Meeting of Oncology Nurses of Republic of Serbia

November, 8 – 10, 2007, Belgrade, Serbia

44th Annual Meeting of Oncology Section of Serbian Medical Association and 21st Annual Meeting of Oncology Nurses of Republic of Serbia were organized in cooperation of the Institute of Oncology and Radiology of Serbia and Oncology section of the Serbian Medical Association, in Sava Center, Belgrade, November, 8 – 10, 2007.

Oncology meeting started with ceremonial opening and plenary lecture by Dr. Slobodan Čikarić titled *80 years long struggle against cancer in Serbia* and continued with cocktail party, supported by sponsor *Pfizer*.

First symposium was dedicated to aromatase inhibitors in the treatment of breast cancer. Dr. S. Vasović pointed on side-effects of hormonal therapy of breast cancer. Dr Joseph Gligorov from Paris (France) presented adjuvant antihormonal strategies in menopausal women with breast cancer. Dr V. Kesić highlighted effects of hormonal therapy of breast cancer on endometrium, and Dr. V. Mijucić lectured about tromboembolic complications of hormonal therapy of breast cancer.

Session entitled *Pharmacotherapy of cancer pain* organized by Medical Science Academy of Serbian Medical Society was particularly successful thanks to brilliant lecturers: Prof. Dr. D. Beleslin, Prof. Dr. S. Apostolski, Dr. N. Milićević, Dr. S. Vučković, Dr. S. Šušnjar, Dr. S. Bošnjak, Dr. D. Radosavljević, Dr. I. Palibrk and Dr. M. Steingraber (Germany). Impressive audience of about one hundred and fifty participants enjoyed excellent lectures referred to: pathophysiology of pain, cancer pain and challenges due to addiction, general principles of applying analgesics, particularly morphine, in patients with cancer pain, opioid and non-opioid analgesics and radiotherapeutic aspects of cancer pain treatment.

Since Serbia has one of the highest incidence of cervical carcinoma among European countries, the main theme of this Oncology meeting referred to this malignancy. Dr. A. Bekić and Dr. V. Kesić presented an illustrative overview of cervical cancer epidemiology in Serbia and worldwide, which pointed on emergency of screening and early detection of this cancer. The lecture on HPV vaccination by Dr. A. Mandić was particularly useful and interesting for researchers because HPV vaccines are up-to-date prophylactic procedures for cervical cancer. The significance of 3D brachytherapy for locally advanced cervical carcinoma (Dr. V. Plešinac), preoperative radiotherapy for stage Ib2, followed by radical surgery (Dr. M. Erak), contemporary surgical treatment of cervical carcinoma (Dr. V. Pažin), and innovative therapeutic protocol for this malignancy (Dr. V. Plešinac) were also the lectures of great interest.

A session was dedicated to diagnostic procedures of breast cancer. Dr. G. Chaupier from Paris (France) gave an overview of mammography screening of breast cancer in France. Dr. A. Bekić and Dr. Z. Milošević presented epidemiology and mammography screening of breast cancer in Serbia. Dr. I. Drinković from Zagreb (Croatia) and Dr. D. Bogdanović from Oncology Institute of Vojvodina illustrated significance of ultrasonography diagnosis of breast cancer and MR mammography. Non-palpable lesions in breast can be diagnosed by stereotaxic vacuum aspiration biopsy, as Dr. B. Jakovljević lectured. At the end of session Dr. T. Falun from Sweden presented lobar concept of breast cancer.

Special session referred to evaluation of scientific work based on citations, which highlighted the best ways of choosing scientific journal appropriate for publishing articles (Dr. Lj. Vučković – Dekić), as well as importance of citation of authors and their articles (Dr. S. Brkić, Medical faculty of Novi Sad; B. Avramović and I. Klajn, *Library of Matica Srpska*, Novi Sad). S. Filipi – Matutinović from University Library *Svetozar Marković* from Belgrade presented Journal Citation Reports 2000-2006: distribution analysis of impact factors.

One educative seminars and one workshop were held during this oncology meeting. The first one entitled *Communication with patients* offered a possibility to cancer patients to share their experience during the disease and through an interactive communication to try to find out answers related to social aspects. Dr. M. Matorčević underlined the importance of sources of support in community. In order to improve protocols of cancer patients' treatment, it is necessary to continue with further clinical investigations of new drugs (Dr. S. Vasović), which requires active participation of patients in clinical trials (Dr. S. Radulović). Dr. Z. Menković presented experiences of patients' Oncology club in Leskovac. Dr. A. Bekić reported results of training course of smoking, held in the Institute of Oncology and Radiology of Serbia, this year.

Workshop entitled *Positioning and immobilization in up-to-date radiotherapy* was intended to radiology technicians.

Authors who published their results in IF journals and who presented them on ASCO Meeting 2007, had an opportunity to describe the same through

the special session *Impact factor – the best articles, ASCO highlights and target therapy*.

Poster presentation included five sessions: Carcinoma of portionis vaginalis uteri; Articles presented on previous science meetings; Target therapy; Breast carcinoma and Miscellaneous. In these five sessions 82 posters were presented.

Nursing programme included oral and poster presentations dedicated to the role of oncology nurse in emergency states, in health care, diagnostics and treatment modalities of cervical cancer.

In conclusion, this meeting introduced several novelties, such as workshop, patient' session and bibliometrics, thus contributing greatly to the success of this meeting, which attracted much more participants than the previous ones.

Aleksandra Erić