

Project no. 026357

Project acronym: HISERBS

Project title: HEALTH IMPROVEMENT IN SERBIA THROUGH  
REINFORCEMENT OF BIOMEDICAL SCIENCE AND TECHNOLOGY

Instrument: SIXTH FRAMEWORK PROGRAMME  
PRIORITY 1

Thematic Priority: LIFE SCIENCES, GENOMICS AND BIOTECHNOLOGY FOR  
HEALTH

SPECIFIC SUPPORT ACTION

**Title of reports: PUBLISHABLE FINAL ACTIVITY REPORT**

Period covered: from 1 to month 36

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Duration: 36 months

Project coordinator name: Diana Bugarski

Project coordinator organization name:  
Institute for Medical Research

Malignancies are one the major causes of mortality in Serbia with increasing incidence. This project was designated for further reinforcement of three biomedical research institutions in Serbia (Institute for Medical Research, Belgrade- IMR, Institute of Molecular Genetics and Genetic Engineering, Belgrade-IMGGE and Institute for Application of Nuclear Energy, Belgrade - INEP) through specific support actions (thematic priority 1. of FP6 - Life sciences, Genomics and Biotechnology for Health and FP6-2004-INCO-WBC/SSA), to be used for combating this dramatic trend by implementing of up-to-date diagnostic and therapeutic products and procedures. The above mission required engagement of various experts in basic research, laboratory practice and clinics. Therefore, mobilization of human and material resources in Serbia, as well as networking with other EU research centers, was carried out in the progress of the project. Visits by distinguished experts in health care and biotechnology from EU, as well as training of Serbian researchers in EU centers were achieved and will continue. EU medical diagnostic standards were introduced and further follow up will be performed. The project attracted the best young researchers and created new jobs.

Researchers from all partner institutions were assigned to specific tasks (pathologies). Teams were established for: **Acute Leukemia, Myeloproliferative Disorders, Breast Carcinoma, Pancreatic Carcinoma, Gastrointestinal Tumors and Thyroid Gland Tumors**. Each work group planned and performed steps within their task, thus mobilizing and networking both human potential and available equipment of the partners.

In the course of establishment and introduction of working protocols, researchers with the strong scientific background and experience with diverse and complementary methodologies were networked within the Project forming a Centre.

Networking of HISERBS multidisciplinary team through standardization of current methodologies, with health care services in Serbia was initiated. All the relevant medical institutions in Serbia were invited to take part in the networking of the researchers in the field of diagnosis and follow up of malignant diseases. The list of the laboratory diagnostic protocols currently used in Serbia was completed thanks to the information provided by the majority of the institutions.

Cooperation with several scientists from EU centers with the strong scientific background and experience was established in order to develop internationally accepted reference methods and certified reference materials, for diagnosis and follow up of malignant diseases.

## **Project execution**

### **1. Mobilization and networking of researchers and equipment of three Serbian institutes: Institute for Medical Research, Belgrade (IMR), Institute for Molecular Genetics and Genetic Engineering, Belgrade (IMGGE) and Institute for Application of Nuclear Energy, Belgrade INEP (INEP).**

The sets of agreements between partner institutions were made with the aim to establish “Coordination centre” for reinforcement of biomedical science and technology to implement cooperation between researches from diverse fields and institutions as a result of networking based on the HISERBS project. The Partnership Agreement was made effective by 1<sup>st</sup> of September 2006.

### **2. Standardization and implementation of modern diagnostic approaches in combating malignancies**

Thanks to the establishment of multidisciplinary teams for each pathologies as well as the connection with other relevant health care services in Serbia, screening of the currently used laboratory protocols in Serbia for diagnosis and follow-up of malignant diseases (leukemia, carcinoma of the breast, pancreas, thyroid gland, prostate and gastrointestinal tract) has been successfully accomplished, and the list of laboratory diagnostic protocols currently used in Serbia for malignant diseases has been made.

Thanks to the connection with several scientists from EU centers with the strong scientific background and experience with diverse methodologies used for diagnosis and follow up of malignant diseases, internationally accepted laboratory diagnostic protocols for leukemia, carcinoma of breast, pancreas, thyroid gland, prostate and gastrointestinal tract have been introduced in laboratory practice in Serbia.

The coordinated activities of HISERBS project resulted in establishment of unified modern diagnostic protocols in combating malignancies, which combined different methodological approaches recommended by various International Societies or Consortiums with expertise in the field.

- Internationally accepted laboratory diagnostic protocols for myeloproliferative disorders consider role of JAK-STAT signaling in the pathogenesis and diagnosis of myeloproliferative disorders and molecular origin of chronic myeloproliferative disorders as suggested by American Society of Hematology.
- Recommendations given by the European Consortium Biomed -1 Concerted Action Group were followed for RT-PCR protocols for analysis of the most common translocations in leukemia. As for Real time PCR analysis of fusion gene transcript for the most common translocations in leukemia, the introduced laboratory diagnostic protocols are based on recommendations given by Europe against Cancer Network Program.
- The recommendations given by The National Academy of Clinical Biochemistry (Practice Guidelines and Recommendations for use of serum tumor markers in the clinic) and European Group of Tumor Markers were considered.

Newly introduced laboratory procedures contributed to improvement of diagnosis and follow-up of various malignancies:

### ***Leukemia and other hematological malignancies***

Up-to date diagnostic protocols for detection and follow-up of molecular genetic markers indispensable for successful treatment of pediatric acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloproliferative neoplasms were introduced and standardized.

### ***Breast carcinoma***

Serum tumor markers for breast cancer, CA 15-3 and CEA, but also some other serum markers (ferritin and markers of inflammation), were evaluated during postoperative monitoring (in order to detect local recurrence or metastasis) or monitoring systemic treatment in patients with advanced disease. The data proved that, despite the limitations of serum markers, application of dynamic parameters, with proper interpretation of results, and in combination with other clinical parameters, could provide relevant data useful in treatment of cancer patients. Additionally, tissue expression of cyclins, A cyclins and the B family of cyclins, was followed. Prognostic significance of human *HER2* gene, as well as of germline mutations in *BRCA1* and *BRCA2* was evaluated in women with breast cancer in Serbia.

### ***Pancreatic cancer and gastrointestinal tumors***

In the field of pancreatic carcinomas it was shown that tissue immunolabeling for Deleted Pancreatic Cancer Locus 4 (DPC4) protein provides a useful tool to examine genetic progression in the adenocarcinoma of the pancreas. For prediction of malignant behaviour of gastrointestinal stromal tumors (GISTs) alteration of cell-cycle proteins, in particular G1-S regulators, such as p16INK4, p27, cyclin D3, and p53, was evaluated. Additionally, members of the IGF/IGFBP family were shown useful in discriminating between primary and metastatic liver malignancy. Determination of serum IGF-II, IGFBP-1 ratio may aid in estimating the compensatory capacity of the liver affected by a tumor.

### ***Prostate cancer***

Prostate specific antigen (PSA) is one of the most frequently used tumor marker due to high incidence of prostate cancer and other prostate diseases. The most important are separate determinations of free fraction (fPSA) and total amount of PSA (tPSA), and subsequent calculation of fPSA/tPSA ratio, which enables differentiation between PCa and BPH in patients with moderately elevated PSA concentrations in the blood. Immunoradiometric assays for detection of blood concentration of PSA (IRMA-PSA), and free fraction of PSA (IRMA-fPSA) have been developed, as well as modification of IRMA-PSA assay for determination of urine concentration of PSA. These tests are now widely accepted in routine practice in many Serbian laboratories. Dynamic approach in determination of these serum markers has provided additional value for the patients.

### ***Thyroid gland cancers***

In the field of thyroid malignant diseases, activities were focused on identification and evaluation of serum and tissue tumor markers (thyroglobulin, calcitonin galectin-3, thyroid peroxidase, HBME-1 and cytokeratin-19) of thyroid malignancy which would facilitate differential diagnostics of thyroid tumors or could be useful in follow-up of thyroid carcinoma patients and their clinical management.

Standardization and validation of the newly introduced laboratory procedures was performed and working protocols were established and introduced. The normal values of the selected markers were determined for the population of Serbia.

HISERBS project activities contributed to great extent to the laboratory diagnostic standards. As a result of activities through HISERBS project, local standards have been established where previously non-existent, and verified and improved in most of the areas covered by the project. In the field of tumor markers, local practice has been evaluated through interlaboratory comparisons with some of the highly respected reference laboratories from EU, which confirmed high quality of local practice. New contacts in the field have made possible establishment of firm connections with external quality assurance bodies and participants for future actions, and has resulted in improved service for the patients in Serbia through implementation of specific follow-up protocols.

Additionally, HISERBS project activities contributed and are complementary to the activities of policy makers. In the course of the project, Ministry of Health of Serbia has started a wide campaign in the fields of prevention of malignant diseases. There is a possibility that some of the conclusions reached through realization of this project may influence national policy on combating malignancies, particularly in field of follow-up of cancer patients, and determining efficiency of therapy used. In case of a wider regional participation similar effects could be achieved regionally.

All this has high value and relevance particularly for the local community and health sector of Serbia.

### **3. Training and mobility**

The objectives related to training and mobility were of a particularly high significance for HISERBS project. Exposing researchers from Serbia to the latest developments in technology and science of laboratory diagnostics in EU institutions was of critical value for achieving progress in this field. It was achieved through: technology related training (on-site and in EU partner institutions), visits of experts from EU partner institutions and training of researchers in EU partner institutions.

Collaboration was established with:

- Andrea Biondi, MD PhD, Centro Ricerca Tettamanti Clinica Pediatrica Università di Milano-Bicocca, Ospedale San Gerardo, Monza, Italy
- Andreas Scorilas, PhD, Department of Biochemistry and Molecular Biology, University of Athens, Athens, Greece
- George Patrinos, PhD, Erasmus University Medical Center, Faculty of Medicine and Health Sciences, Department of Cell Biology and Genetics, Rotterdam, The Netherlands
- David Grimwade, MD, PhD, Department of Haematology, Imperial College at Hammersmith Hospital, London, United Kingdom
- Dr Radek Skoda, Department of Biomedicine, University Hospital Basel, Basel, Switzerland
- Keith Miller, MD, PhD, Department of Histopathology, University College London Medical School, London, United Kingdom
- H.Reinauer, INSTAND e.V. Düsseldorf, Germany, as WHO - Collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine.
- Prof Michael Seckl and Richard Harvey, PhD, Department of Medical Oncology, Charing Cross Hospital London, UK
- Andrew Renehan, MD, PhD, Department of Clinical and Experimental Haematology, Paterson Institute for Cancer Research, Christie Hospital, University of Manchester, Manchester, UK
- Prof dr Siniša Volarević, Medical School, University of Rijeka, Rijeka, Croatia

Experts from EU partner institution visited Serbian institutes (partners in the HISERBS project) in order to transfer knowledge and information of the latest technologies used for diagnosis of different malignant diseases. Twelve researchers engaged at HISERBS project realized short training (from 2 weeks to 2 months) programs in EU partner institutions.

There is a significant value added through participation of the institutions and individuals from the EC, reflected in faster and more efficient achievement of the objectives and goals of the project. In addition, thus established personal and professional contacts will facilitate potential future participation in other scientific initiatives.

#### **4. Dissemination of knowledge**

One of the key project goals was dissemination of the results of the project to either interested professionals or general public with respect to the most specific and efficient laboratory diagnostic tests and protocols for malignancies. These activities had high impact on health sector in Serbia. The success of these objectives was monitored by number of visitors of our open days, attendance of the conferences, number of visitors of the web site.

The comprehensive presentation of HISERB project was organized and included in The 45th Annual Meeting of Oncology Section –Serbian Medical Association and the 22nd Annual Meeting of Oncology Nurses of Republic of Serbia as special scientific meeting of Academy of Medical Sciences.

As a part of dissemination activities, HISERBS project participants have organized a meeting with regional participation in May 2009 The Round Table entitled “Molecular Markers in detection and Follow-up of Malignancies: FP6 HISERBS project Experience” was a part of the 8<sup>th</sup> Balkan Meeting on Human Genetics ([www.studiohrg.hr/human-genetics2009](http://www.studiohrg.hr/human-genetics2009)).

The results of HISERBS Project at local and international level were presented during the Research Information Event held in Belgrade on June 29<sup>th</sup>, 2009, organized by Ministry of Science and Technological Development of Serbia and European Commission.

Two Open Days, dedicated to ‘Molecular Markers in Diagnostics and Follow-up of Hematological Malignancies’ and ‘Serum Tumor Markers in Diagnosis and Follow-up of Malignancies’, were organized and the results of HISERBS were presented to both professionals and general public.

Two types of leaflets (“Molecular-genetic markers in diagnostics and follow-up of hematological malignancies” and “Serum tumor markers”), both in Serbian and English, were distributed to professionals and general public from Serbia and WBC. Increase of public awareness through dissemination of leaflets is continuously performed.

The book with description of the current methods and approaches, introduced for diagnosis of selected malignancies during the progress of the FP6 project, was published. The title of the book is: *Savremeni pristupi u laboratorijskoj dijagnostici tumorskih markera: iskustvo projekta FP6 HISERBS* (Current trends in tumor marker diagnosis: experience of FP6 HISERBS project). This book will be used for further dissemination of the project results.

The web presentation [www.hiserbs.com](http://www.hiserbs.com) was created and updated regularly. This enabled further dissemination of the project results and EU contribution. Increase of public awareness through dissemination of leaflets is continuously performed.

Media coverage and advertisement of the HISERBS project has been provided through the announcement in the Politika, the most influent daily newspaper in Serbia, as well as in the educational-scientific magazine "Context 21", which is broadcasted as a part of the scientific program on the National Television of Serbia (Serbian Broadcasting Corporation), as well as through Croatian National Television, HRT2.

## **5. Reinforcement of human potential and equipment.**

Through HISERBS project human and material recourses in Serbian institutions were reinforced (equipment upgrade, increase in job opportunities, reduction of brain-drain, knowledge transfer from EC), thus making Serbian science eligible for integration into ERA. Employment of young researchers dedicated to this project, and acquisition of the up-to date equipment reinforced our abilities and had great impact on the overall success of the project. New technologies related to the acquired equipment increased training and mobility needs, both in the country and in EC, and improved our abilities to fully validate specific diagnostic protocols and implement EC standards.

New equipment was acquired as the result of FP6 HISERBS project specific support: Partec flow cytometer CyFlow SL, Seymor Glass Slide Labeling System and Autostainer Universal staining system, Applied Biosystems 7500 Real-Time PCR system, BioSafety devices (Biosan, Talron Biotech L.T.D.), Biologocal safety cabinet (HERASAFE KS 12 with UV light, Heraeus Kendro Laboratories) and Multilabel detector system PerkinElmer Victor 3V.

As the result of FP6 HISERBS project support, three young scientists were employed and started their scientific carriers through PhD programs, training within their host laboratories and training in the EU partner laboratories. Thus, HISERBS project contributed to creation of new jobs and reduction of brain-drain.

## Conclusions

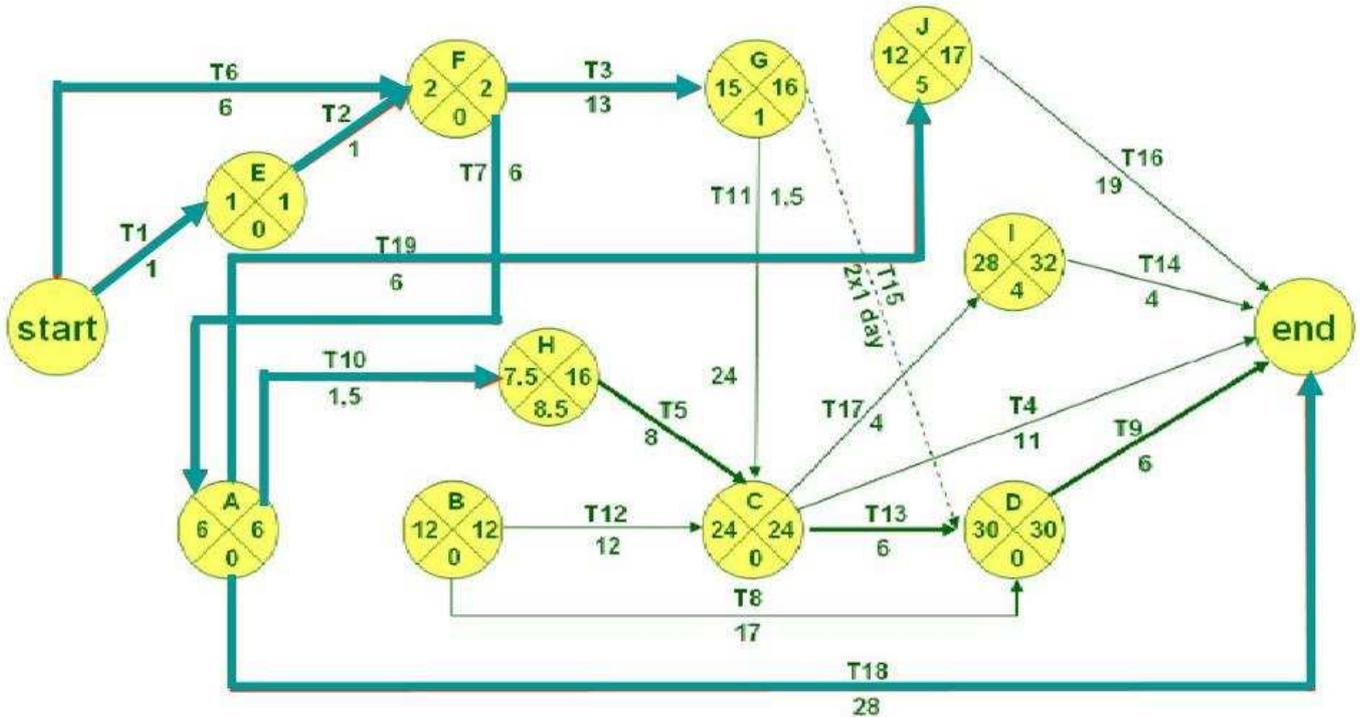
One of the most prominent HISERBS project achievement is local networking and reinforcement of biomedical research institutions in Serbia (creation of the “Cordination centre” for reinforcement of biomedical science and technology). At the same time, HISERBS had lasting effect on mobilization of Serbian biomedical science and technology resources. The HISERBS project was the basis for established communication with a number of institutions in Serbia of importance for public health (Ministry of Health, Republic of Serbia, Academy of Medical Sciences of Serbia, Republican bureau for health insurance of Serbia, Committee for Rare Diseases, Committee for administration of “Gleevec”, Republican bureau for health insurance of Serbia, the Serbian Leukemia Net (SELNET)).

HISERBS project activities were oriented to attract institutions from WBC with common interest to join the network (8<sup>th</sup> Balkan Meeting on Human Genetics, local and international meetings with participation of scientists from WBC, diagnosis performed for patients from WBC). The HISERBS project is a starting point for local and regional networking in the field of biomedical science and technology.

Additionally the realization of this project brought closer researchers, teams and institutions from Serbia to the European Research Area (ERA). Exposing researchers from Serbia to the latest developments in technology and science of EU and international cooperation trough this project, traced future mutually beneficial efforts and promote equitable partnership in research between EU and Serbia. Moreover, HISERBS partner institution, IMGGE, has joined FP6 Collaborative Project „European Leukemia Net“ (ELN) in 2008. It is the most significant European network involved in diagnosis, follow-up and management of leukemia. Thus, the main goal of HISERBS project, facilitation of integration of Serbian researchers in to ERA, was achieved.

The overall impact of activities enabled by HISERBS FP6 project is in finding the possibilities for further implementation of the Project objectives, dissemination of the knowledge, networking of local Serbian institutions and upgrading to EU standards.

Graphical presentation of completed tasks



List of realized Task from the 1-36 months:

T1 Common identification of activities, T2 Establishment of joint workgroups for specific activities, T3 Standardization of current methodologies, T4 Establishment and introduction of working protocols, T5 Establishment of common data basis, T6 Listing of laboratory diagnostic methods in use in Serbia, T7 Selection of novel laboratory diagnostic procedures for introduction/development, T8 Standardization and validation of the newly introduced laboratory procedures, T9 Determination of normal values of the selected markers for the population of Serbia, T10 Technology related training, T11 Visits of experts from EU partner institutions T12 Training of researchers in EU partner institutions, T13 Organization of conference with local participation, T14 Organization of conference with regional participation T15 Open day, T16 Creation of web-site. T17 Increase of public awareness through dissemination of leaflets, T18 Human potentials, T19 Equipment