PROJECT:
INSTITUTE OF SCIENCE AND TECHNOLOGY FOR CANCER CONTROL

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NATIONAL INSTITUTE OF CANCER MINISTRY OF HEALTH

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World and Brazil Cancer Situation

Cancer has been responsible for more than 12% of all causes of deaths in the world. The number of deaths from cancer in 2007 was 7.9 million people and will increase to 12 million around 2030. UICC (International Union Against Cancer) has estimated that in 2020 there will be 15 million of new cases in the world. Of these, 53% would be in developing countries, among them Brazil.

Global cancer load are rapidly increasing with a growth basically led by world population aging and by exposure to risk factors for cancer. This is a direct result of large global changes that occurred during the last decades, and which alter people’s health situation, determined by new life styles and new consumption patterns. The Brazilian National Institute of Cancer (INCA) estimates that in 2008 there will be 466.730 new cases of cancer in Brazil.

In 2004 there was a record of 141 thousand deaths in Brazil and SUS registered 423 thousand admittances and 1.6 million ambulatory appointments from cancer in 2005. Federal expenses in oncological care duplicated between 2000 and 2004.

Brazilian population has undergone intense demographic changes and an increasing fraction has been currently situated in an advanced age zone, exactly the most affected by most common forms of cancer. Factors related to our continental dimensions, with its evident regional differences, our complex ethnic character, as well as the industrial development and environment changes impact, make an approach for outbraving cancer in Brazil indispensable.

In this context, cancer has turned to be considered a public health problem, not only for the developed world, but especially for developing nations. Given the estimations, it is urgent that actions for cancer care attention have attained consideration among health area managers and researchers.
CANCER POLICY IN BRAZIL

The Ministry of Health, conscious of its role, established National Cancer Policy (PNAO, PT/GM n° 2439 from December, 05, 2005), which systemizes cancer as a public health problem, a disease that has been identified as the second cause of death in Brazil, despite the previous efforts for its control.

INCA, Ministry of Health’s office responsible for PNAO, claims that “cancer control strategies face problems that affect since the mechanisms of health policy formulation, until social mobilization, including organization and development of health actions and services, and, knowledge conception and diffusion activities” (MS/INCA, 2004).

PNAO, which is structured in state and regional nets, based on progressive health care line, has been trying to break the “pyramidal” organization, proposing an Oncological Care Net (RAO) arrangement, in its wider spectrum of cancer care.

To respond to this great challenge of health system in outbraving cancer increasingly incidence, there has been a need to join a research component to health actions sampling, well marked in Oncological Care National Policy. The same logic of Net organization has turned to occupy this scientific scenery, the focus of this present proposal, which articulates competences in different institutions and country areas, complementing the necessary performance in several aspects of natural history of the disease.

In health research promotion the amount of knowledge production that has applicability for the population as well as how much technological innovation and incorporation affect population health actions must be taken into consideration. Health research has lacked technology and innovation aggregation. To associate technology to science in public health, even though characterized as a great challenge, will bring a great impact in the conduction of knowledge production. Little industry participation in Brazilian research scenery and low number of patents compared with our scientific publication (Morel, 2005) have well expressed how much Brazil needs to invest in technology. The development of new drugs, particularly in our country, with ground flora and marine fauna richness may mean a differential in searching for cancer control.

Another aspect to be considered is technology evaluation aiming incorporation in SUS. Not always is the knowledge generated in other countries with ethnic and genetic differences suitable for the Brazilian population profile, what makes incentive
for evaluation of technological incorporation necessary. These considerations are well framed for drugs, on which the incorporation of technology generated and/or appraised in the country is fundamental for the optimization of public resources in health. It is necessary to qualify professionals, in the clinical research area, who can contribute in this context.

The MORE-HEALTH (MAIS-SAÚDE), program of growth acceleration of Brazilian Ministry of Health, in its axis 3, has considered the Health Industrial Complex, prioritizing financing for Technological Nets, Clinical Research Nets, Vivarium Nets of high performance and projects on border themes with impact on industry and health service. In this context, cancer has been considered and the program has pointed the creation of Research and Education Macro-Regional Units, coordinated by INCA. In elaboration process, this proposal is in line with this project, on which Researchers Net organization, where consolidated groups are associated to emerging groups, allows to evaluate and operationalize the embryo of these Units.

**INCA as Head-office institution**

Aiming better developing its role of executor and coordinator public office of cancer control of national policy in Brazil, the National Cancer Institute has the mission of performing integrated national actions for cancer prevention and control, through strategic vision of plenary accomplishment of its governmental role, contributing to improving population quality of life.

INCA (www.inca.gov.br), an institution with 70 years of existence, has been characterized as a regional and national reference-institution in oncological care, with 2,400 professionals fully concerned on cancer control. Initially created for performing care activities, it was over the years, incorporating to its profile prevention, research and education actions, which has enabled it to have an important role to influence the natural history of cancer in various stages of cancer development.

INCA organization structure comprises seven Coordinates, being the first four directly involved with institutional aiming activities: (i) Prevention and Surveillance (CONPREV), (ii) Research (CPQ), (iii) Assistance (OACS), (iv) Education and Scientific Dissemination, (v) Human Resources, (vi) Administration (COAD), (vii) Strategic Actions (COAE), and a Communication Division (DCS).
INCA tradition as an education and research institution has been always associated with cancer area care, turning, from Constitution of 1988 and Health Organic Law promulgation, to cover the whole care line, with Health promotion, Prevention, Diagnosis, Treatment and Palliative Cares in cancer.

In this group, appearing as an element that unifies and moves forward on all levels of care line, the quality of management in health has come as another preponderant factor for cancer control. To reach countless dimensions of cancer management in Brazil, when articulating Oncological Care Net, INCA has adopted as strategy the actions and products diversity, in order to enlarge its coverage, to reach a maximum of territorial extension, pursuing to reach all types of environment, equipped or not, with technological resources. This is INCA’s technical areas nature, which operate in synergy: education, research, assistance, prevention/surveillance and management.

Prevention and Surveillance in Cancer

Besides performing epidemiological research on cancer, INCA is responsible for implementing Control, Prevention and Surveillance actions, coordinating activities of:

- Population-based Cancer Registry, Hospital-based Cancer Registry and Estimative of Cancer Incidence in Brazil.

- National Program and Iberian-American Network for Tobacco Control.

- EXPANDE Project for High Complexity’s Centers installation in cancer care in Brazil.

- Brazilian Control Program of Cervix Cancer and Breast Cancer

- Quality Assurance Program in Radiotherapy.

Cancer Care at INCA

Multi-professional assistance, specialized in treatment of patients with malignant neoplasia and correlated disorders, has five units: Cancer Hospital I (HC I), Cancer Hospital II (HC II), Cancer Hospital III (HC III), Cancer Hospital IV (HC IV), and Bone Marrow Transplant Center (CEMO). The responsible area plans and
directs the activities of this hospital units' complex, in aspects of regulatory, technical standards, invoicing, humanization programs, and hospital accreditation.

Numbers of care for cancer patients at INCA show the extent of these actions; in 2006, there were 7,200 new enrollments, 254,502 ambulatory appointments, 15,119 admittances, 11,795 surgeries, 35,966 chemotherapies, 160,407 radiotherapies, and 83 bone marrow transplants (INCA Annual Report, 2006 – www.inca.gov.br).

Bone Marrow Transplants Center, besides performing research through Hematological Neoplasias and Bone Marrow Transplant scientific program, bases the Brazilian Registration of Bone Marrow Donors – REDOME, the Brazilian Registration of Bone Marrow Receivers – REREME, and centralizes the consultations to bone marrow donors’ international banks. The Bank of umbilical cord and placental blood (BSCUP) was the first Brazilian public bank, integrating the Brazilian Network of Umbilical Cord Blood Banks –BRASILCORD, which is coordinated by INCA.

In the hospital units, activities of specialized human resources formation and clinical research are developed, notably clinical trials for various tumor types. Nowadays, 63 clinical studies on head and neck cancer, lung cancer, gastrointestinal cancer, genitor-urinary cancer, breast cancer, paediatric cancer and hematological cancer are in progress.

Research at INCA

In research actions on cancer, INCA acts as an active agent for Production of Knowledge and Human Resources Training in Research, besides performing activities as Policy Formulator and Research on Cancer Forwarder.

INCA Human Resources policy instituted Science and Technology Careers Plan (Law 8,691/1993), which created the specific career of researcher, which besides enabling the admission of health professionals integrally dedicated to knowledge production, allows functional ascension through degrees and productivity.

Institutional planning of research activities at the National Institute of Cancer (INCA) has been accomplished by Research Coordination where the research institutional priorities regarding the following topics are established: (i) strategic research lines for the Institution, (ii) researchers incorporation, (iii) resources reception, (iv) partnerships and external cooperation, (v) support to lato sensu and
strictu sensu Post-graduation activities and (vi) policies of researches scholarships, in a joint action with Education and Scientific Dissemination Coordination.

INCA has a set of 28 research groups registered in CNPq Lattes Platform, organized in 10 Scientific programs (Table 1) and members of Molecular Diagnosis Networks of Rio de Janeiro/Faperj and Brazilian National Network of Clinical Research.

**INCA Scientific Programs**

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<tr>
<th>Genomic Counseling</th>
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<td>Cellular Biology</td>
<td>João Viola</td>
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<td>Pharmacology</td>
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<td>Oncohaematology</td>
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<td>Experimental Medicine – Immunology</td>
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<td>Oncologia e Hematologia Pediátricas</td>
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<td>Clinical Research</td>
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**TABLE 1:** INCA’s Scientific Programs with their respective coordinators.

Research model at INCA (Figure 1) focuses on technical-scientific and technological innovation in cancer development, pursuing to contribute to increase patient’s survival and quality of life. Based on research priorities establishment, it is orientated by epidemiological data relative to cancers that more affect Brazilian health system. Acting in basic, translational, clinical and epidemiological areas, through their different research groups, INCA also aims to establish research/assistance integration, to interact with national and international researchers’ groups and to actively contribute for researchers’ formation with technical capacity and critical analysis on Brazil and World cancer situation.
Figure 1: INCA’s research model. The scheme includes targets, actions and strategies for knowledge production and human resources formation on oncological research.

INCA’s research infrastructure involves a complex of research laboratories, with high technology equipment, organized in multi-users structures, namely: Genomics Unit, Proteomics, Bioinformatics, Optical and Electronic Microscopy, Tumors and DNA Bank with Molecular Pathology Laboratory and an Animal Resource Center for experimental research on cancer.

Brazilian Tumors and DNA National Bank (BNT) has been working since 2006 as a structure that makes possible for tumors and normal samples pairs to be collected and stored, besides blood of patients with the most prevalent tumors in Brazil, aggregated to an epidemiological questionnaire, which enriches the sample quality. Based on ethnic and scientific criteria for users supplying, it has, at this moment, 5,800 organized samples on computerized system. A Brazilian net organization of tumors bank is now on expansion process.

INCA’s Animal Resources Center comprises a 300 m² structure, divided in 3 connected areas, each of which with environments for breeding and production, maintenance, quarantine and animal experimentation, including experimental
surgery. A bank of experimental animals’ embryos and a NB3 Laboratory for experiments on gene therapy is being elaborated with resources collected in 2008.

The Clinical Research Division comprises an extensive infrastructure for following-up, monitoring, and management of clinical trials performed at INCA, and it’s a member of Clinical Research National Network.

The Committee for Ethics in Research allows the ethical and scientific quality of the studies developed in the institution.

As a formulator of research on cancer policy, INCA organized the research on cancer priorities seminar in 2005, in a joint action with the Science and Technology Department/MS, and CNPq, which created a funding edictal for neoplasias with values’ resources of 6.3 million, on which 81 researchers were supported (edictal ST-CNPq/MS-SCTIE-Decit, 06/2005). As a deployment, INCA is organizing a seminar for this edictal results evaluation, at the end of 2008.

As forwarder, it attended PPSUS, in 2006, in Rio de Janeiro, in a joint action with Decit/MS and Faperj, financing the Molecular Diagnosis on Cancer Net in RJ. Similar initiative should happen in 2008/2009.

**Education and Scientific Dissemination at INCA:**

The Education and Scientific Dissemination Coordination is responsible for elaborating the policies on fields of human resources training and qualification for work and dissemination of technical-scientific knowledge, in national sphere, oriented to cancer control in Brazil.

INCA believes that production of knowledge on cancer is only likely to be achieved by means of adhesion to permanent education strategy in a full manner, incorporating its logic in the very structure of oncological care network, in its different levels of approach, from prevention to research.

**Education in Lato Sensu Level**

The approach has been based on integral care quality and on SUS’ orderer principles. From this point of view, education and work articulation must guide oncological care training and organization, committed to the conjunction of care quality and population’s needs.

Education forms: Residency, Specialization, Improvement, Qualification, Updating, Curricular and Academic Traineeship, and Observation Visits. In its organization, four education areas are articulated: technical, medical, nursing and of large areas of health education as: Psychology, Social Service, Dentistry, Hospital
Pharmacy, Medical Physics, Nutrition, Clinical Pathology, Clinical Engineering, and Physiotherapy.

The courses of Specialization for Nursing in Clinical Research on Oncology and Qualification of Management for Oncological Care deserve prominence for their innovation before other education and research institutions. Inedited in Brazil, the first course focuses on forming in national level, nurses for acting in oncological clinical research. The course qualifies in ethic aspects on Clinical Research, management of data, clinical research fundamentals, and fundamental concepts of scientific methodology, epidemiology, biostatistics, and translational research.

The Qualification of Management for Oncological Care course is a national extent project, aiming to contribute for cancer concern insertion in managers’ work line of different spheres of Unique Health System (SUS). The courses are structured on the work of small tutorial groups in which Intervention Projects are developed in relation to health management of one’s place/area. The project considers the formation of “Decentralized Centers of Management Qualification” when the student returns to his work place and (re)organization of the loco-regional oncological care.

**Education in Strictu Sensu Level**

INCA’s Post-Graduation Program on Oncology (PPGO-INCA) (http://pgoncologia.inca.gov.br) aims to educate highly qualified professionals, in a critic and innovative way, in Master and Doctorate degrees, to develop research, education and cancer technological development activities.

Annually offering 20 positions for Master’s degree and 15 for Doctorate’s degree, PPGO-INCA has count with 22 permanent lecturers and 10 collaborators, being structured in 3 concentration areas, 8 research lines and 23 research projects, as follows:

**Concentration Area: Clinical/Epidemiological**
- Research lines: *Clinical Research on Neoplasia*
- Research lines: *Tumors Epidemiological Research*

**Concentration Area: Translational**
- Research lines: *Pharmacology and Pharmacogenomics*
- Research lines: *Bone Marrow Transplant and Cellular Therapy*
- Research lines: *Translational Molecular Research*
Concentration Area: Basic

- Research lines: *Molecular Biology of Neoplasias*

- Research lines: *Cellular Biology of Neoplasias*

- Research lines: *Development Mechanisms of Tumors*

Qualified production of this program in 2007 showed satisfactory indexes, with 45 complete articles in international circulation scientific journals. From these articles, 12 had student’s participation, besides 2 student’s participation from Capacitance Program. From permanent lecturers, 12 published 3 or more Qualis A articles, 50% have a Productivity Scholarship from CNPq and 36% have Post-doctorate degree.

Now classified as concept 5 by CAPES, PPGO-INCA has instituted interaction policies with other post-graduations courses with concepts inferior than ours and located in areas with less scientific density or far from the great research centers, allowing exchange between students and researchers for training on advanced technologies existents at INCA. In 2007, INCA was awarded by PROCAD Edictal from Capes (01/2007 – Oncological Care Program) with R$ 250,000 to aid superior education level personnel of Post-Graduation Maternal-infantile Health Programs of The Federal University of Maranhão.

We currently organizing the implementation of the program of Doctorate degrees post-Medical Residency Program, in a logic of linkage between Lato and Stricto sensu that will allow greater interaction between assistance and scientific development in cancer.

**Program of Capacity and Training on Cancer Research**

This is a singular program in researcher training on oncological area, in which graduation and master’s degree students from Health and Biological Sciences areas are inserted in Research groups linked to PPGO-INCA through Improvement Stage I (for graduates) and II (for masters), for a maximum period of 18 months. Currently, 27 trainees are being financed with INCA’s scholarships. Another special program is the Training in Research after the Medical residency Program. This is also a strategy of interaction between assistance and research for the training of professionals in oncology, who remains more one year in INCA as a trainee in research laboratories.
Scientific Initiation Program

The Scientific Initiation Program has a strong involvement with undergraduated students from public and private institutions, who perform training at INCA’s research laboratories under guidance and supported by CNPq and INCA’s scholarships (PIBIC program), totaling in 2008, 61 scholarship students.

INCA’s Scientific Initiation Meeting has been annually performed, when the studies are evaluated by a commission with internal and external evaluators.

Scientific Dissemination and Knowledge Management on Cancer

Actions of scientific dissemination on cancer at INCA comprises the management of the Libraries Integrated System, which totals four libraries at INCA’s different units, and availability of information on cancer in its more several means, as:

− Creation of the Thematic Area “Cancer Control” of Virtual Health Library of the Ministry of Health – BVS/MS (www.saude.gov.br/bvs/controlcancer), available for Brazil and Latin America, constituting a new channel of produced information representation and dissemination for Institute for Research on Cancer members.

− The Publication of Brazilian Journal of Cancerology – it is the official body of INCA’s scientific dissemination, indexed to LILACS basis and available in CAPES’s Journals Portal, with multidisciplinary approach and quarterly publication; available on printed and digital versions at INCA’s website.

− Thematic and Microthesaurus glossary on cancer and oncology – it contributes for standardization and improvement of the language used by Ministry of Health in federal sphere of Unique Health System, specifically aiming to disseminate the terminology on cancer and oncology and to structure indexation and recovery of information on this theme, turning communication and this language understanding more effective among peers.

Knowledge management also comprises platforms development through information technology, enabling databases systematization, providing subsidies for decision-making and cancer control actions prioritization, involving managers and researchers. At the beginning of 2008, INCA acquired a Business Intelligence (BI) tool, which allows managerial reports creation with different visions and formats, accelerating Priorities Establishment and Decision-making Process. At INCA, this
tool will promote a data analysis in areas such as Planning, Controller, but especially for Prevention actions, in the same data repository, from information crossing for global management. The user will be able to have autonomy to create one’s own reports based on operations performed on databases maintained by INCA. In the scientific research field, CNPq’s Lattes platform allows the delineation of a registered research groups’ mirror, but without specific view and profiles to each knowledge area. In this project, we see the possibility of developing a platform for abilities of research on cancer in Brazil identifivation.

**Communication and Cancer**

INCA's communication policy has leaned over establishing new guidelines of actions based, among other factors, on qualitative research, which will guide communication strategies for cancer control, important component of the Oncological Care Policy.

Focusing on this, there are now four projects in progress:

- Patient’s information level on cancer actions in SUS
- Research on Brazilian People Conceptions on Cancer
- Cancer in Media: A Public Health Matter – its evolution in the past 10 years

**The Project**

To face the impact on the increasing demand of cancer approach, nothing is more urgent than a policy faced to knowledge production and training of human resources in oncological area, besides the dissemination of scientific knowledge in this area.

The great challenge for the coming years is to establish national oncological research network logic, pursuing to identify emergent groups in areas in which research on cancer is incipient and allowing the involvement of national critical mass of researchers on health in cancer matters. From generated products in each one of the projects established in the networks, it is proposed to advance in cancer approach, not only contributing to the evaluation of actions previously established, but also by proposing methodologies that can more consistently contribute, for
prevention and diagnosis, optimizing the cost-effectiveness relation and improving prognosis and treatment, providing greater security and survival for patients.

INCA together with researchers from UFRJ, UERJ, FIOCRUZ, PUC-RS, UFRGS, UFPB, National Museum-UFRJ, UNIFESP, Mogi das Cruzes University, São Carlos University and international collaborators, has proposed to constitute a structure of scientific interaction (Figure 2) focused on contributing to effectively influence the cancer natural history course in Brazil.

**Institute of Science and Technology for Cancer Control: interactions**

Cancer care allows a range of interventions, which involves epidemiological, basic, translational and clinical research; in this sense, this proposal comprises a multidisciplinary and multi-user project turned to scientific knowledge production and human resources training on oncological research, also contributing to progress in knowledge and communication on cancer management, through the following objectives:

Figure 2: The internal circle represent the head quarter Institution where interactions amongst all the groups already exist and are represented by the net. Outside the circle are the extramural institutions and the colors represent a few of the existing interactions.
**General Objective:** To contribute for technical-scientific development on cancer, assisting to oncological care demands in Brazil, through the strategy of network formation, able to confer synergy to knowledge production, human resources formation, publication of scientific knowledge and its application to society and government.

**Specific Objectives:**
1. To study oncogenesis mechanisms, tumoral establishment and metastasis, through cellular, molecular, biochemical and immunological tools and tumoral microenvironment modulation.

2. To study gene-environment interactions for breast and cervix cancer, melanoma, and infantile leukemia.

3. To study biological markers for diagnosis, prognosis, and therapeutic response for solid tumors (esophagus, breast, prostate, retinoblastoma) and leukemia.

4. To establish a structure for the development of new antineoplastic drugs, through multidisciplinary approach.

5. To test synthetic products with antineoplastic potential.

6. To perform clinical trials for new diagnostic and therapeutic strategies incorporation on cancer assessment.

7. Epidemiological evaluation of cervix cancer, focusing on prevalence of HPV estimation through specific type, identifying the factors associated to progression risk or precursory lesions and tracing cost-effectiveness study.

8. The impact of the national actions for tobacco control through epidemiological studies.

9. To extend the training of human resources on research, covering the different approaches of cancer care.

10. To elaborate platforms of knowledge on cancer management to subsidize priorities establishment and decision-making on oncologic area.

11. To produce knowledge to subsidize the communication policy on oncologic care.
12. Amplify the scientific interactions, in the perspective of evaluating and operationalizing the implementation of Macroregional Units for education and research in cancer.

The Institute of Science and Technology for Cancer Control has a general structure as represent in the Figure 3. As strategy in the project, an emerging research group with medium sized equipment will be incentivated. The priority was achieved by the group from PUC-RS with the purchase of a cytometer.

Institute of Science and Technology for Cancer Control: general project structure

Figure 3: The project uses 4 different themes (middle boxes) to study three different groups of cancers (top box) and generate 4 diferent products (bottom box).

The C&T Institute on Cancer will have an organizational structure (Figure 4) constituted by a coordinator, Hector Seuanez, INCA’s researcher, 1A of CNPq, counseled by four researchers of different participant institutions, to wit: Francisco Sampaio, from UERJ, 1A of CNPq; Wilson Savino, FIOCRUZ, 1A of CNPq; Vivian Rumjaneck, UFRJ, 1C of CNPq; and Eliana Abdelhay, INCA, 1c of CNPq. The international consultant chosen to follow the project will be Pierre Hainaut, from IARC, responsible for the molecular carcinogenesis cluster (www.iarc.fr).
Institute of Science and Technology for Cancer Control: working structure

Coordinator

Executive-financial Secretary

Board of Managers

Consultive Council

External Council

Research and Scientific Production

Qualification and Development of human resources for research

Information & Communication management

Tumor cell biology

Cancer Epidemiology

Biomarkers for Diagnostic, Prognostic and treatment

Development of anti-tumoral agents: experimental, pre-clinical and clinical assays

Courses: Distance Semi-presencial Lato sensu and Stricto sensu levels, Managers training

Communication and Knowledge management's on Cancer

Figure 4: Organizational structure of the Institute.
THEME 1: TUMOR CELL BIOLOGY

Tumorigenesis involves cellular changes that lead to loss of proliferation and cell death control mechanisms. After losing control over cell division and/or death, malignant-cell population turns into a cell mass which is too big to get nutrients through simple diffusion mechanisms. In this context tumor-mediated neoangiogenesis and micro environmental changes are critical to keep tumor growth. At one side new blood vessels supply malignant cells with nutrients and at the other side the disfunction of cell adhesion mechanisms and alteration of cell migration lead to metastasis. Even in the case of hematological tumors, the formation of tumor mass often occurs, as well as inadequate migration, leading to unwanted manifestations.

Malignant cells do not always localize at immune-privileged sites. During all described phases of tumorigenesis, the immune system can be in contact with the tumor cell but seems not to respond to nor recognize it in an efficient way. It may respond, but is often downmodulated by tumor-bearing or tumor-micro-environment products.

This sub-project aims to focus on oncogenesis, tumor establishment and metastasis and on the possible mechanisms of tumor intervention, using immunologic and micro-environment modulatory tools.

Subproject 1: Oncogenesis: regulation of tumor development

Rational

Oncogenesis and further illness appearance are associated to several genetic, environmental and personal conditions. Obesity and inflammation, for instances, represent important risk factors for the development of some cancer types, although being poorly understood. Obesity-associated inflammation is probably an important feature, considering its increasing relation to tumor development. Intracellular lipid accumulation into lipid bodies has already been demonstrated in colon cancer cells, as well as in epithelial H-Ras-transformed cells (Accioly et al., 2008). Lipid bodies are dynamic and functionally active organelles playing new functions, their intracellular compartments being involved not only in eicosanoid formation but also in the storage of cytokines, kinase-proteins, GTPases and enzymes responsible for lipid
metabolism. These suggest that lipid bodies play a broad role on cellular signaling, cell-to-cell interaction and on the cellular lipid metabolism (Bozza et al., 1996; Yu et al., 1998; Yu et al., 2000; Brasaemle et al., 2004; Wan et al., 2007). We have demonstrated that leptin produced by fatty tissue activates the PI3K and mTOR pathways in macrophages, thus modulating lipid metabolism and formation of lipid bodies (Maya-Monteiro et al., 2008; Maya-Monteiro e Bozza, 2008). PI3K and mTOR are also involved in cellular proliferation and may contribute to give light to the relationship between lipid bodies and cancer. We also intend to focus on other proliferation control pathways, proposing that the nuclear factors of activated T-cells (NFATs) display an oncogenic and suppressor role. Recently, we have shown that gene promoters responsible for the regulation of cell cycle and cell death display binding sites for NFAT proteins, thus playing a central role in the control of cellular differentiation and transformation (Viola et al., 2005). We have also demonstrated that NFAT1 downmodulates cell cycle and represses A2 cyclin gene transcription (Caetano et al., 2002; Carvalho et al., 2007). Moreover NFAT1 protein seems to restrain proliferation by inhibiting CDK4 kinase expression and inducing p21 expression (Santini et al., 2000; Baksh et al., 2002). On the other hand, over expression of NFAT2 induces cellular proliferation and the increase of cyclins and c-Myc proto oncogene expression, thus demonstrating that this protein is a cell cycle activator (Neal and Clipstone, 2003). We have some results suggesting that NFAT proteins may display a dual effect on proliferation control and cell death, where NFAT1 protein could be a tumor suppressor gene and NFAT2 protein, an oncogene, both playing a central role in cellular transformation (Robbs et al., 2008).

Beyond the loss of proliferation and cell death control, misrule of cellular differentiation can sometimes lead to malignization, as occur in some cases of hematopoietical malignancies. In the particular case of platelet formation, megakariocyte differentiation follows an orchestral pathway that initiates by the megakarion formation and ends at final fragmentation. A candidate molecule mediating in megakariocyte differentiation is acetylcholinesterase (AChE). AChE can be expressed in non-cholinergic tissues and in several hematopoietic cell types, like erythrocytes and megakariocytes, thus suggesting a putative non-cholinergic function to this enzyme beyond its classical role on the ending of acetylcholine action. We have recently demonstrated a role for AChE-R isoform on MKs lineages maturation (Guimarães-Sternberg et al., 2006). We also showed that transgenic mice
overexpressing this variant display normal levels of granulocytes, monocytes and lymphocytes and high levels of platelets, thus reflecting the selective thrombocytic effect of AChE-R and its regulatory role on this cell type differentiation (Pick et al., 2006).

**Objectives**

1. Investigate lipid and proteic heterogeneity of lipid bodies in neoplastic cells;
2. Investigate the role of lipid bodies as a “bridge” organelle between lipid metabolism and inflammation;
3. Investigate leptin effect on altering lipid metabolism, lipid bodies formation and cellular proliferation of intestinal epithelial cells;
4. Investigate the putative activation role of leptin intracellular signaling pathways over the PI3K-Akt-AMPK-mTOR-mediated pathways in cellular proliferation of intestinal cells;
5. Assess interactions between lipid bodies and proliferation and cell death pathways;
6. Identify putative genes differentially regulated by NFAT1 and NFAT2;
7. Ascertain if lymphocytic lineages are more susceptible to NFATs-mediated transformation than other ones;
8. Correlate NFAT1 and NFAT2 expression with therapeutic follow up and survival of patients;
9. Analyze the role of JAK/STAT pathway on megakaryopoiesis, considering that it seems to modulate AChE expression;
10. Assess AChE role on AML-M7;
11. Assess regulation of AChE variants expression by transcription factors of NFAT family during megakaryocyte differentiation;
12. Clarify AChE-S ubiquitination and degradation in the process of MK differentiation.

**Experimental approach**

Analysis of differential gene expression during cell cycle and apoptosis will be assayed by microarray and protein analysis, named NFAT1, NFAT2 and proteins involved in lipid bodies biogenesis and regulation, will be performed with different
tumor samples from patients by tissue microarrays and immunophenotyping. Selective expression of NFAT1 and NFAT2 proteins will be investigated in genetically modified mice by conditioned deletion/expression (Lox-Cre) and through the constructions of their constitutively active molecules (CA-NFAT1 and CA-NFAT2).

AChE activity will be determined in plasma of patients carrying the studied diseases and of control individuals by the Ellman reaction (colorimetric method). Real time PCR will be used to validate results from microarray assays and to quantify different AChE isoforms in blood and bone marrow samples.

The projects dealing with human samples have been submitted to and approved by the Research Ethical Committee of the National Cancer Institute (INCa).

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**Subproject 2: Angiogenesis, metastasis and tumor microenvironment**

**Rational**

Tumor cell establishment, nourishment and further metastasis enclose angiogenesis (from proliferation of pre-existing vessels or incorporation of endothelial progenitor cells – EPCs – from bone marrow), adhesion of tumor cells and their transmigration throughout endothelium to reach the blood stream. The tumor itself supports the formation of new vessels and modifies the extracellular matrix favoring this process to occur. We have shown that the heparin binding N-terminal domain (HBD) of TSP-1 displays angiogenic properties *in vitro* and *in vivo*, in opposition to the whole molecule that is angiostatic (Bernstein et al, 2002, Nunes et al, 2008). We also assessed TN-C role on angiogenesis regulation. Curiously, gliomas have high TN-C content and display high vascular density, although these vessels are poorly junctional and hyper permeable (Jain, 2007). We observed that the EGF-like domain of TN-C from glioma matrix do generate highly proliferative endothelial cells, although these do not organize themselves in tubular structures. Both proteins (TSP-1 and TN-C) interfere in adhesive functions of sindecan-4, which can turn into a new target to therapeutic interventions on angiogenic process. Studying gliomas will bring us important information about angiogenesis control, but not about metastasis-related phenomenon, since gliomas are non-metastatic tumors.

As a first event, before metastasis onset, cells loose their anatomic organization, lacking polarity (in the case of epithelial tumors) and tight contact with
neighboring cells. The apical junctional complex (AJC) is responsible for the maintenance of epithelial cell-to-cell adhesion (Balsa and Matter, 2003) and is composed by tight junctions (TJ) and adherent junctions (AJ) (Nelson and Nusse, 2004). Both structures are disrupted on the epithelial-mesenchymal transition (EMT), which occurs during normal development and also during the beginning of epithelium cancer (Thiery and Sleeman, 2006). Several studies suggest that this event and tumor progression involve EGFR activation (Kovacs et al. 2002), as well as other things like altered expression and post-translational changes of junctional proteins (Bates and Mercurio, 2005; Tobioka et al. 2004; Oliveira et al. 2006). On the other hand, GTPases of the Rho family (Rho, Rac and Cdc42) and protein kinases have been implicated in CJA disruption, as well as in the organization of actin cytoskeleton (Gonzáles-Mariscal et al. 2008). However both cellular mechanisms responsible for modulation of these events have been studied separately, thus lacking clarification if a putative correlation between them exists, in order to mediate epithelial cancer regulation by CJA and actin cytoskeleton.

Another step towards metastasis establishment is malignant cell transmigration throughout vascular endothelium, now showing alteration of its adhesion mechanisms to tissue. Interference on these processes, by inhibition of tumor cells adhesion to endothelium by natural molecules with heparin-like activity, such as polysaccharides from marine invertebrates (Borsig et al, 2007) or snake venom, has been partially described (Cominetti et al, 2004; Ramos et al, 2007 and 2008) and represents a promising therapeutic tool.

Another matter to consider in relation to cellular migration, although not always related to metastasis, is the microenvironment where tumor establishment and growth occur. For instance, patients with LL-T evolve with mediastinal mass, whereas LLA-T patients may show it or not. Besides, LLA-T involves bone marrow commitment differently from LL-T, although both leukemias represent malignization of T cell progenitors. Distinct localization sites may be related to molecules involved in adhesion/migration of lymphocytes in thymus. Sphingosin-1-phosphate receptor 1 (S1P1) play a critical role on migration and mainly on thymocytes evasion from thymus to peripheral lymphoid organs. Low levels of S1P1 agonists, sphingosin-1-phosphate (S1P – physiologic agonist) and FTY720 (synthetic agonist), are able to inhibit thymocytes evasion (Rosen et al., 2003) and also T cell precursors lacking S1P1 can differentiate normally, but are not able to leave the thymus (Matloublan et
Thus molecules responsible for cell adhesion may be site-related and there is also evidence for the involvement of some of them in proliferative processes. APRIL (A proliferation inducing ligand) belongs to TNF family, stimulates tumor growth and binds to sulphated proteoglycans found on cellular surface. We have shown that transgenic mice for APRIL develop hyperplasia prone to malignancy similarly to LLC in humans. Also, APRIL transgenic mice show accumulation of cells in lymphoid organs such as spleen, mesenteric lymphonodes and Peyer patches, with infiltration of neoplastic cells in kidneys and liver (Planelles et al., 2004) related to B1 cells increase. These results lead us to some questions about molecular features involved in establishment and localization of tumor masses.

Differently from APRIL-induced hyperplasia which can turn into a malignancy, HTLV-1 infection can generate adult T-cell leukemia without evident and acute proliferative phenomenon. This same virus can induce a neurological commitment (HAM-TSP) dependent on infected T lymphocytes. Migration of infected lymphocytes to peripheral nerves or their arrest in the lymphohematopoietic system can be hypothetically explained by differential expression of specific adhesion molecules by infected cells. We have preliminary results showing differential expression of semaphorin 3 A receptor (neurophilin 1) in HTLV-1-infected cells. Besides, adhesion properties to extracellular matrix proteins depend on the infected cell lineage, suggesting a putative differential modulation of the involved molecules during adhesion and migration processes.

The investigation of cellular interactions explores molecular modeling techniques involving the use of computational methods, based on three-dimensional structures of the participating molecules. The combination of comparative modeling, molecular docking and molecular dynamics techniques enables a detailed analysis of molecular mechanisms as well as the development of potential agonists or antagonists, which may change into therapeutic compounds to be used in treatment of metastatic processes, particularly in leukemia and lymphoma migration.

**Objectives**

1. Assess mechanisms of sindecan-4 action in response to TSP-1 and their structural domains bearing angiogenic activities in mature and endothelial cells progenitors;
2. Assess the role of TN-C from tumor matrix on the modulation of endothelial cell cycle and angiogenic differentiation, using glioma models;
3. Assess EPCs recruitment and angiogenic modulation by tumor factors, in order to identify new molecular targets and develop adequate approaches to block key stages of tumor grow;
4. Identify cell signaling pathways involved in the lack of cell-to-cell adhesion and correlate theses events with EMT acquisition and tumor progression in colon-rectal cancer;
5. Identify alteration in actin cytoskeleton and correlate these events with EMT acquisition and tumor progression in colon-rectal cancer;
6. Investigate if the molecular changes found in CJA can be tracers and promissory candidates for therapy against tumorigenic phenotype in epithelial cancer;
7. Assess mechanisms involved in tumor cells-endothelial cells interaction, using breast and prostate tumor models;
8. Characterize integrin expression of β1 family in human tumor cell lineages infected or not by HTLV-1 and their adhesive and migratory capacity facing extracellular matrix components;
9. Assess the expression profile of neurophilin 1 and semaphorin 3A receptor in HTLV-1-infected cell lineages and analyze the putative modulatory role of HTLV-1 on the adhesion and migration of infected and non-infected T cell lineages, under semaphorin 3A stimulus;
10. Analyze the putative modulatory role of semaphorin from HTLV-1-infected or non-infected tumor cells on adhesion and migration of normal T cells;
11. Analyze S1P1 and S1P1 ligands role on human T cells development, the role of these molecules in interactions between thymocytes and thymic microenvironment (including other molecules-mediated interactions) and also in cell death, proliferation, cell cycle and cell migration;
12. Analyze S1P1 and S1P1 ligands role on LLA-T and LL-T development and a putative relationship between their molecular expression and preferential localization of tumor cells;
13. Investigate the involvement of chemokines, their receptors and ECM receptors in peritoneal B1-cells migration in APRIL-transgenic and control mice;
14. Investigate the involvement of chemokines and ECM molecules in tumor mass formation in mesenteric lymphonodes and Peyer patches in APRIL-transgenic and control mice;

15. Built molecular models by comparative modeling of integrins involved in the described processes, such as α4β1 (fibronectin receptor), α6β1, α3β1 and α6β4 (laminin receptors) complexes and do molecular docking between peptidic inhibitors and the proposed three-dimensional models. New potential inhibitors will be then constructed and functionally tested using distinct models.

**Experimental Approach**

Human umbilical vein endothelial cells (HUVECs) and EPCs isolated from peripheral blood will be used in the following assays: tubulogenesis (three-dimensional culture on fibrin gel and matrigel); endothelium-tumor interaction (static and dynamic conditions; shear stress; transendothelial migration in culture systems with porous insets; cell interactions with native matrixes: tumor x subendothelial matrix and endothelium x tumor matrix).

Tumor cells: breast (MCF-7 and MDA-MB-231) and prostate (PC-3 and DU-145) carcinoma, glioma (astrocytoma U373 MG and multiform glioblastoma (MGB) lineages), human colon adenocarcinoma (Caco-2, HCT-116, HT-29); human lymphocytes lineages (CEM, JURKAT, MOLT4); HTLV-1-infected human lymphocyte lineages (CIB, HUT, C91PL) and human thymic lymphoma lineage SIL-ALL.

RNA silencing will be performed in endothelial cells in order to decrease sindecan-4 expression and in glioma cells, to decrease TN-C expression. The same technique will also be used with extracellular matrix proteins and their receptors.

Angiogenesis model: matrigel implants containing different angiogenesis modulators and/or blocking antibodies or peptides will be used.

Tumoral angiogenesis/vasculogenesis model: EPCs transfected with luciferase gene will be injected into tumor-bearing nude mice. Incorporation of cells in tumors will be monitored following intravenous injection of luciferin. For microvascular density analysis, matrigel implants and experimental tumors will be analysed by standard immunocytochemical techniques.

CJA and its signaling pathways will be assayed using CJA-disrupting agents: TPA, EGF, PGE₂, LiCl₂, (LPA and calcium depletion) and specific inhibitors of distinct
signaling pathways (MAPK, EGFR, Src, PI3K, PKA, Wnt, ROCK), and also specific antibodies against these molecules.

Overexpression of molecules identified as key agents in CJA signaling pathway, and molecules of the CJA itself, will be performed in order to confirm the observed results.

Proliferation, migration and invasion assays will be analysed by BrdU incorporation and flow cytometry, wound healing and matrigel, transwell migration and immunocytochemistry.

Distinct experiments will be monitored using conventional and confocal immunofluorescence microscopy, immunoprecipitation and immunoblotting. Cryofracture, electronic microscopy and measurements of transepithelial electrical resistance will also be performed.

Subproject 3: Immunological Therapies

Rational

After malignant transformation tumor cells are still capable of evading from immune response. The presence of specific tumor antigens is a rare event, and when present, does not often elicit an efficient response (reviewed by Willimsky e Blankenstein, 2007). Even when specific tumor antigens are presented, tumor cells had evolved escape mechanisms that prevent recognition (lack of presentation of the tumor antigens against which individual's tolerance is not operant) or the tumor itself inhibits immune response. A typical situation exists where the immune response is extremely efficient against tumor: the transplant of allogeneic hematopoietic progenitors. This represents the very first form of cellular therapy, with the first successful transplant dating 40 years ago. Besides being an efficient anti-tumor treatment, it brings along an unwanted collateral effect that is the Graft Versus Host Disease (GVHD). This disease is responsible for a morbidity/mortality of 70% of patients carrying malignancies and submitted to transplantation (Goker et al., 2001). Unfortunately graft versus tumor (GVT) effect is closely related to GVHD (Sehn et al., 1999) and the elimination of lymphocytes causing disease eliminates the desired anti-tumor response (Huff et al., 2003). We have been working on two therapeutic
strategies: one directed against the mechanisms of tumor escaping and other related to the elimination of GVHD.

Tumors potentially express selective immunological targets, but the majority of them can still escape from immune destruction. We must be able to generate long term or memory responses against tumor antigens. Since dendritic cell (DC) is the main cell of innate immunity capable of eliciting T cell response, it has been used as a tumor “vaccine” with some encouraging results in pre-clinical and clinical studies. In spite of extensive investigation, little is known about dendritic cells physiology and how can they be modulated by tumor microenvironment. In human cells, stress situations increase DC activity as well as expression of ABC transporters. The latter are involved in differentiation, activation and migration of dendritic cells (Randolph et al., 1998; Robbiani et al., 2000; Schroeijers et al., 2002; Pendse et al., 2006 and Van de Ven et al., 2006), thus representing potential targets to “vaccines” development with human cells. Using an animal experimental model, we created a system to assess DC activity, where CD8+ and CD4+ T cells are evaluated in response to antigen presentation by activated DCs. Our preliminary results show tumor antigens presentation by DCs in lymphonodes (LN) with moderate co-stimulation (CD86), which leads to specific CD8+ and CD4+ T cells proliferation and their differentiation into memory cells. In spite of this, these cells are not able to prevent tumor growth. Introducing lipopolyssacaryde (LPS) in the system, CD86 expression by DC and T cell proliferation increase, but tolerance reversion and tumor clearance only occur after adjuvant administration in a very specific time gap that is immediately before tumor growth burst.

Considering GVHD elimination, we have demonstrated that granulocytes from donors of G-CSF-treated hematopoietic progenitors for 5 days show an inhibitory activity against T lymphocytes in vitro (Vasconcelos et al., 2003). These results were transported to the animal model in mice, where we were able to show that injection of G-CSF-treated granulocytes (in vivo or in vitro) prevent acute experimental GVHD (Vasconcelos et al., 2006). On the other hand, the mechanisms involved in these reactions are still obscure, considering that granulocytes are short-lived cells and that we have observed GVHD inhibition to occur for over a one year period. In addition, the great majority of transplanted patients carries malignancies and one does not know if the immunosuppressor effect of granulocytes prevent graft anti-tumor reaction.
Objectives

1. Assess the expression levels of ABCB1 and ABCC1 in human DCs during differentiation processes by GM-CSF and IL-4 and activation processes by TNF or LPS;
2. Evaluate modulation of CD86 in DCs and in cells treated with inhibitors of ABC transporters;
3. Assess modulation of maturation/differentiation processes and activation of human dendritic cells in response to several stressful and differentiating agents like UV and thrombin;
4. Assess activation level of P-p38 in human DCs in response to the different stimuli studied;
5. Assess modulatory effect of tumor cells supernatant over human DCs activation;
6. In an antigen-specific experimental system, evaluate the effect of intra-tumor injection of adjuvant (LPS) on reversion of tolerance against antigens exclusively expressed by the tumor;
7. Assess the effect of tumor cytokines over DCs and their consequent capacity of T cell activation;
8. Evaluate the role of chemokine receptors on DCs migration from tumor to lymphonodes;
9. Assess the inhibition mechanisms of GVDH by G-CSF-treated granulocytes and check their effect over tumor rejection in experimental models;
10. Investigate the possibility to generate human inhibitory granulocytes in the absence of treatment for 5 days in vivo in order to initiate a clinical assay.

Experimental Approach

Human dendritic cells studies will be held from monocyte differentiation after being cultured for 5 days in a GM-CSF and IL-4-containing medium. On the 5th day of culture, TNF-α will be added and the cells will be cultured for 48h for activation. The agents that will be evaluated as potential modulators of differentiation and activation of dendritic cells will be added at the same moment of the corresponding cytokines. CD14, CD1a and CD83 will be assessed by flow cytometry, along with other molecules involved in the function of these cells. In functional terms, phagocytosis,
allostimulation and cytokine secretion will be evaluated. Kynase p38 involvement will be measured by P-p38 levels by flow cytometry.

In order to evaluate break of tolerance during anti-tumor response, C57Bl/6 mice transgenic to T cell receptor recognizing OVA or Ea will receive OVA- and EaRFP-transfected B16F10 melanoma cells (B16 redOVA). From one to five days after this procedure, tumors will be injected or not with LPS or cytokines and proliferation and production of IFN-γ by antigen specific T cells will be analyzed. The expression of stimulatory and inhibitory molecules (PD-1) will be evaluated in DCs, and cytokine production will be measured by real time PCR in lymphonodes and tissue. The importance of IL-10 produced by tumor in T cells tolerization will be tested in IL10 -/- mice as receptors and silenced in the tumor (siRNA). The role of CCR5, CCR7 and TLR4 will be evaluated in mice genetically deficient for these molecules. During all treatments, functionality of generated memory cells will be tested with tumor cells challenge.

Gran-G effect over anti-tumor response in the allogeneic model of transplants of hematopoietic progenitors will be evaluated in the F1 progeny of irradiated B6 chimeras (DBA x B6). These chimeras will receive different numbers of P815-GFP molecules (generated from retrovirus transduction). Anti-tumor response will be assayed by flow cytometry of peripheral blood.

In order to test the inhibitor effect of granulocytes in human GVHD, we first need to establish gran-G acquirement from the bone marrow donor. Bone marrow will be treated in vitro with different quantities of GM-CSF during different periods of time (until 18 hours) and further analyzed for their inhibitory capacity over T lymphocytes, generation of low density granulocytes, phagocytic capacity and oxidative metabolism. This study has already been submitted to and approved by the Research Ethical Committee of the National Institute of Cancer (INCa).

References:
Jain RK Nat Rev Neurosci 8:610 (2007)
Aims

Qualitatives
- Identify new therapeutic targets and establish new therapeutic approaches through the comprehension of: the function of lipid bodies in inflammatory and neoplastic processes; the molecular mechanisms mediated by the NFAT family proteins on regulation of lymphoid cell growth and their relationship with malignant transformation of cells from the lympho-hematopoietic system; angiogenesis regulation; the disturbing mechanisms of the apical junctional complex in epithelial tumors; cellular migration over proteins of extracellular matrix and computational molecular modeling;
- Establish new immunotherapy protocols for malignancies and GVDH treatments preserving anti-tumor response.

Quantitatives
Production and human resources
- 19 PhD
- 29 MsC
- 36 Papers in indexed journals

Previous research experience:

Wilson Savino Researcher - He has a degree in biological sciences from the University of the State of Rio de Janeiro (1974), master's degree in histology and embryology by the Federal University of Rio de Janeiro (1979) and Doctor of Science (Cell Biology and Tissue) from the University of Sao Paulo (1982). He presided the Brazilian Society of Immunology in the biennium 1993-1995. He is currently a member of the Brazilian Academy of Sciences researcher and holder of the Oswaldo Cruz Foundation, which directs the Laboratory of Research on Timo. He has experience in the field of Immunology, with emphasis on Cellular Immunology, working mainly on the following topics: 1. Physiology of cell migration in the limph-
Patrícia Bozza - Researcher - She has graduated in Medicine in 1990, the Faculty of Medical Sciences, University of the State of Rio de Janeiro, and received the title of Doctor of Science (concentration in Pharmacology) in 1993 by the Program of Cellular and Molecular Biology Institute of the Oswaldo Cruz. In 1994, Patricia was named Pew Fellow Latin American and developed the post-doctorate at Beth Israel Hospital, Harvard Medical School. Patricia Bozza is a researcher of the Institute Oswaldo Cruz and 1 A researcher of CNPq. Patricia was International Scholar of the Howard Hughes Medical Institute in the period 2002-2006, and coordinated the Brazilian committee of the Pew Program in biomedical sciences. The group of research led by Patricia is dedicated to the study of cellular and molecular mechanisms of leucocyte activation and generation of inflammatory mediators in response to infection and other forms of inflammation.

Vivian Mary Barral Dodd Rumjanek - Professor (UFRJ) - She has graduated in the Biological Sciences Medical Solicitation by UEG, currently UERJ - the University of Rio de Janeiro State (1969), Master by the University of London (1973) and doctorate at University of London (1976). She is currently a Professor of the Federal University of Rio de Janeiro. She has experience in the field of Immunology, with emphasis on Cellular Immunology and Tumoral Immunology. She operates mainly in studies of regulation of the immune system and the problem of resistance to multiple drugs (MDR) in tumor cells.

Adriana Cesar Bonomo - Associate Professor (UFRJ) / Researcher (INCA) - She has graduated in Medicine from the Federal University of Rio de Janeiro (1985), master's degree in Biological Sciences (Biophysics) by the Federal University of Rio de Janeiro (1989), Ph.D. in Biological Sciences (Biophysics) by the Federal University of
Rio de Janeiro (1994) and post doctorate in NIH (1990-1994). Currently is associate professor of the Federal University of Rio de Janeiro, special researcher of the National Institute of Cancer, - reviser of the Brazilian Journal of Medical and Biological Research, - Journal of Cell Biology, - Journal of Leukocyte Biology, Plos one. Today is the coordinator of the PIBIC at INCA and responsible for the training program in human resources for research and chief of experimental medicine also at INCA. She has experience in the field of Immunology, with emphasis on Cellular Immunology, working mainly on the following topics: transplants, T cell, tolerance, autoimmunity and bone marrow.

Cristina Beatriz Cazabuena Bonorino – Associated Professor (PUC-RGS) - She has graduated in Biological Sciences at Federal University of Rio Grande do Sul (1988), master's degree in genetics and molecular biology - Department of Genetics (1991) and doctorate in genetics Immunology - and Ufrrs University Of Colorado (1995). She made her post-doctorated at the National Jewish Center for Immunology in Colorado, in models of autoimmunity, and at the University of Minesotta, in memory T cells. She is currently an associated professor at the Pontifical Catholic University of Rio Grande do Sul, receiving a productivity fellowship from CNPq since July 2008. She has experience in Cellular Immunology, and coordinates the laboratory of Cellular and Molecular Immunology at the Institute of Biomedical Research of PUCRS, acting mainly in the following topics: development of vaccines, tumor immunology and autoimmunity.

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(INCA), 1D of CNPq and Research Scientist of the State of Our FAPERJ. He was head of the Division of Cellular Biology of INCA in the period 2000 to 2008. He is a member of the Brazilian Society of Immunology (SBI), the American Association of Immunologist (AAI) and the Brazilian Society of Cell Biology (SBBC). He was the first secretary of the SBI in the biennium 2002-2003. He is a member of the Editorial Board of Cancer Immunology Immunotherapy (2006-present) and the International Journal of Oncology (2008-present). He is a reviewer of Blood, Cancer Immunology Immunotherapy, Oncogene, The FASEB Journal, Journal of Leukocyte Biology and The Journal of Immunology. Supervised 8 dissertations of Master, 3 of Ph.D and oversaw 1 Post-Doctoral. He has experience in the field of Immunology, Cellular and Molecular Biology, with emphasis on Molecular Immunology, working mainly in the following subjects: immune response, gene expression, cell cycle and cancer.

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Jose Andrés Morgado Diaz Researcher (INCA) - He has a degree in biology from Universidad Nacional de Trujillo - Peru (1981), Master (1991) and Ph.D. (1996) in biochemistry from Federal University of Rio de Janeiro, and Post Doctorate by the Oswaldo Cruz-FIOCRUZ from Rio de Janeiro. He is currently a research associate at the National Cancer Institute where he heads the Group of Structural Biology Research and collaborator of the Federal University of Rio de Janeiro and the University of Maringa State. He is developing projects trying to identify ways of signalling that mediate the loss of cell-cell adhesion epithelial cell cancer and its influence on the development of tumorigenesis. He has experience in Morphology and Cell Biology, with emphasis on cell biology of cancer, working on issues of transduction of signals, and invasion and adhesion protein glycosylation in epithelial cancers.

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Cecilia Jacques Gonçalves de Almeida - Researcher - She has graduated in Biological Sciences at Federal University of Rio de Janeiro (1991), received a Master's degree in Biological Sciences (Biophysics) by the Federal University of Rio de Janeiro (1996) and Ph.D. in Biological Sciences (Biophysics) by the Federal University of Rio de Janeiro (2002). She made her post-doctorate at the Lab of Caveolae, originally located at Albert College of Medicine, NY and at the Kimmel Cancer Center, Thomas Jefferson University, Philadelphia. Her experience is concentrated in the areas of molecular and cellular biology, the study of cellular responses and inflammatory processes of macrophages.

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Suse Dayse Silva Barbosa – Researcher – She has a degree in biomedicine from the University of Rio de Janeiro (1988), master's degree in Cellular and Molecular
Carla Eponina de Carvalho Pinto – Researcher - 486.155.527-20, 12/07/1958. She has graduated in veterinary medicine (Pathological Anatomy Veterinary) by the Fluminense Federal University (1985), master’s degree in veterinary medicine (Veterinary Pathology) by the Fluminense Federal University (1991) and doctorate in molecular biology - Universidad Autonoma de Madrid (2004). She is currently an associated professor at the Fluminense Federal University and researcher visitor to FIOCRUZ / RJ. She has experience in the field of Immunology, with emphasis on Immunopathology, acting mainly on the following topics: laboratory animals, molecules involved in cell migration, chronic inflammatory processes, type I diabetes, diabetic non obese mice (NOD), and studies on the physiological and pathological role of APRIL using transgenic mice for APRIL.

Daniella Arêas Mendes da Cruz – Researcher - 018.036.397-25, 06/02/1973. She has a degree in biological sciences (Medical Modality) from the Federal University of Rio de Janeiro (1996), master’s degree in biology Parasitic by the Oswaldo Cruz Foundation (2000), Doctor of Biology Parasitic by the Oswaldo Cruz Foundation (2003) and post-doctorate from Paris University Descartes, Paris-France (2004-2008). She is currently a researcher in public health from the Oswaldo Cruz Foundation. She has experience in the field of Immunology, with emphasis on Cellular Immunology, working mainly on the following themes: neuroimmunoendocrinology, cell migration, neuropilins, semaphorins, extracellular matrix, chemokines, thymus, chagas disease.

Désio Aurélio Farias de Oliveira – Researcher
Leandra Linhares Lacerda – Researcher
Ernesto Caffarena – Graduated Student.
Eduardo Samo Gudo Júnior - Graduated Student
Klaysia Moreira Ramos Pedrosa - Graduated Student
Marco Augusto Stimamiglio - Graduated Student
Cecília Rocha de Souza - Graduated Student.

Schedule:

Year 1, semester 1
- analysis of protein and lipid content of lipid bodies after stimulation by several agents;
- generation of vectors containing NFAT1 and NFAT2 coding cDNAs;
- analysis of transfected cells expression by microarrays;
- standardization of CD34+ progenitor cells cultures from umbilical cord blood;
- generation of recombinant proteins to be used in *in vitro* assays;
- standardization of ubiquitinization and protein degradation techniques *in vitro*;
- standardization of transfection protocols;
- determination of cholinergic status in selected patients;
- validation of AChE-hRUL138 interaction;
- adoptive transferring of CFSE-labeled cells;
- analysis of anti-tumor T cells differentiation in memory cells;
- analysis of DCs phenotype and antigen presentation in experimental models;
- analysis of expression and activity of ABC, ABCB1 and ABCC1 transporters in several developmental phases of human dendritic cells;
- establish the role of stressing agents like heat and hypoxia in DCs maturation, differentiation and activation, correlating them to the expression of ABC transporters;
- assess the formation of extracellular traps by gran-G;
- standardization of the GVHD model in the presence of tumor;
- establish differences in the migration of several lineages of HTLV-1-infected cells;
- analysis S1P1 and S1P1 ligands role in human T cells development, including interactions between thymocytes and thymic microenvironment (also interactions mediated by other molecules) and events of cell death, cell cycle and cellular migration.
- Abrogation of syndecan-4 expression in endothelial cells (HUVECs and EPCs), by using siRNA technology;
- Selection of tumor cell lines (astrocitomas; glioblastoma multiforme) expressing low TN-C levels – or none - or to produce them by siRNA techniques.

- study of the effect of the same modulators in tumor adhesion to the native sub-endothelial matrix, produced *in vitro*

**Year 1, semester 2**

- analysis of protein and lipid content of lipid bodies after stimulation by several agents;
- assess cell signaling induced by leptin;
- analysis of transfected cells expression by microarrays;
- validation of genes identified by microarrays by real time PCR;
- characterize functions of transfectant proteins (XAF, NFAT, JAK2 and JAK2V617F);
- correlate NFAT expression with AChE-S and AChE-R variants in CD34+ differentiated cells;
- determination of cholinergic status in selected patients;
- validation of AChE-hRUL138 interaction;
- assess the formation of extracellular traps by gran-G;
- evaluate anti-tumor response in GVDH;
- standardization of human gran-G generation *in vitro*, from bone marrow cells;
- analysis of anti-tumor T cells differentiation in memory cells;
- analysis of DCs phenotype and antigen presentation in experimental models;
- analysis of T cells survival and related cell death mechanisms (Fas, etc);
- move forward the study of stressing agents, correlating them to the expression of ABC transporters;
- assess the effect of the differentiating agents sodium butirate and retinoic acid on DCs maturation and differentiation, as well as their effects on these cells activation;
- analyze EGFR and p38 pathways in terms of produced effects by differentiating agents in DCs;
- analyze S1P1 and S1P1 ligands role in LLA-T and LL-T development and the relationship between their expression and their preferential localization in tumor cells;
- identify chemokynes and ECM molecules that could be involved in the formation of tumor masses in mesenteric lymphonodes and Peyer patches of Tg animals for APRIL;
- design new inhibitors of cellular migration by computational comparative modeling.
- Study of the pro-angiogenic effects of TSP-1 and fibronectin fragments/peptides in silenced endothelial cells - adhesion, tubulogenesis, proliferation and migration assays.
- study of the effect of adhesion modulators, such as TSP-1, snake venom desintegrins and marine invertebrate polysaccharides, in the adhesion of breast and prostate tumor cell lines to endothelial monolayers in vitro (in both static and dynamic conditions – e.g., under mimetic blood flow conditions).

Year 2, semester 1
- assess cell signaling induced by leptin;
- leptin role in tumor induction;
- assess the role of signaling molecules and their relationship with lipid bodies;
- analysis of lymphoproliferative phenotype of NFAT1 -/- and NFAT2 -/- animals;
- analysis of lymphoproliferative phenotype of CA-NFAT1 and CA-NFAT2 animals;
- assess NFAT role in induction of alternative splicing of AChE gene associated to the differentiation of myeloid lineage;
- determination of expression of AChE isoforms in selected patients;
- assess AchE-S degradation in vitro and in vivo;
- analyze the effect of gran-G in anti-tumor response in GVHD model;
- move forward the clinical assay with gran-G in order to test inhibition of acute GVHD;
- assess the cytokines involved in anti-host and anti-tumor responses;
- test different adjuvants in DCs (cytokines and stressing agents);
- analyze the effect of products from tumor microenvironment in DCs differentiation and activation;
- search for micro-particles in tumor cells supernatants and analyze their effect in DCs differentiation and activation;
- analyze the effect of thrombin (activated by micro-particles and normally found in tumor microenvironment) in DCs activation;
- identify chemokines and ECM molecules that could be involved in the formation of tumor masses in mesenteric lymphonodes and Peyer patches of Tg animals for APRIL;
- design new inhibitors of cellular migration by computational comparative modeling.
- investigate the regulation of cell signaling pathways, in syndecan-4-silenced endothelial cells, as compared to wild-type cells.
- study the proliferative and differentiation behavior of endothelial cells (HUVECs or EPCs, both wild-type and silenced for syndecan-4 expression) seeded on tumor matrices containing low and high levels of TN-C.
- correlate proliferation/cell cycle and tubulogenic modulating properties of tumor TN-C to specific structural domains of this protein, by using a panel of blocking-function antibodies

**Year 2, semester 2**

- assess the role of signaling molecules and their relationship with lipid bodies;
- identification of therapeutic targets related to lipid bodies biogenesis and activity;
- analysis of lymphoproliferative phenotype of NFAT1 -/- and NFAT2 -/- animals;
- analysis of lymphoproliferative phenotype of CA-NFAT1 and CA-NFAT2 animals;
- assess NFAT role in induction of alternative splicing of AChE gene associated to the differentiation of myeloid lineage;
- determination of expression of AChE isoforms in selected patients;
- assess AchE-S degradation *in vitro* and *in vivo*;
- move forward the clinical assay with gran-G in order to test inhibition of acute GVHD;
- assess the cytokines involved in anti-host and anti-tumor responses in GVHD model;
- analysis of AChE-S ubiquitination/degradation in MKs differentiation;
- role of TLR4, CCR5 and CCR7 on the efficiency of DCs vaccination;
- establish the effect of extracellular ATP on DCs viability, differentiation and activation;
- verify which cytokines found in tumor microenvironment could be responsible for some effect in DCs differentiation and activation;
- identify chemokines and ECM molecules that could be involved in the formation of tumor masses in mesenteric lymphonodes and Peyer patches of APRIL Tg animals;
- design new inhibitors of cellular migration by computational comparative modeling and further synthesis and testing of the peptides identified by this approach.
- Investigate the cell signaling pathways in silenced endothelial cells seeded onto TN-C- rich and poor glioma matrices
- investigate the interaction of EPCs (wild type and silenced) with mature endothelium (HUVECs) in vitro, and its modulation by tumor factors, in static conditions;
- investigate the modulation of endothelial cell cycle (expression/activity of ciclin/CDK complexes; expression of CKIs etc) in HUVECs or EPCs - both wild-type and silenced for syndecan-4 expression - seeded in tumor matrices.

**Years 3-5**
- identification of new therapeutic targets and prognostic tracers based on the knowledge of lipid bodies biogenesis;
- analyze the expression of NFAT1 and NFAT2 proteins in different lymphoma samples from patients by tissue microarray and correlate their expressions with the patients therapeutic response and survival, in order to decide if NFAT proteins can be used as prognostic tracers in the case of lymphoproliferative diseases;
- analysis of AChE activity in patients carrying JAK2 V617F mutation; analysis of the expression of XAF mRNA in bone marrow of patients carrying JAK2 V617F mutation; analysis of the expression of AChE mRNA in bone marrow of patients carrying JAK2 V617F mutation; mapping of AChE-S ubiquitinization sites; LPS-induced platelet response in NFAT1 knockout animals; analysis of the expression of AChE in knockout mice forNFAT1 proteins;
- clinical assay with gran-G in order to inhibit GVHD;
- analyze the mechanisms of GVHD inhibition by gran-G considering intestinal micro flora;
- design and testing of cellular therapies including DCs and lymphocytes in the animal model (pre-clinical);
- design and testing an experimental therapy in humans;
considering the results obtained during the first two years using DCs from normal individuals, we will use the same parameters in: (a) DCs from patients with cancer and thrombosis, and (b) DCs from patients with histiocytosis of Langerhans cells;
- design and testing of the inhibitory agents obtained from computational modeling assays, based on experimentally generated data;
- modulation of the migration patterns of hematopoietic malignancies from the identification of mechanisms that lead to this differential migration.
- Study of the role of tumor TN-C silencing in a model of glioma implantation in rats
- Study of the recruitment and incorporation of human EPCs into tumors implanted into nude mice, by bioluminescent monitoring (model set up and study of adhesion modulators).

Local and international collaborations

Local
- Hugo Castro-Faria-Neto – IOC/FIOCRUZ
- Jonas Perales – IOC/FIOCRUZ
- Richard H. Valente – IOC/FIOCRUZ
- Rossana Melo – Department of Cellular Biology – UFJF
- Christianne Bandeira-Melo – Institute of Biophysics Carlos Chagas Filho/UFRJ
- Roger Chammas, Department of Oncology USP.
- Gustavo Amarante-Mendes, Department of Immunology, USP.
- Martin Bonamino – INCA (National Institute of Cancer, Brazil)
- Ilana Zalcberg Reanult - INCA (National Institute of Cancer, Brazil)
- Bruno Lourenço Díaz – Institute of Biophysics Carlos Chagas Filho/UFRJ
- Wanderley de Souza - Institute of Biophysics Carlos Chagas Filho/UFRJ
- Celso Vataru Nakamura – Department of Clinical Analysis, State University of Maringá
- Heloísa Selistre de Araujo – Department Physiology Science /UFSCar
- Vivaldo Moura Neto – Department of Anatomy, UFRJ
- Julio Scharfstein - Institute of Biophysics Carlos Chagas Filho/UFRJ
- Leila Maria Lopes Bezerra – Cellular and genetics biology department/UERJ
- Paulo Antônio de Souza Mourão – Medical Biochemistry Department/UFRJ
- Prescilla Emy Nagao Ferreira – Cellular and genetics biology department/UERJ
- David Saltovich – Nephrology laboratory- PUC-RS
- Carla Alho – Genetics Laboratory- PUC-RS
- Gilberto Schwartzman – Clinical Hospital of Porto Alegre
- Joao Santana, USP (São Paulo University), Ribeirão Preto
- José Barbuto, ICB-USP
- Laboratory of Inflammation Instituto Oswaldo Cruz, Fiocruz.
- Basic Research Center, National Institute of Cancer, Brazil
- Bioinformatics section, National Scientific Computation Lab, Petrópolis;
- Ludwig Institute of Cancer Research, São Paulo
- Department of Genetics FMRP/USP-Ribeirão Preto;
- Departament of Cellular Biology, FMRP/USP-Ribeirão Preto;
- Department of Immunology, FMRP/USP-Ribeirão Preto;
- Institute Biomedical Science, Federal University of Alagoas, Maceió

International

- Peter F. Weller – Harvard Medical School – United States
- Guy Zimmerman – Utah University - United States
- Anjana Rao - The Center for Research, Harvard Medical School, United States
- Chantal LeGrand – INSERM U533 Hôpital Saint Louis, Université Paris 7
- Anne Woods - University of Alabama at Birmingham, United States
- Cathérine Boisson-Vidal INSERM, Faculté de Pharmacie Université Paris
- Michel Crépin INSERM U 553 Hôpital Saint Louis
- Marc Jenkins - Universidade de Minnesota, United States
- Vince Guerriero, University of Arizona, United States
- Barry Rouse, da University of Tennessee, United States
- William Heath - Australia
- Immunology Department, National University of Rosario, Argentina;
- Immunology Department, National Healthy Institute, Moçambique,
- Cellular biology Department, University Complutense of Madrid, Spain,
- Institute of Molecular Genetics of Montpellier, CNRS, France;
- Hôpital Pitié-Salpetrière, Inserm UMR 787, Paris, France
- Hôpital Necker, CNRS UMR-8147, Paris, France

**Financial Support (last 5 years):**

João Viola


-CNPq, Edital MCT-CNPq/MS-SCTIE-DECIT-Healthy/Neoplasia 06/2005 (401130/2005-3). Title: “Involvement of lipid bodies and lipid metabolizing enzymes (PLA2, COX-2 e FACL-4) in colon adenocarcinoma”. Support researcher (Principal Investigator: Dr. Patrícia Bozza), 2005-2007. R$90.000


-International Centre for Genetic Engineering and Biotechnology (ICGEB), Collaborative Research Programme (CRP/BRA04-02). Title: “Involvement of NFAT Transcription Factor in Cell Cycle Regulation”. Principal Investigator, 2005-2008. US$60.000


-FAPERJ, Edital research support PPSUS (E-26/171.474/2006). Title: “Involvement of NFAT Transcription factors in the regulation of the cell cycle and tumorigenesis”. Principal Investigator, 2008-presente. R$40.000

Adriana Bonomo
-FAPERJ, Edital APQ2 – Events organization support 2008/01 (E-26 / 111.315 / 2008). Title: “15th International Histocompatibility and Immunogenetics Workshop (15IHIWS)”. Principal Investigator, 2008. R$18.000,00

-FAPERJ, Pensário – Support to thematic studies (E-26/110.374/2007). Title: “Cellular and molecular bases of inflammatory response in infectious diseases and cancer: Identification of biomarkers and new therapeutic targets”. Collaborator (Principal Investigator: Dr. Patrícia Bozza), 2007-.R$ 50.000,00 (Researcher part)

-CNPq, Edital Universal 2007 Faixa C-Edital MCT/CNPq 15/2007 - Universal - Faixa C - De R$ 50.001,00 até R$ 150.000,00 (471108/2007-3). Title: “Immunology and hematopoieses: Search of new regulatory strategies”. Principal Investigator, 2007-2009. R$ 77000,00


- SWB – Swiss Bridge Foundation - International cooperation – Subproject 5. Title: “Phase I/II trial on the inhibitory effect of G-CSF induced low density granulocytes (LDG) over acute Graft Versus Host Disease after allogeneic stem cell transplantation and studies on LDGs mechanism of action.”. Principal Investigator, 2006-2008. RS360,000,00


José Andrez Morgado:


-FAPERJ/MS. Cellular and molecular aspects on the cell-cell adhesion in Colo-rectal Cancer. Identification of Target molecules as potential therapeutic agents 2006, R$ 31,000,00

-FIOCRUZ. Ministry of Health – Project: INCA 2006 Cellular and molecular aspects on the cell-cell adhesion in Colo-rectal Cancer. R$ 120,000,00


-FAPERJ, Process: E-26/170.000/2003 Study of the organization and junctional complex function regulation in colon adenocarcinoma cells. R$ 12500,00

-CNPq, Process: 412043/2003-3 Study of the regulation and function of the junctional complex in colon adenocarcinoma cells. R$ 25000,00

Vivian Rumjanek:


Veronica Morandi:
- CNPq – Edital Universal 2006- proc.485188/2006-6 “Role of sindecan-4 e da thrombospondin-1 in angiogenesis in endothelial transfected cells model” - R$27000,00

-CNPq Support to International cooperation 2006 “Phenotypic modulation of adult endothelial and progenitor cells” — CNPq/INSERM – proc. 490757/2006-5 R$36000,00

-CNPq – Edital NEOPLASIAS 06/2005 – proc. 401188/2005-1 “Interaction of tumor cells with the sub-endothelial matrix and with the endothelium”- - R$ 70.000,00

-CNPq Edital to the Stem Cell studies. 024/2005 – proc “New Molecules pro-angiogenic for the a optimization of human circulating endothelial progenitors culture” –. 552412/2005-8 R$ 60.000,00

-CNPq – Edital to neoplasis studies, 06/2005 – proc. 401188/2005-1 “Interaction of tumor cells with the sub-endothelial matrix and with the endothelium –R$ 17.000,00

-PRONEX/CNPq (edital FAPERJ 04/2006) (colaborator) “Glycobiology: Polysaccharide effects in vascular biology”, Principal Investigator: Prof. Paulo Antônio de Souza Mourão/UFRJ R$ 75.000,00

-“Inflammation and cancer”, Project approved according to the edital of support to state entities/FAPERJ, (collaborator) Principal Investigator: Prof. Thereza Christina Barja-Fidalgo/UERJ R$ 45.000,00
- “Intracranial tumors: Image, surgery, classification, cellular and molecular biology”, project approved according to Pensa Rio/FAPERJ (collaborator) coordinator: prof. Vivaldo Moura Neto/UFRJ. R$ 67,000,00

- “Glycobiology: Polysaccharide effects in vascular biology”, approved according to PRONEX/CNPq (edital FAPERJ 04/2006) (collaborator) coordinator: Prof. Paulo Antônio de Souza Mourão/UFRJ

Cinthya Sternberg:

-Support APQ1 FAPERJ 2007- E-26/110.167/2008; number 2007.4064.1
Total: R$ 20,000,00 (Consume: R$ 17,000; Equipments: R$ 3,000,00)

Cristina Bonorino:
-Memory generation of anti-tumoral T CD4+ in vivo - Edital Neoplasis, CNPq, 2005 – R$ 70,000,00

-Study of neuro cell protection neuro of the skin from the UVA/UVB radiation. O Boticário, 2005, 212,000,00.

-Cellular therapy for breast cancer patients – optimization of the conventional therapy– Edital Procóredes III, FAPERGS, 2006, R$ 35,000,00

-Development of a immunessupressor based on Hsp70 of Mycobacterium tuberculosis Finep, 2008, 120,000,00

Patrícia Bozza:
- PAPES V-FIOCRUZ  Title: Biogenesis and new functions of the lipid bodies in cell signaling: Contributions for the inflammation and cancer. (Principal Investigator: Dr. Patrícia Bozza), (2008-2010). R$ 60.000,00

- CNPq, Post-doctorade national programme (Ed 342007 L3 PD 558697/2008-9) Title: Cellular and molecular basis of biogenesis and lipid bodies in macrophages function: Contributions to innate immune response (Principal Investigator: Dr. Patrícia Bozza), 2008-2012. R$ 261300,00

- CNPq, Edital Universal - (485333/2007-4) Title: The receptors toll role in the immunomodulatory activity of lipids of Schistosoma mansoni. (Principal Investigator: Dr. Patrícia Bozza), 2007-2008. R$ 119.000,00


- FAPERJ, Cientista do Nosso Estado - Title: “Heterogeneity and function of lipid bodies in macrophages: Contributions to atherosclerosis and innate immune response”. (Principal Investigator: Dr. Patrícia Bozza), 2007-2008. R$ 48.000,00


- PRONEX/MCT/FAPERJ, Title: Fisiopathologic Mechanism and new therapeutical approach of lung inflammatory diseases: From the bench to the clinical practice. (Principal Investigator: Dr. Walter Zin), (2006-2008). R$ 600.000,00

- Edital MCT-CNPq/MS-SCTIE-DECIT - Health/Neoplasias 06/2005 (401130/2005-3) Neoplasias title: Involvement of lipid bodies and lipid metabolizing enzymes (PLA2,
COX-2 e FACL-4) in colon. adenocarcinoma (Principal Investigator: Dr. Patrícia Bozza). R$ 90.000,00

- PAPES IV-FIOCRUZ (400131/2006-4) Title: Participation of the lipid bodies in the innate immune response of the host facing intracellular parasites (Principal Investigator: Dr. Patrícia Bozza), (2006-2007). R$ 60.000,00

Wilson Savino:
- CNPq/Faperj – Pronex - Laminin e cellular migration in the lympho-hematopoietic system; functional role and therapeutic potential. Principal Investigator 2008 – 2011 R$ 470.000,00

- CNPq – Pró África 2008 – 2010 Lymphocyte migration in the HTLV-1 vírus the infection R$55.000,00 Principal Investigator

- CNPq – Pró Sul 2006 – 2008 Migration and death of T Lymphocyte in the experimental Chagasic infection R$ 40.000,00

- CNPq – Universal 2008 – 2010 Participation of the Galectin-3 in the migration and death of Thymocytes in physiologic conditions and in the experimental Chagasic infection R$39.503,00 Principal Investigator Déa Maria Serra Villa Verde

- CNPq – Universa l2006 - 2008 Thymocyte/thymic epithelial human cells interaction: gene expression profile. R$35.000,00 Principal Investigator

- Faperj – APQ12006 - 2009 Migration and death of T lymphocytes in Lymphoid animal organs infected by Trypanosoma cruzi R$17.500,00 Principal Investigator Juliana de Méis

- Faperj – Cientista do Nosso Estado 2007 - 2009 Thymocyte/thymic epithelial human cells interaction: gene expression profile. R$ 54.000,00 Principal Investigator

- PAPES – Fiocruz 2008 - 2010 Duchenne’s muscular dystrophy: study of the Inflammatory process R$60.000,00 Principal Investigator
- PAPES – Fiocruz 2008 - 2010 Participation of galectin-3 in the migration and death of thymocytes in experimental physiologic conditions and in the experimental Chagasic infection R$60.000,00  
Principal Investigator Déa Maria Serra Villa Verde

- PAPES – Fiocruz 2008 – 2010 Caspase activity and migration of T lymphocytes in experimental infection by *Trypanosoma cruzi* R$30.000,00  
Principal Investigator Juliana de Méis

- Inserm Fiocruz 2008 – 2009 Duchenne’s muscular dystrophy: study of the Inflammatory process R$15.000,00  
Principal Investigator Suse Dayse Silva Barbosa

- INCA Fiocruz 2006 - 2008 Molecules involved in the human stem cell addressing R$190.000,00  
Principal Investigator

- INCA Fiocruz 2006 – 2008 Cellular and molecular interactions in the physiopathology of the infection by HTLV-1: Study model of lymphocytic migration and transfer of viral material. R$350.000,00  
Principal Investigator

**Participation in Graduate programs:**

**João Viola:**
- Post-graduation Program in Oncology, National Institute of Cancer (INCA), Permanent Professor.
- Post-graduation Program in Biophysics, Institute of Biophysics Chagas Filho, UFRJ, Collaborator Professor.
- Post-graduation Program in Morphology, Institute of Biomedical Sciences, UFRJ, Collaborator professor.

**Patrícia Bozza:**
- Post-graduation in Cellular and Molecular Biology / IOC-FIOCRUZ – Permanent Professor.
- Post-graduation in Medical Clinic (Pneumology) UFRJ –Collaborator Professor
- Post-graduation in Immunology ICB/USP- Collaborator Professor

Adriana Bonomo:
- Post-graduation Program in Oncology, National Institute of Cancer (INCA), Permanent Professor.
- Post-graduation Program in Microbiology, Institute of Microbiology Professor Paulo de Góes, Permanent Professor.
- Post-graduation Program in Biophysics, Institute of Biophysics Carlos Chagas Filho, UFRJ, Collaborator Professor.

Cristina Bonorino:
- Post-graduation Program in Cellular and Molecular Biology – PUCRS – Permanent Professor.

Veronica Morandi:
- Post-graduation Program in Biology/UERJ Permanent Professor.

Wilson Savino:
- Post-graduation Program in Oncology, National Institute of Cancer (INCA), Permanent Professor.
- Post-graduation in Cellular and Molecular Biology / IOC-FIOCRUZ – Permanent Professor.
- Post-graduation in NeuroImmunology – UFF Collaborator Professor.

Déa Maria Serra Villa Verde
- Post-graduation in Cellular and Molecular Biology / IOC-FIOCRUZ – Permanent Professor.
- Post-graduation in Genetics and molecular biology – Unicamp - Collaborator Professor.
- Post-graduation in Parasitary Biology – Fiocruz- Collaborator Professor.
- Post-graduation in Veterinary medicine- UFF - Collaborator Professor.

**Suse Dayse Silva Barbosa**

- Post-graduation in Cellular and Molecular Biology / IOC-FIOCRUZ – Permanent Professor.

**Carla Eponina de Carvalho Pinto**

Post-graduation in Cellular and Molecular Biology / IOC-FIOCRUZ – Permanent

**Vivian Rumjanek:**

- Post-graduation program in Biological Chemistry, Institute Medical Biochemistry UFRJ Permanent Professor.
- Post-graduation Program in Biophysics, Institute of Biophysics Carlos Chagas Filho, UFRJ Permanent Professor.

**José Morgado:**

- Post-graduation Program in Oncology, National Institute of Cancer (INCA), Permanent Professor.

**Cinthya Sternberg**

- Post-graduation Program in Oncology, National Institute of Cancer (INCA), Permanent Professor.
- Post-graduation in NeuroImmunology – UFF –Permanent Professor

**Institutional infrastructure:**

National Institute of Câncer, Brazil (INCA): PCR machines (6), horizontal and vertical electrophoresis systems, PCR preparation hoods, clinical centrifuges (5), CO-2 incubators, Biosafety hood class II-A2 (4), microcentrifuges (3), refrigerated microcentrifuge, heat block, speed-vac, Reichter-Jung cryostat, orbital shakers, air-heated shakers, refrigerators, freezers (4), -80°C freezers (4), refrigerated centrifuge
(2), direct and inverted microscopes (contrast phase and fluorescence), dissection microscope, fluorimeter, Milli-Q water purification system, computers, laser and inkjet printers.

Institutional equipments and facilities: Animal facility with pathogen free animals and inter-room isolation for infection control. Cell and animal manipulation laboratory with safety hoods and clinical centrifuge, several mouse strains available. FACScalibur flow cytometer with two lasers and sorter. Beta counter, MEGA-BACE and 377 ABI DNA sequencers, Real-Time PCR system, electronic-transmission microscope, ultracentrifuge, microarray system, tissue microarray, laser capture microscope, Nanodrop spectrophotometer, BL-II safety room with 2 class II-B2 safety cabinets, air-lock and restricted access. We are acquiring a confocal imaging system with multiphoton system (expected in 05/2009), an in vivo bioluminescence IVIS system (expected in 02/2009) and a new BL-II+ laboratory and mouse embryo bank laboratory, both placed in the animal facility (expected in 10/2009)

UERJ: 02 laminar flow safety hoods, 01 refrigerated centrifuge, 02 CO-2 incubators, 01 inverted microscope with digital image capture system, 01 optic fluorescence microscope Nikon, with digital image capture system, 01 optic microscope Nikon, 03 micropipettes, 01 multichannel pipette, 02 electrophoresis system, 02 ELISA readers, spectrophotometer, 01 spectrophotometer UV/Vis. Multiuser center: Transmission Electron-microscope Zeiss model 906; Scanning Electron-microscope LEO model 1450VP; metalizer CRESSINGTON model SPUTTER COATER 108, Critic point PELCO, model CPD2 Critical Point Dryer; Confocal Microscope Zeiss model LSM 510 META.; Flow cytometer FACS-Excalibur Bur

PUC-RS: Tecniplast ventilation unit for cages for 100 cages storing 10 animals each, exclusive room for rats/hamsters in the bioscience university biotherapy, Autoclave, Milli-Q water purification system, CO2 incubator, 2 laminar flows, PCR machines; DNA gel systems (2), electrophoresis system (3), refrigerated centrifuge Eppendorf 01, refrigerated clinical centrifuge - 01, mini-centrifuge - 01, centrifuge baby FANEM- 01, tube centrifuge- 01, authomatic pipetor - 02, inverted microscope; freezer -80, Sorvall centrifuge, air-heated shakers, cold room, fluorescent
microscope Zeiss ax70 3 colors; Inverted microscope.

FIOCRUZ: Cell culture room with laminar flow (3), CO2 incubator (2) e inverted microscope; ELISA reader with fluorescence capacity; microscope of fluorescence system; luminometer, luminex, eletroporator, FACS, 02citocentrífuges; refrigerated centrifuge; animal manipulation safety hoods; horizontal and vertical electrophoresis systems; spectrophotometer,; recipients of liquid nitrogen for the storing of samples and isolating stands with controlled environment to the animal in experiment maintenance. Besides that, the Pharmacodynamics and Physiology Department, where the Immunopharmacology laboratory is has equipment of common usage as mass spectrophotometer HPLC system, ultracentrifuge; Protein sequencer; cold room, beta counter, cytosensor. The laboratory is connected to the universal net of computers through the optical fiber net with quick Access, and the professionals and students have wide Access to the CAPES site, and also have an important journal library and books from the FIOCRUZ library.
THEME 2 - MOLECULAR MARKERS AND CÂNCER

Sub-project 1: Molecular markers of early diagnosis and susceptibility to cancer

Rationale: Esophageal squamous cell carcinoma (ESCC) comprises over 90% of esophageal tumors in the World. This tumor is particularly present in developing countries, and Brazil is one of the high incidence areas (Pakin et al., 2001). In Brazil, ESCC is the 4th most incidence tumor among men and the seventh among women (INCA, 2008). ESCC is highly fatal and despite the recent surgical, and chemio/radiotherapy improvements, the prognosis for esophageal SCC is still poor, with a 5-year survival rate below 5% (Ribeiro et al, 1996). The main reason for the poor prognosis is that ESCC is usually detected at late stages (over 60% of tumors in Brazil are detected at stages 3 and 4) (Estimativa 2008: Incidência de Câncer no Brasil). However, patients with early diagnosis, in which tumors are restricted to the mucosa or submucosa, have 5 year survival rates of 90% and 45%, respectively (Skinner et al, 1982).

Endoscopic and cytologic screening programs for early detection of esophageal cancer have not been economically or clinically successfull. This situation emphasizes the urgent need for novel markers to improve early detection and create new opportunities for drug-based therapies (Zhou et al, 2005). Among the risk groups that present the highest potential for early diagnosis of CEE are patients with head and neck cancer, who present a 5-year survival rate above 50% if they do not have a second primary CEE. However, many studies show that around 30% of patients with NHSCC will have a synchronous CEE or esophageal pre-neoplastic lesion (Mcguilt et al., 1982, Shiozaki,H. et al, 1990; our unpublished results).

Wang et al (2006) have shown that the serum proteomic profile of patients with precancerous esophageal lesions is a promising biomarker that could be applied as an early screening tool. Similarly, tissue-specific genomic instability (Eisenberger et al., 2003) and epigenetic alterations (Carvalho et al., 2008) can be used as serum early biomarkers seen through the loss of microsatellite and hypermethylation of the promoting regions of specific genes, respectively. The analysis of alterations in low
penetrance genes can also help to identify those individuals who are at higher risk to develop ESCC (Ribeiro Pinto et al., 2003, Rossini et al., 2007).

Recently, our group analyzed the GBP-2 (Guanylate binding protein-2) expression suggesting that GBP-2 may represent a marker of interest in ESCC (Guimarães et al., 2008). Splicing process might play a major role in carcinogenesis. In this line, our group predicted four splice variants of the GBP-2 gene with bioinformatic methods, using a platform developed by our group which integrated transcriptome and proteome data (patent application process 020070063423-RJ 15 May, 2007). Three splice variants predicted by bioinformatics (GBP-2e, GBP-2d e GBP-2c) were found to be differentially expressed between cancer cell lines and normal tissues (data not published yet). Whether these splice variants may be useful as biomarkers for esophageal cancer needs to be investigated.

Mutations in mitochondrial DNA are correlated with several types of solid tumors (Brandon et al, 2006). The inheritance pattern of mitochondrial DNA is matrilineal, it is possible to speculate that the phylogeography of cancer may be related to certain haplogroups present in the population. As a whole such investigation might contribute as a tool for the early detection of cancer. There are no studies associating mutations in mitochondrial DNA and the etiology of cancer in Brazilian populations.

Objectives:

To study the mechanisms of tumor development and to identify biomarkers for early detection of ESCC that possess high sensitivity and specificity, both in experimental and clinical areas, with the specific objectives: a) Diagnosis of asymptomatic patients with ESCC that are epidemiologically under risk conditions; b) To try to have clinical treatment based on molecular biomarkers. c) To monitor the therapeutic responses; d) Sequence the full extension of mitochondrial DNA isolated from biopsies of CEE, breast and lung cancer from patients diagnosed with cancer. e) To investigate the correlation between the occurrence of mutations and their predictive value in individuals at risk of acquiring cancer in order to obtain informative markers for the early diagnostic of cancer. f) To determine the phylogeography of mutations by means of identification of individual haplogroups as tool to establish the genetic composition of the affected population.
Experimental Design

Patients (1000) with HNSCC diagnosed at The National Institute of Cancer (INCA) will go through chromoendoscopy to check for early preneoplastic esophageal lesions or CEE. Blood and tissue samples from HNSCC and esophageal lesions will be collected and have DNA and RNA extracted. The analysis for molecular markers in tumors and serum will involve loss of heterozygosis (LOH), using affymetrix chip analysis with confirmation in a broader number of samples through CGH and loss of microsatellite (Eisenberger et al., 2003). Complimentary to this, serum analysis of the promoting regions of genes that are hypermethylated only in the CEE will be done (Carvalho et al., 2008). We will also investigate the TP53 mutation profile as well as polymorphisms in genes involved in pharmacokinetics and pharmacodynamics of alcohol and tobacco components, as well as DNA repair enzymes (Rossini et al., 2007).

The expression of splice variants of the GBP-2 gene will be analyzed by Real time PCR (Sybergreen). Data will be collected and analyzed with ABI PRISM 7000 Sequence Detection System and software system (Applied Biosystem). Blood and urine will be collected from 60 patients with ESCC (from both genders and at all ages) and 60 controls (paired to cases by gender, age and the lack of esophageal lesions). The proteomic profile of their blood and esophageal tissue and tumor will be analysed for potential detection of tumor biomarker. These analysis will be compared with those obtained with experimental animals: two groups of Balb/C mice will be used, with one group (120 mice) being submitted to N-nitrosodiethylamine (NDEA) ad libitum in the drink water for up to 180 days, and one control group, composed by 20 animals. They will be sacrificed at each 30 days (20 animals at each time from NDEA group and one animal from control group) and will have their proteomic profile in the tumor and normal mucosa analysed. The proteomic profile in urine and blood of animals will be investigated at each 30 days.

Alternatively, biopsies of patients with breast and lung cancer at INCA will also be obtained. Haplogroups: DNA sequences obtained will be analyzed using algorithms based on sequences deposited in the MITOMAP data bank (http://www.mitomap.org/). PCR: 32 pairs of primers already described in the literature will be used (Tan et al, 2008). Conformational identification of mutations: PCR amplification products will be fractionated in a TTGE system (temporal
temperature gel electrophoresis), (BioRad Dcode TTGE). DNA sequencing: amplicons bearing mutations will be sequenced in an automatic DNA analyzer (Applied Biosystems, mod 3130, 4 capillaries) using the Big Dye dideoxy termination procedure according to the manufacturer’s recommendations.

All of the tissue samples will be collected under strict quality control procedures for biological samples, following the criteria of inclusion of patients, epidemiological, and clinical data collection of samples according to The National Tumor Bank of INCA.

**Goals:**

**Qualitative:** To improve the survival of patients with ESCC, lung and breast tumors, through the implementation in the clinic routine of biomarkers of early detection of these tumors.

To analyse and develop biomarkers of susceptibility and early diagnosis of ESCC and HNSCC.

**Quantitative:** 5 PhD and 9 MsC thesis, 18 scientific articles.

**Expected results and impacts:**

To identify biomarkers for early diagnosis of ESCC, lung and breast tumors. Impacts: economy for public health system. To apply it in screening programs for early detection of HNSCC and ESCC, improving the cost-effectiveness of these programs. Potential applications in other high risk groups, such as anonymous alcoholics. To increase the survival rate of patients with ESCC, NHSCC, lung and breast tumors.

To carry out therapeutic intervention based on the mechanisms of tumor development. Future impact in clinics through the implementation of these biomarkers on drug design.

Identification of mutations of mitDNA that may be used as tumor biomarkers. Characterization of haplogroups at those individuals affect to reveal cancer phylogeny and individual susceptibility.

**Previous experience in research area**

Dr. Ribeiro Pinto is the head of the group of molecular and clinical studies in esophageal cancer, registered in CNPq. This group has been working for the last ten years investigating different aspects of esophageal cancer, from the multi-factorial
effects of etiological factors in experimental models, analysis of individual susceptibility factors to environment agents, analysis of mechanisms of mutagenesis and DNA damage and repair in humans and experimental assays, genetic and epigenetic mechanisms involved in tumors development, etc…Specifically, Dr. Kruel’s group introduced the esophageal experimental carcinogenesis expertise in Brazil, and has been working in this area since 1990, having published over 10 articles since then. The focus of these experiments have been the evaluation in experimental animals of the risk factors for esophageal cancer. Dr. Guimarães showed recently that the profile expression of GBP2 possess the potential to be used as an esophageal tumor marker (this was done with a Swiss Bridge Foundation Project). In the last ten years, this CNPq group has published over 20 papers, two book chapters, 11 PhD and 24 MsC thesis in the area of esophageal cancer. Dr. Rumjaneck has taken part in in many different cancer projects in collaborations with Dr. Claudete Esteves Klumb, Dr. Marcos Paschoal, and Vivian Rumianek, with many papers published in the area.

References
Estimativa 2008: Incidência de Câncer no Brasil.
http://www.inca.gov.br/estimativa/2008/
Parkin et al., Eur. J. Cancer: 37, S4-S66 (2001)
Rossini et al., Carcinogenesis, 28: 2537-2542 (2007)
Chronogram (for two years and resumed for the following three)

Year 1, 1st semester: Clinical exams and collection of biological samples in patients with HNSCC and/or ESCC, bresat and lung tumors. Extraction of DNA and RNA from these samples. Experimental carcinogenesis.

Year 1, 2nd semester: Clinical exams and collection of biological samples in patients with HNSCC and/or ESCC, bresat and lung tumors. Extraction of DNA and RNA from these samples. Proteomic profile analysis of experimental carcinogenesis.

Year 2, 1st semester: Array of genomic instability of HNSCC and ESCC. Proteomic profile analysis of experimental carcinogenesis. GBP2 gene expression analysis. MitDNA amplification (parcial amplification of 300 bp fragments).

Year 2, 2nd semester: Array analysis. CGH and microsatellite sequencing of DNA tissue samples. Proteomics analysis of experimental assay and of clinical samples. GBP2 gene expression analysis. Sequencing of MitDNA fragments.

Year 3: CGH and microsatellite sequencing of DNA tissue samples. Proteomic profile analysis of clinical samples. GBP2 gene expression analysis. Sequencing of MitDNA fragments.

Year 4: Hypermethylation analysis in serum and tissues. Proteomic profile analysis of clinical samples. TP53 mutation analysis. Annotation of haplogroups, mutations, frequency, etc.. obtained from mitDNA.

Year 5: Polymorphism analysis. Interpretation of results and publications.

Financed projects in the last 5-years

CNPq: Universal 2004 (R$ 21700,00) and 2007, Produtividade em Pesquisa 2003 (bolsista 2) and 2006 (bolsista 1C and 1D).

Fundo de Pesquisa do Hospital das Clínicas de Porto Alegre: 3 projects financed in 2004, 1 in 2005, 2 in 2006, and 1 in 2007, with the amount per project between R$ 5000,00 and R$ 10000,00.

Capes: Project CAPES/COFECUB.

International: Swiss Bridge Foundation (2003-2008) - Oesophageal cancer project Swiss Bridge Foundation 2008 (275000 Swiss Francs).

Fundação Ary Frauzino (Program of Oncobiology FAF).

Local and International Interactions

Nationals:
Gastrocentro (Dr. Nelson Andreollo) da Universidade Estadual de Campinas, Serviço de Gastroenterologia (Sergio Gabriel da Silva Barros), Hospital das Clínicas, Universidade Federal do Rio Grande do Sul. Instituto Fernandes Figueira, (Dr. Dante Pagnoncelli) FIOCRUZ.

Internationals:
International Agency for Research on câncer (IARC), Lyon, France (Dr. Pierre Hainaut, Dr. Zdenko Herceg), Uppsala University, Sweden (Dr. Matti A. Lang), University College London, UK (Dr. Peter F. Swann), Universita degli Studi di Milano, Milão, Itália (Professor Luigi Bonavina), University Southern California, USA (Dr. Tom DeMeester). Dr. Yegor Vassetzky, Director of Research in Chromatin and Cancer, Institute Gustave-Roussy, Villejuif, Paris, France (project CAPES-COFECUB 287/2008).

Post-graduation Programs involved with:
- Post-graduation Program in Oncology (rated as 5) from National Institute of Cancer (Dr. Ribeiro Pinto is the Head).

- Post-graduation Program in Biology (rated as 6), and Post-graduation Program in Clinical and Experimental Physio-pathology (rated as 6), from State University of Rio de Janeiro.
- Post-Graduation Program in Surgery and Medicine (rated as 4) from Federal University of Rio Grande do Sul.

- Post-graduation Program in Biological Chemistry (rated as 7), from Federal University of Rio de Janeiro.
Sub-Project 2 – Prognostic and predictive biological markers

With the detection of a cancer additional information about treatment and treatment response are fundamental for planning therapeutic strategies. It can be carried out identifying characteristics of neoplastic cells or molecules produced by these cells providing data about therapeutic alternatives, disease progression, and treatment resistance. For instance, one of the important features of acute myeloid leukemia (AML) is the occurrence of cytogenetic and molecular abnormalities that are strongly predictive of response to induction therapy (Mrózek et al, 2001). Nevertheless although all the refinements in the diagnosis of cytogenetic abnormalities or mutation detection in AML and the advances in therapeutic approaches which improved the outcome of these patients the survival rate among adult AML patients is only 30%. This statement shows the demand for new findings that promise to improve cure rate (Grinwade et al. 2001). In this regard the use of recent technological advances to improve our capacity of treatment of adult AML patients and identify new biomarkers for prognoses and therapeutic outcome (focusing in patients with normal cariotypes) are mandatory.

In Chronic Myelogenous Leukemia (CML) the use of cytogenetic and molecular techniques are providing an incensement in therapeutic achievement providing data for patient therapeutic management by evaluation of tumor-load, a paramount parameter for prognosis. (Hochhaus, 2003; Gabert et al, 2003). Additionally to these approaches are the development of strategies for mutation detection at Tyrosine kinase-domain of BCR-ABL associated with treatment resistance to Gleevec – a tyrosine kinase inhibitor (Azam et al, 2003). Therefore, quantitative methods for mutation detection at BCR-ABL kinase-domain to evaluate treatment failure are fundamental for the establishment of therapeutic algorithms customized for each patient accordingly to a clinical-biological profile, and to differentiate distinct mechanisms of resistance against Tyrosine-kinase inhibitors (Branford et al, 2004).

In other tumors, like ovary and prostate cancer, biomarkers able to predict metastatic disease have to be described, once metastasis represent the most frequent cause of cancer death for patients with these tumors. Describing functional roles of gene products involved on metastasis formation, gene expression profiling of splicing isoforms and the humoral immune response against tumor associated
antigens (TAA), correspond to promising strategies for the description of new biomarkers and therapeutics approaches (Brito et al, 2008). Additionally in prostate cancer, the lack of a safe method for assessing the prognosis for these patients has led researchers in the field to search for new techniques. The prostatic stroma has a pivotal role in the pathogenesis of prostate adenocarcinoma (Condon 2005; Chung et al, 2005). In a recent study (Zhao et al, 2007) was suggested that ENPP2/autotoxin and the lisofosfatidic acid are the mediators between stroma and epithelium that are implicated in the prostate carcinoma. Recent also studies have shown that proteoglycans and their lateral glycosaminoglycan chains (GAG), as well as free GAG hyaluronan, play an important role in prostate pathology, what make them useful for diagnostic and prognostic purposes (Ricciardelli et al., 1997; 1998; Zellweger et al., 2003). Recently was showed that extracellular matrix of periacinar stroma permits a paracrine action of epithelial factors on the neighbors muscle cells (Babinski et al., 2007). Previous data about the accumulation of chondroitin sulphate proteoglycans around the acinar prostate support this hypothesis (Cardoso et al., 2004).

DNA metilation of the CpG islands in promoter regions of genes has emerged as an important inactivation mechanism of tumor suppressor genes in various malignant diseases (Laird, 2003). This strongly represents an alternative pathway to gene mutation or deletion for the loss of tumor suppressor genes. There is now an increasing evidence for the relevance of hypermethylation-associated gene silencing in the pathogenesis of human cancers, and markers for aberrant methylation seem to represent a promising avenue for monitoring the onset and progression of malignancies (Fiegl et al., 2005). To date, the role of aberrant methylation in childhood NHLs has not been investigated exhaustively, and previous studies have mainly focused on the p16/INK4a and p15/INK4b genes (Klangby et al, 1998; Lindström et al., 2001). These observations prompted us, to investigate the prognostic significance of the genetic and epigenetic events in a series of childhood NHLs.

In hereditary syndromes cancers strategies to identify and characterize mutations favoring tumor development are essential for genetic counseling. A pressing problem in risk assessment for individuals carrying mutations in BRCA1 is the dearth of information regarding the cancer association of hundreds of missense variants found in the population, called unclassified variants (UCVs). Individual UCVs
are usually very rare and, in some cases, specific to different ethnic groups, making meaningful epidemiological studies extremely hard to conduct. Previous work, showing the excellent correlation between results from a test based on the ability of BRCA1 to activate transcription and the genetic data, provides the proof of principle for such an approach (Carvalho et al., 2007, Karchin et al., 2006). On the other side, a continuous progress in mutation detection methodologies is required to characterize the ample spectrum of deleterious changes. Moreover, the identification of germ-line mutations in some hereditary tumor, like retinoblastoma, are very important for reproductive counseling (Barbosa et al, 2008).

There are yet many basic questions about the tumorigenesis process. It is a multistep process during which incipient cancer cells acquire a myriad of genetic and epigenetic alterations. Such alterations confer numerous changes in cell behavior, including inappropriate survival and proliferation (Hanahan & Weinberg, 2000). The emergence of RNA interference (RNAi) as a mechanism to suppress gene expression has revolutionized mammalian genetics allowing the identification of gene functions on a “forward genetic approach”. More recently, “short-hairpin RNA” (shRNA) libraries targeting all the expressed genome of organism has began to facilitate decoding of gene functions on a genome scale.

**Objectives**

The main purpose of this project is to characterize biological markers for tumor progression and therapeutic response, describe and evaluate functional aspects of genetics variants of tumor suppressor genes (related to hereditary cancer) and identify new tumor suppressor genes.

The objectives are:

1. To introduce proteomic approaches in the study of homogeneous adult AML patients according to their clinical, morphologic, immunophenotypic and genetic profile in order to identify biomarkers with prognostic relevance, especially in those patients with normal karyotype where markers have never been found;
2. To define the response of CML patients to different tyrosine Kinase inhibitors using multiple diagnostic methods and to dissect its biological heterogeneity by applying and combining in a comprehensive analysis different technologies, such as Real Time PCR (RQ PCR) and sequencing for mutation detection at BCR-ABL Tyrosine kinase domain;
3. To Investigate the aberrant promoter methylation pattern of genes PIG7/LITAF, NOXA and BIM encoding proteins implicated in apoptosis regulation; to correlate the methylation status of individual genes with clinicopathological parameters and to determinate the association among methylation patterns and overall survival and event-free survival of NHL patients;
4. To correlate the possible stroma and epithelium acinar components modifications in the prostate cancer with Gleason score.
5. To investigate the functional roles of differentially expressed genes between primary and metastatic prostate cancer and ovary cancer to characterize markers with predictive and/or metastatic-disease outcome potential.
6. To evaluate by functional assays unclassified variants from BRCA1 and its relationship with breast and ovary tumor susceptibility;
7. To conduct a mutation detection survey, at sequence level and for chromosome rearrangements in RB1 from paraffin embedded tumor and fresh tumor samples of Retinoblastoma patients. To find familial cases of retinoblastoma and to identify related individuals at potential risk of developing tumours and / or transmitting RB1 mutations, and to include these patients in Genetic Counselling programmes
8. To identify genes with tumor suppressor activities using assays based on RNA interference with a retroviral shRNA library for mouse genome.

**Experimental Design**

**Proteomics of Acute Myeloid Leukemia**

This project is a collaborative effort to take advantages of recent technological advances to improve our capacity of treatment of adult AML patients identifying and validating new prognostic biomarkers capable of predicting prognosis and the therapeutic response especially in those patients with normal karyotype where markers have never been found. To achieve this objective we will add proteomic analysis, for which we have the necessary infrastructure and expertise, to our arsenal of cellular and molecular analysis routinely used in our institution to diagnosis AML as cytomorphology, immunophenotyping, conventional cytogenetics, fluorescence in situ hybridization and molecular biology.

For this analysis bone marrow or peripheral blood leukemic cells will be analyzed for their protein profile including protein modifications by several proteomic approaches as 2D gel electrophoresis and matrix-assisted laser desorption/ionization
time of flight mass spectrometry MSMS and Multidimensional protein identification technology (MudPIT) analysis. These profiles will be stratified according to patient FAB subtype, cytogenetic group, genetic profile, risk group and therapeutic response. As controls we will analyze a cohort of bone marrow mononuclear cells from healthy donors. Proteins differentially expressed in each class of patients will be considered as biomarkers. All biomarkers of interest will be confirmed for their altered expression in several other AML samples by Western-blot whenever antibodies where available and by Real-Time RT–PCR. The correlation of proteomic data with all other criteria used routinely to diagnose adult AML patients in our institution will contribute to stratify new subtypes and will indicate new targets to the development of rational drugs leading to a better cure rate for these patients.

**BCR-ABL Mutations and Treatment Resistance**

CML patients. Eighty-five patients of the Hematology Service (Instituto Nacional de Câncer - INCA), Rio de Janeiro are already enrolled in an ongoing molecular study. We expect to include some 30 new CML patients/year from INCA, as well as some 75 patients from other Centers. The total number of patients expected to be followed-up during this project is ~190 patients/year. Each sample will be processed after red cell lysys. c-DNA will be synthesized from 2 μg of total RNA. The absolute quantitation of BCR-ABL transcripts will be carried out by a validated TaqMan® assay in a Applied Biosystems 7000 thermocycler, including specific probes labeled 5'FAM-3'TAMRA and primers for P210 b3a2 e b2a2 isoforms. BCR-ABL levels will be estimated by the BCR-ABL/ABL ratio in comparison with a baseline group. Quinase Domain mutations will be identified by AS-PCR and direct sequenced in a MegaBACE 1000 automated sequencer. A semi-quantitative approach will be used to relatively quantify mutant and non-mutant clones, through a RFLP-based method and subsequently confirmed by pyrosequencing, in collaboration with Drs Jaspal Kaeda and John McVey, from the Hammersmith Hospitals Trust, London, UK.

**Aberrant Methylation Pattern in NHL**

Patients and controls: 80 untreated pediatric patients with diagnosed NHLs (60 B-cell and 20 T-cell) will be included in this study. The selection of cases will be based on the availability of paraffin-embedded tissue (PET) blocks for molecular and immunohistochemical analysis. Twenty peripheral blood samples from healthy
persons will be used as control to assess the methylation status of the target genes in normal lymphocytes.

Methylation-specific PCR assays: Genomic DNA will be extracted from PET samples. Extracted DNA will then subjected to chemical treatment with sodium bisulfite as previously described (Herman et al., 1996). The presence of bisulfite-modified DNA in each sample will be determinate by amplification of a fragment of B-actin gene. Promoter methylation status will be analyzed by methylation-specific PCR (MSP) using methylated and unmethylated gene-specific primers for the genes PIG7/LITAF, NOXA (p53 inducible genes) and BIM involved in the apoptosis pathway.

Immunohistochemical (IHC) analysis on tissue microarrays: The expression of PIG 7/LITAF, NOXA and BIM protein will be analysed by IHC on tissue microarrays (TMA) using a TMA device (Beecher Instrument, SilverSpring, MD, USA) and antibodies for LITAF, NOXA and BIM proteins.

Statistical analysis: Pairwise correlations among genes and between methylation status of individual genes and clinicopathological parameters will be investigated by chi-square test and Fisher’s exact test. To determinate the association between hypermethylation of individual genes and overall survival and disease-free survival the log-rank test of Kaplan-Meier will be used. All the analyses will be carried out with SPSS software package, version 11.5.

**Biomarkers for Cancer Prostate and Ovary Cancer Progression**

Genes that will be studied in this project will be selected based on literature data and on previous results from our group and also by the “biopanning” strategy. The validation of gene expression profiling will be performed using qRT-PCR, immunoblot, immunohistochemistry by tissue microarray and in silico. Functional assays will be performed by stable protein overexpression and interference RNA in established tumor cell lines. The biological effects of these mentioned treatments on cell proliferation, migration and invasion will be evaluated as representing events of tumorigenesis and tumor progression of these neoplasia. Tumor associated antigens will be tested in immunological assays using a serum bank from both neoplasia.

**Stroma and epithelium acinar components modifications in the prostate cancer and Gleason score**

Thirty prostates from radical prostatectomy patients will be separated into groups according to Gleason score. Group 1: Gleason 2-6, group 2: Gleason 7,
group 3: Gleason 8-10. The followed techniques will be used: 1- Histochemistry followed by morphometry and stereology - Picrosirius red with and without polarization to show collagen; Weigert resorcin-fuscin with and without previous oxidation with oxon to show elastic fibers and Masson trichromic to show collagen and muscle fibers. 2- Immunohistochemistry followed by morphometry and stereology. Specific antibodies will be used to stain of collagen types I, II, IV and VI and the non collagen protein such as laminin, fibronectin and entactin. 3- Transmission and Scan Electron Microscopy: The prostate tissues will be fixed with 2.5% glutaraldeide and processed with routine technique. 4- Proteoglycan, glycosaminoglycan and collagen biochemistry study: The followed parameters will be studied: 1) tissue molecules concentration; 2) proportion of the different glycosaminoglycans chains. 5- RNA interference assays to determine the possible role of genes differentially expressed in the prostate cancer and its relation with Gleason score. 6- Western blot to analyze the expression of proteins differentially expressed in the prostate cancer and its relation with Gleason score. 7- Real time PCR to analyze the expression of genes differentially expressed in the prostate cancer and its relation with Gleason score.

**Functional evaluation of BRCA1 unclassified variants**

In order to overcome the limited cover of TA we will test a variation of the transcription-based assay to assess the impact of several UCVs (identified in the Breast Cancer information Core Database) on the activity of BRCA1 C-terminal region extended to exon 12 and part of exon 11, and use the available genetic data to cross-validate our test. In this analysis we will test the activity of fusions of heterologous DNA binding domains (DBD; LexA or GAL4) to the C-terminus of BRCA1 (in the context of amino acids 1315 to1863) carrying missense mutations in yeast and mammalian systems. The ability to activate transcription is assessed using a reporter driven by a promoter containing LexA or GAL4 binding sites (Carvalho et al., 2007).

**RB1 Mutations in Retinoblastoma**

Samples from 30 patients per year, diagnosed with retinoblastoma at Instituto Nacional de Câncer, will be included in this study. Exons of RB1 gene will be amplified by PCR from paraffin embedded tumor samples and analyzed by SSCP. Fragments with differential migration will be sequenced. Pathogenic sequence changes will be also analyzed in DNA samples isolated from blood to characterize
constitutive mutations. Fresh tumor samples will be evaluated for chromosome rearrangements.

**RNA interference and characterization of Tumor Suppressor Genes**

We propose the use of a genome-wide loss of function screen for growth factor independent survival using a short-hairpin RNA” (shRNA) libraries that targets all the expressed mouse genome. For this library, the cloned shRNAs are framed by naturally occurring mir30 sequences, which results in increased stability and effectiveness (Paddison et al., 2004; Stegmeier et al., 2005; Silva et al., 2008). Initially, we will look for shRNAs that allow FL5-12/BAF 3 cells (non-transformed murine pro-B lymphocytes lineage) to survive growth factor withdrawal, in this case IL-3. “Hits” from the in vitro screen are candidate tumor suppressor genes and will be directly tested in a mouse model of Burkitt lymphoma (Eµ-μc mouse). We will use retroviral vectors to introduce selected shRNAs from the screen into Eµ-μc transgenic liver hematopoietic stem cells and subsequently transplant into lethally irradiated wild type recipient animals. Recipient mice will be monitored for tumor development by blood counts and palpation. And acceleration of tumor development would indicate co-operative effect of the sRNA and the Myc oncogene. Finally, we wish to analyze the clinical relevance of genes identified in the screen and in our in vivo studies. In collaboration with clinicians of INCA’s Hematology Department, we will analyze expression and mutational patterns in human tumors, particularly in Burkitt’s samples. Overall, we will use an innovative genomics approach to identify candidate tumor suppressors in vitro. We will then functionally evaluate genes identified in the screen in a mouse model of lymphoma and analyze expression and mutational patterns in clinical specimens.

**Team**

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**Goals**

**Quantitative**

- To characterize potential prognostic biological markers for different tumors.
- Development of methodologies and strategies for characterization of new prognostic biological markers.
- Transfer of technology from research to diagnosis.
- To provide new technicians trained in new diagnostic technologies.
- Improve the national health system.
- Optimization of target specific therapies focusing cost-benefit aspects

**Quantitative**

- To publish 85 articles.
- To conclude 45 PhD thesis.
- To conclude 30 MsC dissertations.
Results and Impacts

Proteomics of Acute Myeloid Leukemia

The most serious type of hematological cancer is acute myeloid leukemia (AML). Particularly in elders above 60 years old, the survival rate is less than 30% and only a few reach cure. Despite the accurate techniques in diagnosis and sub-classification, an important number of patients do not fit in any of the present cytogenetic and molecular prognostic group. Therefore, there is a great demand for studies that show how to allocate these patients in the favorable and unfavorable subsets of clinical response to treatment. As these genetic and molecular classifications are insufficient for that matter, a new approach to fill this need is the study of AML cellular proteins. In order to do that, proteomics is a promising tool because it becomes possibly to study the functional state of a cell. For instance, it might allow inferring how the AML cells are going to respond to a certain type of chemotherapeutic drug. This will prevent unnecessary toxic effects of unworthy treatment and improve the selection of the most adequate drug to eliminate particular proteomic subtypes of AML.

Aberrant Methylation Pattern in NLH

Although there are some evidence that genetic events like p53 mutations are related to prognosis in specific types of cancers including pediatric NHL, the influence of epigenetics alterations have not been fully explored so far. The outcome of these patients may be difficult to predict based on single genes studies. Our study emphasize the need of amplifying the knowledge of genetics and epigenetics events involved in the pathogenesis and prognosis of pediatric NHL. Furthermore, it may allow the development of novel therapeutic targets and new approachs for monitoring the disease through specific methylation pattern which could be monitored in patient’s plasma samples during treatment.

BCR-ABL Mutations and Treatment Resistance

The diagnostic uniformization of CML molecular response to TK inhibitors as well as the identification of tyrosine kinase domain mutations are predictive factors of therapeutic response with practical repercussion to patients. This study will allow the stratification of patients according the chance of response to a TKI of first generation. Patients with no chance from beneficial with this therapeutic modality will be re-
directed to other modality with more chance to be cured in an initial phase of the disease. At the same time the molecular classification of the resistance can aid to define targets for new therapeutic approaches as T315I.

**Biomarkers for Cancer Prostate and Ovary Cancer Progression**

To characterize genes and proteins relevant for metastasis diagnoses and useful as biological markers or therapeutic targets against disease progression in prostate cancer and ovary cancer.

**Structural, Ultrastructural, Biochemistry and Molecular Characterization of Prostate Carcinoma with Different Gleason’s Scores**

To characterize the role of the different extracellular matrix components and smooth muscle fibers in the prostate cancer and its relation with Gleason score.

**Functional evaluation of BRCA1 unclassified variants**

To improve the cover of BRCA1 transcription activation assay for cancer association determination. Increase the number of BRCA1 missense variants screened for cancer risk association. Disclosure cancer risk association data to improve genetic counseling and treatment.

**RB1 Mutations in Retinoblastoma**

To obtain original data on the incidence and localisation of RB1 mutations in the Brazilian population. To improve the quality of attention to patients and relatives, and to establish a national Retinoblastoma network in Brazil.

**RNA interference and characterization of Tumor Suppressor Genes**

To identify genes evolved in apoptosis and survival signaling through the use of a shRNA library that silences most of the expressed mouse genome. Identify shRNAs selected in the in vitro screen that can cooperate with Myc induced lymphomagenesis in the Eµ-Myc mouse model. Analyze the clinical relevance of genes identified in the in vivo studies using clinical samples.
Previous experience in research

At CEMO (Bone Marrow Transplantation Center at Instituto Nacional de Câncer), where the research and laboratories are coordinated Dr Eliana Abdelhay, we have been studying acute leukemia’s for more than ten years. Several studies dealing with genetic background and trying to define karyotypic profile of Brazilian patients have been performed. In the last years other studies trying to better characterize these patients in terms of their prognostic outcome were done using molecular biology approaches. Approximately two years ago we started a proteomic facility at CEMO to give continuity to our work with CML proteomics (Pizzatti et al 2006). In this first study we compared normal mononuclear cells with chronic phase patients mononuclear cells and could find some interesting biomarkers of this first phase of the disease. Subsequently we analyzed mononuclear cells from patients in chronic and blastic phase. We also analyzed patients sensitive to Gleevec and those who were resistant. The results obtained in these analysis are now being prepared for publication. Also during these two years period we started the analysis of AML cells. Until now we collected 75 protein samples and have analyzed 37. Comparing these 2D profiles some specific differences related to FAB subtype, chromosomal rearrangement, mutational status have been already identified. More than 700 peptides have been identified by MS/MS and a great number of post translational modifications were detected. All the patients are being followed and samples collected for a therapeutic response analysis. To continue this analysis we will finish the analysis and statistical correlations of these 75 samples and will include in this study 100 more patients.

Dr Claudete Esteves Klumb (Klumb CE) MD, PhD graduated by Rio de Janeiro Federal University and has been working at Hematology Service of National Cancer Institute, Rio de Janeiro, Brazil since 1987. During this period, she has been involved with activities straight for diagnosis, follow-ups, prognosis and efficiency of therapeutic protocols of patients with leukemias and lymphomas. After her PhD post-graduation, Klumb CE established a research line focusing on the pathogenesis and prognostic factors of childhood lymphomas, which is developed at the Laboratory of Molecular and Cellular Hematology, headed by Maia RC. This work resulted in 20 publications on international scientific journals (the last three ones, in press) and presentations in local academic events. Klumb CE is professor of Post-graduate Strictu Sensu Oncology Program of INCA where she teaches regular courses, as
well orientates undergraduate and post-graduate students from biological and medical areas. In addition, Klumb CE is a boarder member of Undergraduate Training Program at INCA. The main purpose of this program is to stimulate scientific mind among young undergraduate students in the setting of research laboratories.

The group coordinated by Dr. Ilana Zalcberg Renault are conducting studies in treatment resistance in CML and since 1996 the group carried out studies in: (1) haematopoietic chimerism surveillance in alogeneic transplantation; (2) Post-Transplantation surveillance I Chronic Myeloid Leukemia Leucemia Mielóide Crônica; (3) biologic-molecular classification of Acute Mieloid Leukemia (AML) from adults and children; (4) identification and definition of prognostic subgroups of Diffuse Large B-Cell Lymphoma based on genetic characteristics; (5) Genetic and Epigenetic changes in Multiple Myeloma Alterações Genéticas e Epigenéticas no Mieloma Múltiplo; (6) Study of gene receptors rearrangements in Acute Lymphoid Leukemia (7) Prognostic value of somatic hypermutation in VH-genes in Chronic Lymphocytic Leukemia.

The team members of the Urogenital Research Unity, UERJ (www.urogenitalresearch.org), coordinated by Dr. Francisco Sampaio, dedicate their time to get new knowledge about urogenital system in a basic and clinical view. The research is conducted using different models, including clinical and experimental models with a great variety of methods. The Unity has its own specialized laboratories including anatomic and surgery, experimental surgery, extracellular matrix biochemistry, histochemistry, immunohistochemistry, electron microscopy, cell culture, molecular biology, and so on. Also the Unity has a strong collaboration with other departments inside and outside the University, including other countries, such as USA and France. The important characteristic of this Unit is the strong relation between experimental and clinical research that makes the work stimulant and productive. In the last 5 years the Urogenital team published more than 40 papers and had more than 13 master thesis and 11 doctor thesis approved.

Dr. Etel Pereira Gimba has publications about biomarkers characterization in prostate cancer and kidney carcinoma (Brito et al. Molecular Carcinogenesis 17, DOI 10.1002/mc.20433, 2008; Pontes et al., The Prostate 66:1463, 2006) and works as researcher at Instituto Nacional de Câncer.

Dr. Marcelo Alex Carvalho is very well positioned to undertake the proposed research. The laboratory is dedicated to genes that are
involved in predisposition to breast cancer using an interdisciplinary approach that involves biochemistry, molecular biology, genetics, structural biology and bioinformatics with recognized expertise (see section 4: literature list). Dr. A. Monteiro, from H. Lee Moffitt Cancer Center (Tampa, FL, USA) - who originally described BRCA1 transcription activation property, is our collaborative partner. The group has proposed a framework for the use of the transcription assay to help classify BRCA1 alleles, performing structure-function analysis to predict the outcome of mutations in BRCA1. This is significant because it allows early detection of women predisposed to cancer that currently have no information regarding their risk. We focus on rapidly using information from basic research to provide a direct benefit to the patient. These results will also have considerable significance in identifying new targets for preventive and therapeutic intervention against women's cancers.

The Genetic Counselling Group, at the Genetics Division of the Instituto Nacional de Câncer (INCA), coordinated by Dr. Héctor N Seuanez, has been studying 190 families of patients with retinoblastoma since 2000. This Group is the only one in Brazil dedicated to the identifications of mutations and cytological aberrations in patients with retinoblastoma. Patients are referred by the Paediatric Department of INCA, Hospital de Servidores do Estado do Rio de Janeiro, Faculdade de Medicina de Ribeirão Preto and Hospital das Clínicas de Porto Alegre. At the same time, we carried out retrospective studies in formalin-fixed, paraffin-embedded tumour tissue of the Pathology and Cytopathology Service of INCA. Studies of our Genetic Counselling Group at the Instituto Nacional de Câncer of Brazil were published by Braggio et al. (Journal of Clinical Pathology 57:585-590, 2004), Andrade et al. (Cancer Genet Cytogenet 167:43-46, 2006) and Barbosa et al. (Pediatric Blood and Cancer, no prelo, DOI 10.1002/pbc.21687). Finally, three MSc dissertations were produced by our post-graduate students (Braggio 2002, Andrade 2004, Barbosa 2006).

Dr Ricardo Luis Alves Silva is researcher at Instituto Nacional de Câncer, has experience in the area of Genetics, with emphasis on human and medical genetics, focused, mainly, in the subjects: breast cancer, ERBB2, bioinformatics, molecular markes. He has been trained in the use of in vivo RNAi technology, using the Eu-Myc mouse model, and in genome wide screen using RNAi for the identification of tumor suppressor genes during one year training as “Research Associate” at Memorial Sloan Kettering Cancer Center (New, USA).
References

Cardoso et al. BJU Int. 93:532-538 (2004)

Chronogram

Year 1, 1st semester
- Adult AML sample collection and protein extract preparation.
- Patient and controls sample selection for methylation analysis in NHL.
- Selection of patient samples for resistance studies to CML treatment. RNA extraction, cDNA synthesis and Q-PCR for BCR-ABL transcripts.

- Collection of the samples and histophatological analysis to determine the Gleason score in prostate cancer. Histochemistry followed by morphometry and stereology. Picrosirius red with and without polarization to show collagen; Weigert resorcin-fucsin with and without previous oxidation with oxon to show elastic fibers and Masson trichromic to show collagen and muscle fibers. Proteoglycan, glycosaminoglycan and collagen biochemistry study.

- Sample collection of tumoral tissues from ovary cancer.

- Identification and selection of BRCA1 missense variants localized in the region limited by amino acids 1314-1396 (exon 12 and part of exon 11) in the Breast Cancer Information Core-BIC (http://research.nhgri.nih.gov/bic/). Identification of a BRCA1 missense variants sub-population (in the region limited by amino acids 1314-1396) with available genetic cancer association data.

- DNA isolation from retinoblastoma tumor samples embedded in paraffin. Chromosome analysis and FISH from fresh tumor samples. Multiplex PCR for exon amplification of \( RB1 \), SSCP and DNA sequencing of selected fragments.

- In vitro experiments with shRNAs libraries and FL5-12/BAF 3 cells (non-transformed murine pro-B lymphocytes lineage) growth factor withdrawal conditions.

**Year 1, 2\(^{nd}\) semester**

- Adult AML sample collection, protein extract preparation and bidimensional (2D) gel analysis.

- Patients and controls sample selection, DNA extractions, methylation assay with sodium bisulfite, PCR assays, Tissue Microarray (TMA) construction, immunohistochemistry.

- RNA extraction, cDNA synthesis from CML samples. Q-PCR for \( BCR-ABL \) transcripts. PCR amplification of Tyrosine kinese domain, followed by DNA sequencing and analysis.

- Histochemistry followed by morphometry and stereology from prostate tumors. Picrosirius red with and without polarization to show collagen; Weigert resorcin-fucsin with and without previous oxidation with oxon to show elastic fibers and Masson trichromic to show collagen and muscle fibers.
Proteoglycan, glycosaminoglycan and collagen biochemistry study: The followed parameters will be studied: 1) tissue molecules concentration; 2) proportion of the different glycosaminoglycans chains. Western blot to analyze the expression of proteins differentially expressed in the prostate cancer and its relation with Gleason score. Immunohistochemistry followed by morphometry and stereology. Specific antibodies will be used to stain of collagen types I, II, IV and VI and the non collagen protein such as laminin, fibronectin and entactin.. Real time PCR to analyze the expression of genes differentially expressed in the prostate cancer and its relation with Gleason score. Paper submission.

- Serum and tumor samples collection from patients with ovary and prostate cancer. Selection of genes differentially expressed and expression profile validation by Real-Time PCR. Characterization of splicing isoforms of OPN protein with functional and immulologic assays. Tumoral antigens characterization by immunologic assays.

- Identification of a BRCA1 missense variants sub-population (in the region limited by amino acids 1314-1396) with available genetic cancer association data. Identification of conservative residues among BRCA1 orthologs localized in the region limited by amino acids 1314-1396 (exon 12 and part of exon 11) for design of synthetic variants with a putative neutral and deleterious profile. Generation of BRCA1 selected variants by overlapping extension site-directed mutagenesis.

- DNA isolation from retinoblastoma tumor samples embedded in paraffin. Chromosomal preparations from fresh tumor samples and FISH. Multiplex PCR for exon amplification of RB1, SSCP and DNA sequencing of selected fragments. Sequence data analysis for mutation identification.

- In vitro experiments with shRNAs libraries and FL5-12/BAF 3 cells (non-transformed murine pro-B lymphocytes lineage) in growth factor withdrawal conditions. Selection of shRNAs for retroviral construction and infection into \( E\mu\text{-}myc \) transgenic liver hematopoietic stem cells and subsequently transplant into lethally irradiated wild type recipient animals.
Year 2, 1st semester

- Adult AML sample collection and preparation of protein extract; 2D gel analysis; mass spectrometry; AML proteomic profiles description.
- Patients sample selection, DNA extractions, methylation assays with Sodium Bisulfite, PCR assays, Tissue Microarray (TMA) construction, immunohistochemistry.
- RNA extraction, cDNA synthesis from CML samples. Q-PCR for BCR-ABL transcripts. PCR amplification of Tyrosine kinase domain, followed by DNA sequencing and analysis.
- Immunohistochemistry followed by morphometry and stereology from prostate tumors. Specific antibodies will be used to stain of collagen types I, II, IV and VI and the non collagen protein such as laminin, fibronectin and entactin. Western blot to analyze the expression of proteins differentially expressed in the prostate cancer and its relation with Gleason score. Real time PCR to analyze the expression of genes differentially expressed in the prostate cancer and its relation with Gleason score. RNA interference assays to determine the possible role of genes differentially expressed in the prostate cancer and its relation with Gleason score.
- Identification of a BRCA1 missense variants sub-population (in the region limited by amino acids 1314-1396) with available genetic cancer association data. Identification of conservative residues among BRCA1 orthologs localized in the region limited by amino acids 1314-1396 (exon 12 and part of exon 11) for design of synthetic variants with a putative neutral and deleterious profile. Generation of BRCA1 selected variants by overlapping extension site-directed mutagenesis.
- DNA isolation from retinoblastoma tumor samples embedded in paraffin. Chromosomal preparations from fresh tumor samples and FISH. Multiplex PCR for exon amplification of RB1, SSCP and DNA sequencing of selected fragments. Sequence data analysis for mutation identification.
- Selection of shRNAs for retroviral construction and infection into $E_{\mu}-myc$ transgenic liver hematopoietic stem cells and subsequently transplant into lethally irradiated wild type recipient animals. Selection of clinical tumor samples.

**Year 2, 2\textsuperscript{nd} semester:**

- Adult AML sample collection and preparation of protein extracts; 2D gel analysis; mass spectrometry; AML proteomic profiles description; MULTIPID analysis; data stratification according sample classification.
- Patient’s sample selection, DNA extractions, methylation assays with Sodium Bisulfite, PCR assays, Tissue Microarray (TMA) construction, immunohistochemistry; statistical analysis; presentations in meetings and publications.
- RNA extraction, cDNA synthesis from CML samples. Q-PCR for $BCR-ABL$ transcripts. PCR amplification of Tyrosine kinese domain, followed by DNA sequencing and analysis and data analysis.
- Transmission and Scan Electron Microscopy. Real time PCR to analyze the expression of genes differentially expressed in the prostate cancer and its relation with Gleason score. RNA interference assays to determine the possible role of genes differentially expressed in the prostate cancer and its relation with Gleason score. Presentation of master and doctor’s thesis of students that participate of this project. Papers submission.
- Generation of BRCA1 selected variants (both naturally occurring and synthetic) by overlapping extension site-directed mutagenesis. Missense variants cloning into pLex9 and pGBT9 and sub-cloning into pCDNA3. Functional evaluation of synthetic variants localized in the region limited by amino acids 1314-1396 (exon 12 and part of exon 11) using the transcriptional activation assay in yeast system.
- DNA isolation from retinoblastoma tumor samples embedded in paraffin. Chromosomal preparations from fresh tumor samples and FISH. Multiplex PCR for exon amplification of RB1, SSCP and DNA sequencing of selected fragments. Sequence data analysis for mutation identification. Manuscript preparation for publication.

- Selection of shRNAs for retroviral construction and infection into \( E_{\mu}-myc \) transgenic liver hematopoietic stem cells and subsequently transplant into lethally irradiated wild type recipient animals. Selection of clinical tumor samples and Tissue Microarray.

**Year 3-5**

- For the next three years in proteomic analysis of adult AML, additional 2D gel analysis and mass spectrometry will be carried out to provide an AML proteomic profile. MULTIPID analysis will be carried out. Data stratification will be done accordingly to patient’s FAB subtype, cytogenetic group, genetic profile, risk group and therapeutic response. Selected biomarkers will be confirmed for their altered expression in several other AML samples by Western-blot whenever antibodies where available and by Real-Time PCR. Manuscripts will be prepared during the last three years.

- In the following 3 years we will expand the methylation analysis for other genes potentially involved in the prognosis of paediatric NHL and the methylation results will be matched to protein expression studies by immunohistochemistry. Western blotting analysis will also be performed in selected cases.

- Following the analysis of mutations at Tyrosine kinase domain of BCR-ABL associated with treatment resistance in CML patients, additional clinical data will be collected and new patients will be included.

- For prostate cancer we intent to continue the characterization of genes differentially expressed in the prostate cancer evidenced by biochemistry, western blot and real time PCR techniques. Presentation of master and doctor’s thesis of students that participate of this project and papers submission.
- In ovary and prostate cancers selected biomarkers for metastasis will be validated by in vivo assays. Antigen panels will be tested in serum samples collected in different institutions. Potential therapeutic targets will be evaluated.

- For functional analysis of BRCA1 unclassified variants functional evaluation of synthetic and naturally occurring variants localized in the region limited by amino acids 1314-1396 (exon 12 and part of exon 11) using the transcriptional activation assay in yeast system and in mammalian system, will be carried out. Experimental results using available genetic cancer association data will be validated.

- New patients diagnosed with retinoblastoma will be included for molecular and cytogenetic analysis.

- Concerning the description of genes with tumor suppressor activities experiments with in vitro and in vivo models will be carried out and additictional human tumor samples will be collected to make new Tissue Microarrays. The results will be published.

**Financed projects in the last 5-years**

**Nationals**

- CNPQ - 06/2005 - Estudos cito-moleculares para a identificação de grupos de risco terapêutico e marcadores de prognóstico em pacientes adultos e pediátricos com Leucemia Mielóide Aguda (LMA). Principal investigator: Ilana Zalcberg-Reanault (R$ 70.000,00).


- CNPq- Ed Apoio às Atividades de Pesquisa em Genética Clínica - Rede Nacional de Câncer Familiar. Coordenador: Héctor N Seuanez Abreu (R$ 455.000,00).
- CNPq-Ed Neoplasias 2005/2006 – Identificação e validação de biomarcadores da resposta preditiva ao tratamento em pacientes adultos com leucemia mielóide aguda. Principal investigator: Eliana Abdelhay (R$ 50.000,00).
- CNPq-Ed.Universal 2005/2006- O proteoma como uma ferramenta na identificação de marcadores moleculares de prognóstico em LMC. Principal investigator: Eliana Abdelhay (R$ 30.000,00).
  Principal investigator: Marcelo Alex de Carvalho.
  Principal investigator: Marcelo Alex de Carvalho.
  Principal investigator: Marcelo Alex de Carvalho.

International
- Fundação Swissbridge (Suiça) - Heterogeneidade molecular das leucemias e linfomas- Principal investigator: Héctor N Seuanez Abreu (CHF 838.000,00).

Local and International Interactions

Nationals
- Hospital AC Camargo-SP - Dr. Fernando Soares na área de patologia molecular e microarranjos de tecidos. Interaction with Etel Rodrigues Pereira Gimba.
- Hospital Municipal Souza Aguiar, RJ (Dr. AG Cavalcanti). Interaction with Francisco José Barcelos Sampaio.
- Hospital Universitário Clementino Fraga Filho, UFRJ- Dr. Rony Schaffel, Médico do Serviço de Hematologia. Interaction with Ilana Zalcberg-Reanult.
- Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro (UFRJ)- Dr Mariano Zalis. Laboratório de Infectologia e Parasitologia Molecular. Interaction with Cláudia Esther Rocio Hassan.
- Hospital Universitário da Universidade federal de Juiz de Fora- Dr. Angelo Attala, Médico do Serviço de Hematologia. Interaction with Ilana Zalcberg-Reanult.
- Hospital Universitário Oswaldo Cruz, Universidade de Pernambuco, Recife - Dra Tereza Cartaxo Muniz. Centro de Oncologia Pediátrica -CEON). Interaction with Cláudia Esther Rocio Hassan.
- InCor – SP - Grupo de Biologia Computacional do Laboratório de Genética e Cardiologia Molecular), coordenado pelo Dr. Paulo Sergio Lopes de Oliveira. Interaction with Etel Rodrigues Pereira Gimba.
- Universidade de São Paulo(USP), São Paulo, Departamento de Patologia - Carlos E. Bacchi da. Interaction with Claudete Esteves Klumb.
- Universidade Federal de Urbelândia - Grupo do Dr Luiz Ricardo Goulart Filho, em estudos funcionais de proteínas envolvidas na progressão no CaP (Dr. Carlos Moreno, Emory University, Atlanta, EUA). Interaction with Etel Rodrigues Pereira Gimba.
- Universidade Federal Fluminense- Dr. Adelmo Daumas Gabriel, MD Médico do Serviço de Hematologia. Interaction with Ilana Zalcberg-Reanult.
- Universidade Federal Fluminense, RJ. Dr. HJ Bagetti Filho. Interaction with Francisco José Barcelos Sampaio.
Internationals

- Asociación Española Primera de Socorros Mútuos, Montevideú, Uruguay - Dr. María del Rosario Uriarte, PhD. Interaction with Ilana Zalcberg-Reanult.


- H. Lee Moffitt Cancer Center & Research Institute, Laboratory of Cancer Genetics Tampa, FL, USA. Dr. Alvaro N. A. Monteiro, PhD (pesquisador responsável). Interação Responsável: Marcelo Alex de Carvalho.

- Hospital de Pediatria “Ricardo Gutierrez”, Buenos Aires, Argentina. - Dras Maria Victoria Preciado e Paola Chabay. Laboratório de Virologia. Interaction with Cláudia Esther Rocio Hassan.

- Hospital Hammersmith, Londres UK - Dr. Jaspal Kaeda Departamento de Hematologia. Interaction with Ilana Zalcberg-Reanult.

- Institute of Human Genetics and Anthropology, Jena, Germany - Dr Thomas Liehr. Interaction with Eliana Abdelhay.

- Instituto de Puericultura e Pediatria Martagão Gesteira UFRJ - Dr Marcelo Land,. Interaction with Eliana Abdelhay.

- St Jude Children’s Research Hospital-Memphis, TN - Dr Susana Raimondi-Department of Pathology-. Interaction with Eliana Abdelhay.

- St Jude Children’s Research Hospital-Memphis, TN Dr Raul Ribeiro-Department of Oncology. Interaction with Eliana Abdelhay.

- UNESP (Convênio genérico para estabelecimento de programa de cooperação acadêmica) . Interaction with Francisco José Barcelos Sampaio.

- Universidade de Caen, França - Dr. Serge Carreau. Interaction with Francisco José Barcelos Sampaio.

- Universidade Stanford, CA, Estados Unidos (Dr. H. Zhao e Dr. D. Peehl). Interaction with Francisco José Barcelos Sampaio.


- University Hospital Göttingen, Göttingen, Germany - Prof. Dieter Kube, Department of Haematology and Oncology. Interaction with Cláudia Esther Rocio Hassan.
- University of Cincinnati Medical Center - Grupo Dr. Geord F Weber EUA caracterização de isoformas de “splicing” da proteína OPN. Interaction with Etel Rodrigues Pereira Gimba.
- William J. Harrington Jr.: Professor de Medicina/Divisão de Hematologia/Oncologia, Universidade de Miami, Sylvester Comprehensive Cancer Center, Miami, USA. Interaction with Claudete Esteves Klumb.

Post-graduation programs

- Post-Graduation in Cellular and Molecular Biology (rated as 6), from Fundação Oswaldo Cruz.
- Post-Graduation Program in Biological Chemistry (rated as 7), from Federal University of Rio de Janeiro.
- Post-Graduation in Genetics and Biological Chemistry (rated as 3), from Federal University of Uberlândia.
- Post-graduation Program in Oncology (rated as 5), from Instituto Nacional de Cancer.
- Post-Graduation in Human and Experimental Biology (rated as 5), from State University of Rio de Janeiro.
- Post-Graduation in Biophysics (rated as 7), from Federal University of Rio de Janeiro.
- Post-Graduation in Genetics (rated as 6), from Federal University of Rio de Janeiro.
- Post-Graduation in Physiology and Chirurgical Sciences (rated as 5), from State University of Rio de Janeiro.

Institutional Counterparts
The the Urogenital Research Unity at State University of Rio de Janeiro (UERJ) has its own specialized laboratories including anatomic and surgery, experimental surgery, extracellular matrix biochemistry, histochemistry, immunohistochemistry,
electron microscopy, cell culture, molecular biology, and so on. In the last 5 years the Unit got more than 10 financial grants that could be used to buy new equipments to all the laboratories.

The Research Center of Instituto Nacional de Câncer (INCA) has equipments at that will be used in this project: Automatic DNA Sequencer device MEGABACE 1000 (with 96 capillars), Automatic DNA Sequencer device ABI-Prism 377 (for 64 samples), thermocyclers (8 units for 96 samples), micro-centrifuges (4 units), centrifuge for plates, two freezer at -80°C, cryopreservation system for 10,000 samples. INCA also supports with: equipment maintenance, liquid Nitrogen, Nitrogen, disposable plastic material (tips, tubes, etc), reagents (Tris, EDTA, Agarose, cryotubes, Enzymes, etc).
THEME 3: CLINICAL STUDIES ON ONCOLOGY: BASE FOR DEVELOPMENT AND TECHNOLOGICAL INCORPORATION IN THE ECONOMICAL INDUSTRIAL COMPLEX OF HEALTH

RATIONALE

Clinical studies, whether they are prospective or observational, form the base for the decision procedure in medicine, specially related to new expertise in the area as clinical oncology, in which, every year, there are new drugs and therapeutic options. Many of those options and alternatives arise from a revolution in oncology with the incorporation of knowledge coming from molecular and cellular biology of cancer, creating, each day more, hypothesis, with defined biological bases, to be tested in clinical studies.

Aware of this situation and trying to play its role as a creator of cancer politics in Brazil, the “Instituto Nacional de Câncer” (INCA) has been working on a project for about 10 years related to the development of clinical studies of its own that is innovative at the same time as possible to generate data interesting to the Health Ministry. Besides that, following a world tendency of oncology research, INCA has invested in groups of translational research, that is, that develops projects aiming at a fast transition of the basic science knowledge to the clinical practice. It has the potential to benefit the patients faster and effectively as well as it is connected to directly to the politics and structures focusing the technological development in the health area.

The Health Ministry established as one of its goals the investment and development of the Industrial Economical Complex of Health. That way, there is the interest at a shot period of time of the country to have the condition of not importing technologies but becoming a producer and exporter of medicine and diagnosis kits, impacting on the payment statement of the country. Together with that, INCA has been investing since 2005 in partnerships to implement a multidisciplinary program to develop biotechnology in oncology. For that, huge investments in infra structure has been happening in order to create a Technological Development Center (CDT-INCA). Another mission that falls on the duty of the public sector in this area is to evaluate the incorporation cost of new technologies through measures of cost effectiveness. To sum up, the project on clinical studies has two bases: A) the development of
drugs anti neoplastic using different strategies B) essays to evaluate the incorporation of new therapeutic strategies based on its effect and cost effectiveness.

The project has the potential to transfer results both to the public and to the private sector: Private: once the interesting molecules are identified, those are developed in partnership with the national pharmaceutical industry. For that, mechanisms as “Profarma” from “BNDES” (Bank for Brazilian Development) might be used to consolidate the partnership between private and public sectors. Public: the generated data in the incorporation studies of new therapeutically strategies may subsidize the Health Ministry in the decision making about the technological incorporation. Moreover, the project has the potential to generate patents, once the new molecules are originated naturally or synthetically.

Specific Approaches

Program of Drugs development

INCA has today a nationally and internationally referred center in clinical research. This center is able to conduct studies of phase I, II and III. For this role, the institution is been used for partnerships with Brazilian academic institutions to test molecules candidates to anti neoplastic agents. With that, it has been established the Development Programs of New Drugs from INCA (PDD-INCA) that works in the Clinical research Division from INCA. With this intent, INCA has invested in the creation of a specific infra structure in the graduation of a research group. This group has national institutions as: INCa, Fiocruz, Universidade de São Carlos-SP, Universidade de Mogi das Cruzes-SP, UNIFESP and Museu Nacional da UFRJ, all part of that propose. Furthermore, taking this opportunity, it may be said that the group can be extended with researchers from UFRJ, Universidade Federal from Paraíba and other sectors from INCA (Molecular and Cellular Hematological Laboratory). These new researchers have complimentary projects to the original ones widening the scope of PDD-INCA.

Once formed, this group, having INCA as its coordinator, may act as a coordinator center in the same way as the American NCI (National Cancer Institute). In general, the program is based in 3 bases covering the strategies present in the development of drugs area regarding anti neoplastic medicine in the world:
1) Explore the biodiversity (sea an vegetal)
2) Test synthetic composed with anti neoplastic potential
3) Generate and alter the structure of peptides through computing biology, from targets (bio markers) previously identified. Such strategy is also known as rational design of drugs.

From the identification of promising compounds, INCA will accomplish the following phases of development through partnerships with Fiocruz (animal toxicology), “Farmanguinhos” (medicine formulation) and national pharmaceutical industry (transposition of scale and studies of phase III). The clinical studies phase I, II and III will be made in the clinical study structure already present in INCA, as well as in centers from the net of INCA in Clinical Research (already working and in an expansion phase). With this strategy, INCA, through its partners wants to become a worldwide reference center in the drug development area.

Clinical Essays to evaluate the incorporation of new therapeutical strategies focusing on cost effectiveness.
- Studies on phase III using laser therapy of low potency as a method for mucosites prevention in patients who underwent radiotherapy and chemotherapy for treating tumors in the neck and head regions.
- Impact of Molecular Image in the Radio Therapy Planning and in the Evaluation of Therapeutically Answer of Patients with Cancer in the Uterine Cavity and Anal region: a definite method.

OBJECTIVES
- Consolidate the development of drugs program from INCA and its respective Net
- Develop clinical essays to evaluate new therapeutically strategies that permit to evaluate the incorporation of new technologies focusing on the cost effectiveness.
EXPERIMENTAL DESIGN

1 – Drug Development Program

It is based on 4 steps described below:

Step 1 – Identification of candidate molecules
a) Screening of natural products from different sources such as marine, plants and bacterial products using high throughput platforms.

Marine products: Methodology involves manual collection of samples in different regions according to Museu Nacional standard procedures. Access to the collection points will be made by boat. Samples will fixed in 96% ethanol. Preparations will be identified using Systema Porifera (Hooper & van Soest, 2002), and World Porifera Database (van Soest et al., 2005). Extracts will be prepared from at least 180 sponges collected. Those extracts will be send to INCA for antiproliferative tests and those with some activity will be selected.

Products derived from plants: The therapeutic use of medicinal plants still exists among different populations all over the world. This practice is more diffused in developing countries, where the majority of the poor population does not have access to pharmaceutical drugs (CARRICONDE, 2002). Brazil is the country with the biggest biodiversity and it is estimated that it possess around 20% of the vegetal species of the planet. This genetic patrimony, rather rare in developing countries, has currently an enormous economic value in several fields and it has mainly a huge importance in the development of new medicines (CRAGG; NEWMAN; SNADER, 1997; CALIXTO, 2005).

Flavonoids are products of plant metabolism, found in seeds, citrus fruits, olive oil, tea and red wine. These compounds have profound effects on the function of immune and inflammatory cells as determined by a large number of in vitro and in vivo studies (review by KAWAI et al., 2007). Differences in the chemical structures, bioavailability, distribution and metabolism of flavonoids lead variations in their pharmacological properties. Many flavonoids are now recognized to present antiproliferative, anti-oxidant, anticarcinogenic, antiatherogenic, anti-inflammatory and anti-allergic properties with very low toxicity (review by HAVSTEEN, 2002).
However, the molecular mechanisms underlying their biological effects have not yet been fully identified, suggesting the existence of undetermined molecular targets (MIDDLETON ET AL., 2000, HAVSTEEN., 2002, LOTITO ET AL., 2006). Several studies have disclosed the therapeutic potential of flavonoids, among them it can be highlight their immunomodulatory and antiinflammatory activities. Flavonoids were described to inhibit mastocyte degranulation, decrease the release of histamim, tryptase (THEOHARIDES et al., 2001, MIDDELTON; KANDASNAMI; THEOHARIDES, 2000), leucotriens, prostaglandin D2 and macrophage granulocyte colony stimulating factor (GM-CSF) (CHANG et al., KIMATA et al 2000), inhibit the production of IL-6 and IL-8 by mastocytes and macrophages and inhibit the production of IL-4 and IL-13 by human basophils (HIRANO et al., 2004; OGASAWARA et al., 1986). It has been observed that flavonoids stimulates gama interferon synthesis (SEN, LENGYEL 1992) by both citotoxic and helper T cells (HUGHES, 1999) which are responsible for the immune response against virus-infected cells, bacteria and tumor cells; they inhibit the production of interleukin 5, a cytokine that is produced during inflammatory processes (PARK et al., 1999). Some flavonoids, like quercetin, have been shown to decrease chronic diseases (KNEKT et al., 2002). In vitro screening using flavonoids has shown inhibitory effect on cyclooxygenase and 5-lipoxygenase, enzymes that produces mediators of the inflammatory process (WAGNER, 1989).

On the other hand, the group of Dr. Raquel Maia has analysed modified compounds derived from lapachol. Preliminary results in leukemia cell lines have demonstrated anti-tumor effect including MDR positive cells. This suggests those substances are not substrates for MDR proteins. The compounds LQB118 and its analogues were designed by Prof. Costa PRR do Laraboratório de Química Biorgânica do Núcleo de Pesquisas de Produtos Naturais (NPPN), UFRJ, RJ, Brasil.

Products derived from bacteria: The existence of tumors which are resistant to the drugs used to destroy them indicates the constant need for the development of new bioactive compounds which can be tested against these tumors. One alternative is to study natural substances produced by marine organisms. In fact, drugs such as Dolastatin and Bryostatin were isolated from marine invertebrates. The isolation is however troublesome, for instance, 13 tons of the bryozoan is necessary to purify 1 mg of Bryostatin. Data suggests that Bryostatin is in fact produced by the
invertebrate endosymbiotic bacterium Candidatus Endobugula glebosa, which belongs to the clade Teredinidae. The bacterium Teredinibacter turnerae is the prototype species of this clade and interestingly, unlike other endosymbiotic microorganisms, it can be cultivated in vitro. The genome of this bacterium contains 9 clusters of genes responsible for polyketides production, including genes bry necessary for the synthesis of Bryostatin. The other genes show similarity to genes involved in synthesis of other anti-cancer compound, Bleomycin. Preliminary data in our lab shows that methanolic extracts of T.turnerae inhibit the proliferation of two non-small lung cancer cell lines (A549 and H460). In the present project we propose to study the production of these putative anti-cancer compounds by T.turnerae grown under different growth conditions, where the gene clusters mentioned above are known to be expressed. Further we want to evaluate the effects of these extracts not only on proliferation, but also in other cellular processes such as migration, methastasis, and apoptosis.

b) Development of synthetic molecules (partnership with Universidade de Mogi das Cruzes)

The efficiency of chemotherapeutic drugs is determined by different factors, including the genotype of the tumor cell. Many anticancer agents induce DNA damage that triggers a p53-dependent apoptotic response. The p53 tumor suppressor gene is mutated in up to 50% of human tumors, which may contribute to resistance to various types of therapies. Identifying drugs that induce p53-independent apoptosis is therefore important. The cytotoxicity of taxol has been reported to be independent of p53 function. In addition, various investigational drugs have been described to have p53-independent mechanisms of action. It is not clear whether p53-independent apoptosis is a common phenomenon generated by a large number of drugs or whether p53-independent pathways are exceptional. Studies from a number of laboratories have shown that lysosomal rupture is an early event in various apoptotic processes, including apoptosis initiated by oxidative stress, serum withdrawal, and Fas ligation. Interestingly, it has been demonstrated that p53-induced apoptosis involves early lysosomal membrane permeabilization (LMP). Translocation of lysosomal proteases cathepsin B into the cytosol can induces apoptosis. It was recently demonstrated that immortalization and/or transformation increases the susceptibility of cells to lysosomal death pathways, suggesting that
these pathways are potential targets of anticancer drug development. So, according to this scenario, the main goal of this project is the development of chemotherapeutic agents based in lysosomotropic properties of palladacycles that active p-53-independent apoptosis.

c) Protein modelling through computational biology and bioinformatics.

**Step 2 – Animal toxicology**
Will be developed with Fiocruz (ENSP – Lab. of Professor Francisco Paumgartten and Sérgio Ynuiama), using validated methodology for acute and subacute toxicity as well as mutagenesis and carcinogenesis.

**Step 3 – Formulation**
Will be performed by Farmanguinhos (Fiocruz), according to GMP standards.

**Step 4 – Clinical trials**
Step 4 will be run at INCA and its network. INCA has expertise to run phase I, II e III, as well as PK, Pharmacogenetics and biomarker identification.

2- **CLINICAL TRIALS TO EVALUATE THE INCORPORATION OF NEW TECHNOLOGIES**

- Study Phase III using low power laser therapy as prevention method of oral mucositis in patients who underwent chemoradiotherapy for the head and neck cancer.

There will be 78 patients analyzed, enrolled in the “Instituto Nacional de Câncer”, diagnosed with cancer in pharynx, hippopharynx, and nasopharynx unable to have surgery, submitted to the treatment with radiotherapy and chemotherapy. The patients are chosen at random before the start of the study to receive the treatment with laser (investigation group) and not receiving treatment with laser (control group or placebo). The application will be made with DMC, InGaAlP diode laser, with radiation in the red region of the electromagnetic region (660 nm), power of 100mW, fiberoptic end with 0.246 cm² of section area. It was determined an
energy density of 4 J/cm²/point, punctually applied, at a distance of 1 cm, for 10s per point, making 9 points per region. The oral regions that will be treated are: right and left side, inferior and upper lip, upper and lower lip mucosis side of tongue of both sides, tongue inside, lower mouth part and behind the last teeth area in both sides.

- Impact of the Molecular Imaging in the Radiation Planning and the Evaluation of Response to Therapy of Cervix Cancer and Anal Canal Cancer patients: A Surrogate Method
  
  Patient will undergo studies of molecular imaging (18F-FDG-PET/CT) before and after therapy (chemo + radiotherapy). Imaging analyses will be performed qualitatively and semi-quantitatively (using the standardized uptake value) in order to seek metabolic changes after therapy. The 18F-FDG uptakes (before and after therapy) will be correlated with the molecular/genetic cancer profile using different methodologies (immunohistochemistry, microarray, etc.) On the other hand, images taken before therapy will be used to define radiation planning. These results will be further compared to the current methodology (using CT) applied.

  Expected results: Molecular imaging could be used as a surrogate method to evaluate early response to therapy (unlikely performed by anatomic imaging modalities). In addition, the metabolic imaging could be an ancillary tool in radiation planning (biological tumor volume - BTV).

TEAM
- Carlos Gil Ferreira, researcher at Divisão de Pesquisa Clínica, INCA (Principal Investigator)
- Roberto Gomes de Souza Berlinck, researcher at, Universidade de São Paulo – São Carlos (Principal Investigator)
- Eduardo Carlos Meduna Hadju, researcher at, Museu Nacional, Universidade Federal do Rio de Janeiro (Principal Investigator)
- Antonio Carlos Fávero Caíres, researcher at, Universidade de Mogi das Cruzes (Principal Investigator)
- Marcelo Mamede Lewer, researcher at Divisão de Pesquisa Clinica do INCA (Principal Investigator)
- Carlos Augusto Gomes Soares, researcher at Departamento de Genética da UFRJ (Principal Investigator)
- Ana Lúcia Moraes Giannini, researcher at Genetics Department de Genética da Universidade Federal do Rio de Janeiro
- Franklin David Rumjanek, researcher at Instituto de Bioquímica Médica da UFRJ
- Raquel Ciuvalschi Maia, researcher at Hematology Service, INCA (Principal Investigator)
- Paulo RR Costa, researcher at Laboratório de Química Biorgânica do Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro.
- Alcides JM da Silva, researcher at Laboratório de Química Biorgânica do Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro.
- Marcos Antonio Maurício Scheiner, biologist, Hematology Service INCA.
- Flavia da Cunha Vasconcelos, biologist, Hematology Service, INCA.
- Marcia Regina Piuvezam, researcher, Laboratory at Universidade Federal da Paraíba (Principal Investigator)

GOALS

Qualitative for the Program of Drug Development

- Prospection: Collect 80Kg of poriferans on the coast. Test 5,000 extracts of sea origin. Prepare at least raw extracts from 180 collected samples. Purify and identify pure components.
- Identify new substances, derived from Lapachol that are able to induce cell death in samples with a determined polymorphism pattern.
- Identify potential anti-tumor flavonoids.
- Study the effect of T. turnerae methanolic extracts on the proliferation of tumor and non-tumor cell lines;
- Develop and test innovative chemotherapy compounds with lysosomotropic properties of palladacycles, identifying the most promising ones.
- Study the animal toxicology of promising compounds.
- Develop phase I clinical trials for these compounds.
- Through Computational Biology, do rational design of new compounds.
Qualitative for Assays with new therapeutic strategies

- Standardize the preventive application of low power laser for all patients submitted to radiotherapy in the head and neck region.
- Conduct and conclude the study of the impact of molecular imaging in the radiotherapy planning and evaluation of response in cervix cancer patients. The objectives of this study will be the evaluation of the metabolic alterations that happen during and after different therapeutic modalities and the impact on the radiotherapy planning through molecular imaging.

Quantitative

- Theses and Dissertations: 4 master’s degree and 2 PhD
- Articles: 12 articles published in Qualis A journals
- Patent possibilities

PREVIOUS EXPERIENCE IN RESEARCH (Principal Investigators)

-Carlos Gil Ferreira, MD, PhD, graduated in Medicine at the Federal University of Juiz de Fora (1992), residence in Clinical Oncology at INCA in 1997 and PhD in Experimental Oncology at the Free University of Amsterdam (2001). Currently has an EXP-A fellowship from CNPq and is a Senior Researcher and Chair of the Service of Clinical and Applied Research at the National Cancer Institute (INCA). Experienced in the area of Medicine, with emphasis in Cancerology, acting mainly in the following themes: lung cancer, drug development, molecular biology, apoptosis and tumor banks.

-Antonio Carlos Fávero Caíres, PhD, major in Chemistry from the State University Júlio de Mesquita Filho in São Paulo (1982), Master’s degree in Nuclear Technology from the University of São Paulo (1985) and PhD in Inorganic Chemistry from the State University Júlio de Mesquita Filho in São Paulo (1993). He is currently an Adjunct Professor and researcher at the Mogi das Cruzes University, working as a permanent member of the Graduate Program in Biotechnology. He is experienced in the areas of Chemistry and Biochemistry, concentrating his research in the area of synthesis, characterization and technological applications of organometallic compounds. Of special importance in this sector is the applications and mechanistic
studies of palladium organometallic compounds in the inhibition of enzymes and in the medical area, with the development of efficient compounds to fight cancer and development of biomolecule markers. He works with the methodology of FTIR/ATR and of specular reflectance for the solution of problems and mechanisms in the Biotechnology area. In this area he has already developed work showing the modification of the secondary structure of cystein-proteases by the action of drugs. He is the intellectual mentor of a family of international patents in this area, whose titularity belongs to the São Paulo State Research Foundation (FAPESP in Portuguese).

-Marcelo Mamede, MD, PhD majored from the Federal University of Juiz de Fora, holding a PhD in Nuclear Medicine from the Kyoto University (2004). The PI worked in the field of molecular imaging in important research centers in the United States (National Institutes of Health and Brigham and Women’s Hospital – Harvard Medical School), where he evaluated the diagnostic accuracy in neuroendocrine tumors, the impact on the radiotherapy planning and on the evaluation of therapeutic response in esophagus cancer.

-Carlos Augusto Gomes Soares is an Adjunct Professor 4 at the Department of Genetics (Biology Institute – Rio de Janeiro Federal University – UFRJ) and member of the Graduate Program commission at the Genetics Department-UFRJ) Majored in Biological Sciences – Marine Biology and holds a Master’s degree in Ecology, having concentrated his studies in microbial ecology and marine microorganisms/invertebrates interaction. He obtained his PhD in Genetics, specializing in molecular genetics of bacteria/invertebrates interaction. He obtained his Post-Doctorate at CDC/Vector Transmitted Infectious Disease Division, with studies of the bacteria/ticks/host interface. He currently develops research related to: i) molecular genetics of the interaction of cellulolytic/nitrogen-fixing bacteria, symbiont of Teredinidae mollusks, with a marine biotechnological approach and in the study of biosynthesis of polyketides with potential anti-cancer activity; ii) genetics of bacteria transmitted by ticks and the study of the interface bacteria/ticks/host. He was awarded a prize in 2006 by the Royal Entomological Society of London. He acts as an ad hoc consultant at CNPq, at the Brasília Catholic University, as a reviewer and as an editor of the Oecologia Brasiliensis Journal.
-Raquel Ciualschi Maia graduated in Medicine with specialization in Clinical Hematology and has a PhD in Cellular and Molecular Biology from the Oswaldo Cruz Foundation. She is the PI of the Cellular and Molecular Hemato-Oncology Laboratory at the Cancer Hospital-I at the National Cancer Institute (INCA), acting mainly in the following themes: 1) multiple drug resistance (MDR) in neoplasias, 2) mechanisms of apoptosis induction by drugs in neoplasias, 3) identification of prognosis factors in neoplasias and 4) anti-tumor and anti-MDR effects from new chemotherapy compounds in neoplasias. In parallel with the research activities, she works as a clinical hematologist at the Hematology Service, as a Professor from the stricto sensu Graduate Program in Oncology at INCA and as a collaborating Professor at the Graduate Program in Morphological Sciences at the Anatomy Department of the Biological Sciences Institute from UFRJ. She participates in the Oncobiology program associated to the Biomedical Sciences Institute from UFRJ.

-Antonio Carlos Fávero Caíres, PhD, major in Chemistry from the State University Júlio de Mesquita Filho in São Paulo (1982), Master’s degree in Nuclear Technology from the University of São Paulo (1985) and PhD in Inorganic Chemistry from the State University Júlio de Mesquita Filho in São Paulo (1993). He is currently an Adjunct Professor and researcher at the Mogi das Cruzes University, working as a permanent member of the Graduate Program in Biotechnology. He is experienced in the areas of Chemistry and Biochemistry, concentrating his research in the area of synthesis, characterization and technological applications of organometallic compounds. Of special importance in this sector is the applications and mechanistic studies of palladium organometallic compounds in the inhibition of enzymes and in the medical area, with the development of efficient compounds to fight cancer and development of biomolecule markers. He works with the methodology of FTIR/ATR and of specular reflectance for the solution of problems and mechanisms from the Biotechnology area. In this area he has already developed work showing the modification of the secondary structure of cystein-proteases by the action of drugs. He is the scholar mentor of a family of international patents in this area, whose titularity belongs to the São Paulo State Research Foundation (FAPESP).
-Marcia Regina Piuzevam has degree in Pharmacy and Biochemistry by the Londrina State University (1982), Master of Science (Microbiology) by the Federal University of Rio de Janeiro (1988) under the guidance of Professor José Mauro Peralta and doctorate in Science (Microbiology) by the Federal University of Rio de Janeiro (1994). The experimental part of the Ph.D. (CNPq) was held in Seattle Biomedical Research Institute under the coordination of Dr. Steven G. Reed and Co colaboração of Immunex, Seattle, WA, USA. The Post-Doctoral was held in Department of Immunology, University of Strathclyde, Todd Centre, Glasgow, UK, in the laboratory of Professor James Alexander. She is currently a Professor of the Federal University of Paraiba and researcher level 2 in CNPq. She has experience in the field of Immunology, with emphasis on Cellular Immunology. She has worked on: The medicinal plants and their effects in experimental model of allergic (mice BALB / ce ovalbumin) with collaborations with the UFBA, UFRJ and FIOCRUZ / Rio de Janeiro. The plants under study are: Cissampelos sympodialis Eichl (Menispermacaeae), Amburana cearensis (Fabaceae) and Cida cordifolia (Malvaceae).

REFERENCES
Checinska et al. BMC Cancer (Online) 6:166 (2006)
Costa et al. ONCOLOGIA ATUAL 6: 432 (1996)
Ferreira et al. Oncologia Atual 7:18 (1997)
Soares et al. Critical Care Medicine, v. 34, p. 715 (2006)
Mamede et al. Neoplasia 7: 369 (2005)

**CRONOGRAM**

1**nd** Semester
- Bioprospection of marine products.Bioprospecção de produtos marinhos
- Purification of marine extracts
- Screening of natural products (plant , marine, bacterial derived)
- Functional and Toxicological tests with synthetic molecules
- Functional tests with Lapachol derived products
- Clinical studies with laser therapy
- Molecular Imaging study for RxT planning.

2**nd** Semester
- Bioprospection of marine products
- Purification of marine extracts
- Screening of natural products (plant, marine, bacterial derived)
- Functional tests with palladacycles
- Toxicological tests with Paladacíclicos
- Data collection for laser therapy studies
- Molecular Imaging study for RxT planning
- Functional tests with Lapachol derived products and the influence of gene polymorphisms.
3rd Semester
- Bioprospection of marine products
- Purification of marine extracts
- Screening of natural products (vegetable, marine, bacterial)
- Functional tests with palladacycles
- Fase I study with palladacycles
- Publication of scientific article with laser therapy results
- Molecular Imaging study for RxT planning
- Functional tests with Lapachol derived products and the influence of gene polymorphisms.

4th Semester
- Bioprospection of marine products
- Purification of marine extracts
- Screening of natural products (vegetable, marine, bacterial)
- Functional tests with palladacycles
- Fase I study with palladacycles
- Publication of scientific article with laser therapy results
- Molecular Imaging study for RxT planning
- Finishing of the Molecular Imaging study for RxT planning
- Publication of scientific article about Lapachol and the influence of gene polymorphism.

3rd Year
- Functional tests with marine products
- Functional tests with flavopiridol
- Fase II study with com palladacycles
- Animal toxicology with bacterial products
- Manuscript preparation with data about molecular imaging study for RxT planning
- Synthesys of Lapachol derived molecules.

4th Year
- Functional tests with marine products identified
- Animal toxicology studies with flavopiridol
- Fase I studies with bacterial products
- Fase II study with palladacycles
- Synthesys of Lapachol derived molecules.

5º ano:
- Functional tests with marine products identified
- Fase I studies with flavopirirdol
- Fase II study bacterial products
- Fase II study with palladacycles
- Manuscript preparation with data about molecular imaging study for RxT planning
- Tests with new molecules derived from Lapachol

FINANCED PROJECTS IN THE LAST 5-YEARS


- CNPq – Edital 15/2004 PROSUL. PROJETO EMBARC - Bioprospecção, Taxonomia, Filogenia e Biogeografia de Esponjas Marinhas do Brasil, Argentina e Chile. Principal Investigator: Eduardo Hadju(R$40.000,00).


- FAPERJ – Edital Cientista do Nosso Estado Encontro de Biotas: Composição e Distribuição de Poríferos na Confluência Austral dos Oceanos Pacífico e Atlântico. 2007. Principal Investigator: Eduardo Hadju (R$57.600,00).

- CNPq – Edital 15/2007 PROSUL Capacitação para Pesquisa em Taxonomia, Filogenia e Biogeografia de Poríferos da América do Sul (PROJETO EsponjAS). Principal Investigator: Eduardo Hadju (R$50.000,00)

**POST-GRADUATION PROGRAMS ACTIVITIES**

- Post Graduation in Biological Chemistry, Universidade Federal do Rio de Janeiro (grade 7).

- Post Graduation in Oncology, Instituto Nacional de Câncer (conceito 5).
- Post Graduation in Medical Sciences da Universidade do Estado do Rio de Janeiro (grade 4).
- Post Graduation in Biological Sciences (Zoology), Universidade federal do Rio de Janeiro (grade 4).
- Post Graduation in Genetics, Universidade Federal do Rio de Janeiro (grade 6).
- Post Graduation in Biotechnology, Universidade de Mogi das Cruzes (grade 4).
THEME: CANCER EPIDEMIOLOGY IN BRAZIL

Presentation

The Brazilian Public Health Program, called “Programa MAIS SAÚDE – direito de todos”, represents a radical reorientation of the public health policy of the Brazilian Ministry of Health, in order to consolidate the Brazilian Unified Health System (“SUS”) and to improve the scenario of morbidity and mortality in Brazil. It is an important step towards achievement of the constitutional right to health. The strategic goals of this program include: reduction of social iniquities and expansion of people’s access to public health services; implementation of social and economic policies to promote public health; improvement of people’s awareness of their right to health and of the importance of maintaining healthy habits and behavior; development of the Brazilian industrial health complex; intensification of cooperative actions for health promotion; and expansion and qualification of work power in SUS.

In accordance with this policy, the Brazilian National Cancer Institute (“INCA”), defined its own goals, which are: expansion of the specialized oncologic attention to all regions of the country in order to establish a structured oncologic net in Brazil; development of actions designed to prevent cancer end to promote health; improvement of scientific research and technological evaluation for cancer detection and control in Brazil; stimulation of regional and international cooperation for cancer prevention, detection and control; stimulation of knowledge production and divulgation in the field of cancer, thereby qualifying human resources and public services.

The program of National Institutes of Science and Technology (“INCT”), proposed by the Brazilian National Council For Research (“CNPq”), aims at promoting high quality and ‘at the edge’ scientific research, in combination with qualification of human resources and knowledge transference to the society, to private economic activities or to the government.

The subgroup of research in Cancer Epidemiology in Brazil intends to congregate these goals, proposing a series of studies focusing on the epidemiological relevance and strategic opportunities of the objects.
Rationale

Cancer is usually recognized as a multistep process caused by accumulating gene mutations within a stem cell [Loeb et al., 2000]. These discoveries created the concept of cancer as an endogenous genetic disease. However, the increasing incidence of many cancer types in the past fifty years poses questions on the causal origins of gene mutations that lead to carcinogenesis. Expansion and ageing of the population as well as progress in cancer detection cannot fully account for the observed growing incidence of cancer. Moreover, the rising incidence of cancers is seen across all age categories, including children, and adolescents [Belpomme et al., 2007]. These observations suggest that environmental factors play an important role in cancer genesis.

With regards to environmental causes of cancer, it appears that involuntary exposure to diverse physical, chemical and biological agents present in the surroundings of individuals [Das, 2003; Clapp et al., 2006; Sasco et al., 2003], as well as lifestyle influences, such as smoking, alcohol consumption and diet [Belpomme et al., 2007], play a major role in the occurrence of the disease. Lifestyle influences are more clearly defined and easily evaluate through classical epidemiologic approaches. The identification of environmental carcinogens, on the other hand, is usually not feasible through classical epidemiological methods. Thus, a combination of biological and toxicological data in association with genetic susceptibility evaluation is required to interpret epidemiological studies in the context of molecular gene-environment interactions.

The recognition of risk factors is required in order to establish new cancer prevention policies, aiming at limiting the exposure to avoidable environmental and occupational carcinogens. Likewise, informative campaigns must be oriented to additional important risk factors like diet and lifestyle.

The project presented here is divided in 4 research lines: 1) Study of gene-environment interactions on the risk of different types of sporadic cancer; 2) Characterization of genetic and environmental risk factors associated with the infection by human Papilomavirus and the progression to cervix cancer; 3) Strategies for control of acute toxicity related to tobacco production and marketing; 4) Implementation of a Geographic Informative System, designed for real-time data
transmission on cancer registries and treatment follow-up, with on-line management of epidemiological databanks time-space distribution.

**RESEARCH LINE I: GENE-ENVIRONMENT INTERACTIONS AND RISK OF SPORADIC CANCER**

Biochemical and genetic studies have revealed several targets of carcinogenesis, including oncogenes, tumor suppressor, DNA repair and cancer susceptibility genes [Hahn et al., 2002; Hoyer ET AL., 2002]. These discoveries created the concept of cancer as an endogenous genetic disease. However, data based on the analysis of co-occurrence of cancer in a cohort of identical twins showed a rather low concordance rate, indicating that environmental rather than genetic factors predominate in the etiology of cancer [Lichtenstein et al., 2000]. Indeed, with the exception of certain types of familial cancer, such as adenomatous polyposis coli, the contribution of hereditary factors to the development of cancer appears to be relatively minor [Li, 1995; Easton, 1994; Fearon, 1997]. This conclusion, however, is based on dominant genes and does not apply to recessive traits or combinations of genes, whose contribution to the causation of sporadic cancer cannot be evaluated from family studies [Lander e Schork, 1994].

Familial cancers which result of highly penetrant mutations represent only a few percent of the total cancer cases. Sporadic cancers, which are much more frequent, develop as a result of exposure to risk factors in genetically susceptible individuals [Mucci, 2001]. Individual variations in the activation or detoxification of exogenous carcinogenic factors account for differences in the susceptibility to cancer [Irizaray et al., 2007]. Because the frequency of genetic polymorphisms varies among ethnic groups, the risk for cancer may differ among distinct populations as a consequence of either gene or environmental causes.

The identification of genetic variations with potential influences on sporadic cancer has benefitted from recent genomic and proteomic approaches in molecular epidemiologic studies. However, the confirmation of the biological role of such gene polymorphisms requires support from biochemical and/or toxicological data, as well as evaluation of the prognostic value of such variations in a clinical setting. The
combination of these different approaches will help define the clinical value of genetic information for diagnosis, prognosis and treatment decisions.

The subprojects composing this research line use classical and molecular epidemiologic approaches in order to evaluate the influence of gene polymorphisms as potential risk factors for the development and/or progression of different types of sporadic cancer. The studies also use *in vitro* molecular techniques and/or clinical investigations in order to characterize the biological role of selected polymorphisms. The combination of these complementary approaches may help define the actual clinical value of the genetic information for diagnostic, prognostic or treatment decisions in the therapeutic conduct.

**Ethical Aspects:** All the protocols described hereby were approved by local ethical committees and all participants or their legal representatives signed a written informed consent.

**Subproject I.1: Study of the association between polymorphisms of genes NQ01, CYP17 and CYP19 with breast cancer in young women**

The breast cancer is the leading cause of death by cancer among women Brazil (Brazil / Ministry of Health / INCA, 2003). Despite the higher incidence of breast cancer occur in women from 50 years of age, there has been an increase in the incidence of disease in young women (Cardona and Agudelo, 2007). Between 1998 and 2003 found a significant increase in the percentage of hospitalizations for breast cancer among younger women (up to 29 years of age) in all states in the Southeast region analyzed (Sao Paulo, Rio de Janeiro, Minas Gerais and Espirito Santo). In Rio de Janeiro also noted an increase in the number of deaths from breast cancer in women younger age groups (30-49 years), and the mortality rates of Rio de Janeiro in that age the highest in relation to other states of Southeast Region (Gonçalves and Barbosa, 2006). In view of increased rates of incidence of breast cancer in young Brazilian women, observed in the last decade and the limited information available on the genetic polymorphisms associated with the development of breast cancer in these women, a study was conducted case-control-based hospital in Rio de Janeiro, where they were obtained epidemiological information and biological samples (blood and cells of the oral mucosa) of women with less than 36 years.
Objectives: To determine the magnitude of association between the MspAl polymorphism of the gene CYP17 and breast cancer in young women and explore the occurrence of interaction between this polymorphism and early menarche; determine the magnitude of association between the Arg 264 Cys polymorphism of the gene CYP19 and cancer the breast in young women and explore the occurrence of interaction between this polymorphism and obesity.

Experimental Design: During the period January 1999 to December 2005 was made a case-control study of the base hospital in Rio de Janeiro to examine the contribution of risk factors in the development of breast cancer in women under 35 years of age or less.

Population of the Study: The basis of study comprises the population of women with 35 years of age or less who lived in the southeastern region of Brazil in the period from 1/1/1999 to 31/12/2005. The sample of analysis for this research is composed of 246 cm female breast cancer diagnosed in children younger than 36 years, and an equal number of controls without previous history of cancer.

Data collection: The all participants endorsed a questionnaire was used to assess the history of exposure to risk factors selected. The variables were analyzed hormonal substances, items of diet, medication, radiation, pesticides and other chemicals. Moreover, has built up a heredrograma with the data of breast cancer in the family of the participant, revised up the values of total cholesterol and triglycerides, and estimated to be the body mass index (BMI = weight (kg) / altura2) and waist / hip. At the end of the interview were collected two samples of 5 ml of blood.

Analysis of data: Data are being stored in the database Access 2000 (Microsoft). Then where will the realization of the bivariate analysis of the variables analyzed, seeking to identify the relationships of confounding and / or interaction between them. Later, as will the development of multivariate modeling, through employment of unconditional logistic regression, setting up the magnitude of association of variables through odds ratios with their intervals at 95%. The interaction between genetic polymorphisms analysed with environmental variables selected will be explored through determination of odds ratios of interaction, with the intervals at 95%, upon approach to studies in cases of cancer (case-only study).
The inflammatory enzyme cyclooxygenase-2 (COX-2) is usually undetected in normal tissues, but it can be induced by cytokines, growth factors or tumor promoters. COX-2 is over-expressed in many types of solid tumors, and in breast cancer, its presence is associated with bad parameters, including tumor size, positive nodal status and lower survival (Ristimaki et al., 2002; Spizzo et al. 2003). The mechanisms involved in the regulation of COX-2 expression are not completely understood may be influenced by genetic variations. The analysis of the promoter region (RP) of the human COX-2 gene (PTGS2, locus 1q25.2-q25.3) reveals the existence of many potential regulatory sites. Genetic variants have been described within or next to these regulatory sites and can affect gene transcription (Papafili et al., 2002; Zhang et al., 2005). Beyond variations in the RP, other sites in the 3’-untranslated region (3’-UTR) may also affect COX-2 expression. The 3’-UTR of PTGS2 contains 30 copies of “ATTTA” elements, which generate consensus binding regions for inflammatory proteins that regulate the stability and degradation of COX-2 RNA<sub>m</sub> (Caput et al., 1986; Di Marco et al., 2001, Dixon et al., 2001). However, there have been no molecular studies evaluating the impact of PTGS2 3’-UTR polymorphisms on the stability and degradation of COX-2 RNA<sub>m</sub>. The frequency of PTGS2 gene polymorphisms varies among ethnic groups, which may affect the cancer risk on different populations. Our group charactherized the distribution of PTGS2 gene polymorphisms in the Brazilian population (Piranda e Vianna-Jorge, 2007) and identified four variations with minor allelic frequencies higher than 10%, three located in the RP (-1290AG, -1195AG, -765GC) and one in the 3’-UTR (8473TC).
Objectives: Evaluate the influence of PTGS2 gene polymorphisms on the risk of breast cancer development and/or progression, on COX-2 expression in tumor species and on the control of gene expression in vitro.

Experimental Design: Caseo-control study, comparing the frequency of the four selected polymorphisms, their genotypic and haplotypic distributions among patients with breast cancer and among healthy, age-paired women. The allelic frequencies and genotypic or haplotypic distributions will be compared using Chi-square of Fisher’s exact tests. The association between genotypes and/or haplotypes and the risk of cancer will be evaluated by the Odds Ratio (OR) and the respective 95% interval of confidence (IC95%), with adjustment for possible confounding co-variables. Preliminary results, comprising 216 cases and 131 controls, suggest that polymorphism 8473TC increases the risk for breast cancer among Brazilian women (OR = 1.72; IC95%: 1.08-2.76). In order to confirm this result, considering other co-variables, we estimate that the sample size should be expanded to 600 cases and controls. We will also conduct a case-case study in order to evaluate the association between PTGS2 gene polymorphisms and selected clinical endpoints, such as tumor stage at diagnosis, global and free-of-disease survival, and tumor relapse or progression. We will also evaluate the influence of PTGS2 gene polymorphisms on COX-2 expression levels in tumor species (analyzed by imuno-histochemistry). In order to characterize the functional role of 8473TC polymorphism on the regulation of gene expression and RNA_m stability we will use molecular biology approaches. The influence of 8473TC polymorphism on the regulation of gene transcription will be evaluated via expression of a gene reporter (luciferase) in cells transfected with wild-type (pGL3-RP-3UTR) or variant (pGL3-RP-3UTR-8473) constructs. The influence of 8473TC polymorphism on the RNA_m stability will be evaluated via real-time PCR, in order to quantify the time decay or luciferase RNA_m levels. The combination of case-control and case-case results with the molecular characterization of the impact of PTGS2 gene polymorphisms on COX-2 expression might contribute for the understanding of patophysiological mechanisms involved in the genesis and/or progression of breast cancer.
Subproject I.3: Melanoma Molecular Epidemiology: genetic-environmental interactions and survival of hospital base

The melanoma is the neoplasm cancer with the greatest metastasis potential per milligram of tissue. Despite representing about only 5% of all skin cancers diagnosed, it represents the majority of deaths related to skin cancer. Although it has been observed a change in the epidemiological picture of the incidence and mortality from melanoma in the country, characterized by the elevation of these indicators, there is a relative dearth of epidemiological information about the clinical course of cancer in Brazil, as on the contribution of genetic-environmental interactions associated with its determination in our population.

Objectives: To determine the magnitude of association between selected genetic polymorphisms (folate and vitamin D metabolism) and prior ultraviolet intensity radiation exposure in patients with melanoma; determine the overall survival in hospital-based patients samples with primary melanoma of the skin and primary site indefinitely second staging; determine the patients undergoing surgery relapse-free survival according to the stage and in accordance with the micro staging; determine the overall survival of patients with metastases from the same diagnosis and therapy according to used; describe the distribution of clinical features and staging of patients with melanoma treated during the investigation; compare general survival according to pattern of therapeutic procedures adopted during the investigation.

Experimental Design: An epidemiological study will be conducted with two cohorts of patients diagnosed with melanoma conducted at the Instituto Nacional de Câncer. The first is constituted by the universe of incident cases diagnosed in the period 1996-2005 (historical cohort), formed by the second incident cases diagnosed during the period 2008-2010 (concurrent cohort). It will be developed to study the survival of the following 5 years from the historical cohort, estimated overall survival and
disease free survival. With the concurrent cohort will be held a case to case study, by providing associations ratios odds between selected genetic polymorphisms of the folate (gene MTHFR) and vitamin D (VDRs) metabolism, and ultraviolet radiation exposure history during childhood and teenage, with 95% confidence intervals.

**Target Population:** melanoma cases will be identified from the INCA registry in the period from 1996 to 2005. The estimated number of registered cases annually is around 100 patients with melanoma, which potentially creates a population of about 1000 patients in the series to be studied.

**Collection and analysis of data:** patient, tumor and evolution of the disease informations will be collected by consulting the records and interviewing. It will be used a qualitative model of food frequency questionnaire (QFAQ) for collection of dietary data. From then it will be possible a descriptive analysis of cases through demographic analyses, the frequencies of each of the clinical presentations, the types of treatment performed. There will be analysis of survival through the method of Kaplan Méier, with the analysis of overall survival (from the date of diagnosis until death or loss to follow up), disease free survival (until relapse or loss of follow up), the relapse type for each possible presentations (localized disease, locoregional disease, metastatic disease). These analyses will be carried out to the entire population of the study and for each group defined by staging and the micro staging. Information on median survival, survival at 1, 2 and 5 years will be achieved. With regard to patients with metastases, will be performed an analysis comparing the use of systemic therapy or not, as well as the type of systemic therapy by the method of log-rank, and a multivariate analysis will be conducted using the model of leading Cox in mind other potential confounding factors such as sex, age, performance status and lactic dehydrogenase blood levels.

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Subprojeto I.4: Interação genética e ambiental nas neoplasias malignas na infância

The identification in cord blood samples of somatic abnormal fusion genes (ETV6-RUNX1 AML1/ETO, MLL/AF4 TCR e IgH gene rearrangements) clearly suggest the pre-natal origin of childhood acute leukemia. However, the comparative higher proportion of fusion genes identified seems superior to the annual incidences of acute leukemia in places where cord blood samples were tested. These results lead the hypothesis that a single gene aberration is no sufficient to sustain an abnormal cell clone. The variable period to start a clinical phenotype of the disease support the hypothesis that additional pos-natal risk factors are necessary to disclose the leukemic process (Greaves, 2006). The only exception is the MLL gene rearrangement that has a high penetrancy and it is a marker associated with short latency leukemia, according to modelling model demonstrated in mice experiments (Barabe et al, 2007); others distinct abnormal fusion genes need other additional events as demais fusões (two-hits model). There is a consensus that the origin of a cancer is a multi-causal effects: genetic alterations of xenobiotics metabolisms, DNA repair system, cycle-celular function interacting with external factors can originate pediatric neoplasias. However, direct evidence for gene-environment interaction was not full convincent established, so far. Recently, a association between maternal exposures during pregnancy to substances and infant leukemia raised a question whether such neoplasia is de novo or a secondary leukemia (Pombo-de-Oliveira et al., 2006, Koifman et al., 2008). The similarities found in leukemias and embrional solid tumors regarding genetic biomarkers, age of onset of disease, birth weight, suggest that both group of malignancies have similar gene-environment interactions que (Sharpe et al, 1996). There is an inverse relationship between vitamin and folate intake and infant embrional tumors, nevertheless a study should be perform to test such association in less affortunated children and their genetic background (Goh et al, 2007, French et al 2003). Very few studies have been performed related to genetic polymorphism and environmental exposures. New tools such as DNA micro arrays, identificaton of gene expression profile coordinated with proliferation gene or responses to a especific stimuli are of great values to sustain the trasnlational research in childhood cancer.
Main Goal: to conduct an epidemiology-molecular study on embrinionic tumours in order to indentify biomarkers associated pathogenesis mechanisms, as well as, to predict survival lenght.

Objetivos específicos: To identify somatic gene fusions in cord blood (using Real-time PCR method) and test the hypothesis that additional molecular abnormalities may occurs associated with such gene translocations (using SNPs arrays methods); To determine whether the occurrence of embrional tumours is associated with gene polymorphism of MTHR, MS, TS, RFC and the interaction with folate intake during pregnancy (through a case-control study); To test whether dypirone and benzene substances induce leukemia in offspring of mouse exposed during pregnancy and to evaluate them according to MLL status; To identify gene expression profile according to transcriptional signature; To determine the profile of miRNA specific of leukemic cells; Finally, to identify protein differentially expressed in leukemic cells according to higher and lower prognostic risk.

Desenho do estudo: This proposal study is feasible and structural based on the accomplishments of the current network of scholars that compunds the Pediatric Hematology-Oncology Program. It intends to ascertain about 3,000 cases (2009-2014). A case-control study will be conducted combining biological markers analysis and the maternal entrevies data collected. Well-strutured questionnaire will allowed to investigate the environmental exposures. Characterization of gene polymorphism (direct seqüencing) will performed in index-child and the mother biologic specs. Patients cells samples will be used for Células RNA and DNA extraction. mRNAs will be analysed quantitative and qualitative methods using the Solid platform (Applied Biosystems) (Cloonan et al., 2008). The same sample will used to proteomic analysis separating liquid-phase protein and sequencing those differentially expressed (Hegedus et al., 2005). As regiões genômicas serão recuperadas do DNA total utilizando microarrays desenhados para este propósito (Hodges et al., 2007). Estas regiões serão analisadas através de seqüenciamento massivo utilizando a plataforma Solid (Applied Biosystems) no Centro de Genômica de Alto Desempenho do Distrito Federal, localizado na Universidade Católica de Brasília. Para avaliação do potencial leucemogênico da dipirona, serão utilizados camundongos CD-1, que serão expostos in utero à dypiron (testing group), benzen (positive-controle) ou ao veículo de diluição (controle negativo). The characterization of leukemia in offsprings
will performed by flow cytometry and by RT-PCR (Soszynska et al., 2008; Barabé et al., 2007).

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**RESEARCH LINE II: HUMAN PAPILOMAVIRUS (HPV) AND CERVICAL CANCER**

Cervical cancer is the second most common type of cancer among women and infection by human papillomavirus (HPV) is the main risk factor. HPV infection is a sexually transmitted disease. There are more than 120 different known HPV genotypes. Genotypes are divided into low, medium and high-risk, according to its oncogenic potential. A persistent infection by one or more types of oncogenic HPV is an important etiologic factor for the development of intraepithelial cervical neoplasia (ICN) and for its progression into cervical cancer (Sellors et al. 2003). The main strategy for control of this disease at population level is the periodic preventive examination known as Papanicolaou, followed by appropriate treatment of malignant or precursor lesions.

Therefore, to improve knowledge about HPV genotypes most common in our country, as well as the contribution of risk factors associated with infection, which may result in cervical of cancer, the following research are presented for the next 3 years: 1) Evaluation of strategies for cervical cancer screening in areas covered by the Family Health Strategy Program, 2) HPV genotypes associated to cervical cancer in women admitted to hospitals in the SUS network in selected Brazilian capitals, 3) prevalence of HPV and different genotypes in a cohort of HIV-positive pregnant women, and risk factors associated with persistence of HPV infection after delivery.
Subproject II.1: Evaluation of the strategies for cervical cancer screening in areas covered by the Family Health Strategy Program

The Papanicolaou (Pap) smear is the most commonly used method worldwide for the screening of precursor lesions of cervical cancer. The difficulty in making an impact on the mortality rates from this disease in developing countries in which the Pap smear is the only available screening tool has raised questions with respect to the efficacy of conventional screening. Therefore, emphasis has been placed on HPV-DNA testing. On the other hand, in developed countries that have invested heavily in Pap smear-based screening programs, the occurrence of cervical cancer has decreased. More recently, even in Latin American countries such as Chile, Costa Rica and Mexico, in which screening is well-organized and coverage is high, an impact has been found on mortality from this type of cancer.

Currently, the debate on whether to incorporate HPV-DNA testing into screening programs has been steadily growing, stimulated in part by the commercial availability of the HPV vaccine. Various issues remain to be answered regarding the role of factors related to the progression of cervical lesions and whether the use of this new technology will lead to over-diagnosis or to real effectiveness in screening.

The results of this study may contribute towards evaluating the future strategies to be adopted in cervical cancer screening programs in Brazil aimed at low socioeconomic populations in whom the risk for this disease is consequently greater.

**General objectives:** To evaluate screening strategies for cervical cancer and its precursor lesions and to assess factors associated with progression of the disease, in municipalities of the northeast of Brazil covered by the Family Health Strategy Program.

**Specific objectives:**

a) To determine the prevalence of HPV infection according to specific HPV type;  
b) To identify the factors associated with risk of progression of cervical cancer or precursor lesions;  
c) To compare the cost-effectiveness of cervical cancer screening, using the Pap smear alone or in combination with HPV-DNA testing.

**Experimental design:** A community-based intervention study for diagnostic testing.
a) **Study population:** Women of 18-59 years of age who have already had sexual intercourse and are resident in one of the areas attended by the Family Health Strategy Program.

b) **Procedure:** Cervical smears will be submitted to the Pap test and, in a randomized sample of the women (intervention group), HPV-DNA testing will be performed by polymerase chain reaction (PCR).

c) **Study endpoints:** Cervical intraepithelial neoplasia (CIN) grade 2 or greater according to histopathological evaluation and HPV infection.

d) **Independent variables:** Age, education level, per capita family income, HPV infection, viral load, smoking, and reproductive and sexual history.

e) **Data analysis:** 1) Prevalence estimation of HPV infection according to specific HPV type and analysis of the factors associated with progression; 2) Calculation of sensitivity, specificity and predictive value of the strategies used; 3) Analysis of cost-effectiveness.

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**Subproject II.2: HPV genotypes present in cervical cancer in women admitted to hospitals in SUS network in selected Brazilian capital.**

The role of human papillomavirus in the development of cervical cancer is well established, and the evidence of the oncogenic potential of HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 is considered sufficient (Muñoz, et al, 2006; IARC, 2007). Currently, HPV is considered a necessary cause for cervical cancer, and nearly 100% of all cases are attributable to the viruses (Parkin, 2006). The recent development of the quadrivalent vaccine against HPV 6, 11, 16 and 18, and the bivalent against HPV 16 and 18, can be an important strategy for the control of this cancer. Both vaccines were registered as new products by the National Health Surveillance Agency (ANVISA), receiving permission for marketing in Brazil, with prophylactic indication for girls and women from 9 to 26 years (quadrivalent vaccine)
and 10 to 25 years (bivalent vaccine). Monitoring HPV genotypes associated with cervical cancer, before and after the introduction of the vaccine, in large scale, in the country, is recommended, considering the possibility of the maintenance of infection associated with other HPV types less prevalent today, which are not covered by the available vaccines, and for which cross protection may not occur. (WHO, 2007; Lowdes CN, 2006).

**Objectives:** 1. To describe the distribution of HPV genotypes associated with cases of cervical cancer treated in public hospitals (SUS), before implementation of HPV vaccine countrywide; 2. To describe the epidemiological profile of women in the study.

**Experimental design:** Pathologists in each center will collect tumor samples, measuring 0.5 cm X 0.5 cm X 0.5 cm. Samples will be stored in cryotubes with DNAZOL® and sent to the Division of Genetics of the National Cancer Institute of Brazil (INCA). DNA will be isolated and PCR amplified using primers for the region of the L1 HPV gene. The amplified fragment will be sequenced using automatic sequencers. Amplified fragments will also be used in hybridization against specific oligonucleotides to HPVs with oncogenic high-risk to detect possibility of co-infection. The sequences obtained will be compared to those deposited in sequences public databases to identify the virus type. Comparative and phylogenetic analyses using the programs Arlequin and Mega 4.0 to determine the diversity within each type of virus found, will also be made.

**Epidemiological study:** Women who agree to participate in the study will answer an epidemiological questionnaire covering other known HPV infection co-factors including socio-demographic data, sexual behavior, reproductive history, smoking and history of infection with other STDs

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Subproject II.3: PREVALENCE OF HPV AND DIFFERENT GENOTYPES IN A COHORT OF HIV-POSITIVE PREGNANT WOMEN, AND RISK FACTORS ASSOCIATED WITH PERSISTENCE OF HPV INFECTION AFTER DELIVERY

Infection by human papilloma virus (HPV) is considered a sexually-transmitted disease, and it is detected in approximately 10-20% of the sexually-active population between 15-49 years of age (Nonnenmacher et al. 2002). Currently, more than 120 HPV genotypes are known. They are classified into low, medium and high risk, according to their oncogenic potential. Fifteen of the anogenital HPV genotypes can lead to the development of cervical cancer (Rosenblatt et al. 2006). The persistent infection by one or more types of oncogenic HPV is an important etiologic factor for the development of intraepithelial cervical neoplasia (ICN) and for its progression to cervical cancer (Sellors et al. 2003). Rosenblatt et al. (2006) have reported that immunocompromised patients present a 100 time increased incidence of vulva and anal carcinoma, as well as an increased risk of approximately 14 times for cervical cancer. In women infected by HIV, there is a higher prevalence of HPV infection and of intraepithelial lesions (Levi et al. 2004), these latter being more severe, faster progressing, harder to treat and with an elevated rate of recurrence (Souza et al. 2001). In Brazil, the prevalence of HPV in HIV-seropositive women diagnosed by PCR varies according to the region studied. In Rio de Janeiro, a recent study found a prevalence of 51% (Ginsztejn et al., 2006), whereas Levi et al (2004) reported a prevalence of 87% in São Paulo, accompanied by a high rate of infections with multiple genotypes (Levi et al, 2002; Muñoz et al., 2003). The polymorphism of the tumor suppressor gene *tp53* at codon 72 has been extensively investigated for association of several cancers across the world (Kietthubthew et al. 2003). The first report of the correlation between an increased risk of cervical cancer and
polymorphisms in p53 has been done in 1998 (Storey et al. 1998). These authors detected the genotype p53Arg in 76.1% of cervical tumor cases, compared to only 36.6% in controls. Brazilian studies that evaluated the presence of polymorphism at codon 72 of \textit{tp53} confirmed that the presence of Arg genotype is associated with a higher susceptibility to cervical carcinogenesis in those infected with HPV, and also a higher chance of recurrence in those that has previously developed cervical cancer (Brenna et al. 2004; Souza & Villa, 2003).

**OBJECTIVES:**

**General:**

1. To determine the prevalence of HPV and its different genotypes in HIV-positive pregnant women; 2. To define risk factors for persistence of HPV infection after delivery. **Specific:** To determine the prevalence of HPV infection; to investigate which genotypes are more frequent in that population; to correlate data of oncotic cytology with the genotypes found; to verify the prevalence of the four genotypes covered by the quadrivalent vaccine for HPV; to determine demographic, clinical and genetic risk factors that might be related to the persistence of HPV infection after delivery.

**EXPERIMENTAL DESIGN:** This is a longitudinal, prospective study of HPV prevalence in a cohort of pregnant, HIV-positive women. All patients followed at the “Programa de Assistência Integral à Gestante HIV-positiva” of UFRJ will be invited to participate to the study. Those who agree in signing the informed consent term will be enrolled. An average of 80 HPV-positive patients is estimated to be included per year. The molecular study will be conducted at the Laboratory of Human Virology of UFRJ. Samples will be collected, whenever possible, at the beginning of the third trimester of gestation. Another sample will be collected 4 to 6 months after delivery. CD4 t cell count and HIV viral load tests are routinely done for all HIV-positive pregnant women, in the beginning and around the 34\textsuperscript{th} week of gestation, and are conducted at UFRJ. The presence of other sexually transmitted diseases is also routinely screened at our antenatal service and includes: VDRL and FTA-Abs at each trimester of gestation, direct examination and culture of peripheral swab for \textit{N. gonorrhoeae} and \textit{G. vaginalis}, pH determination and direct examination for exclusion of bacterial vaginosis and direct examination for \textit{T. vaginalis}. After genomic DNA extraction and purification from vaginal swabs, specific primer pairs will be utilized for PCR amplification and determination of HPV genotype. For detection of HPV, a nested PCR will be conducted. The first round will be done with external primers MY09/11 and internal primers GP5/GP6 (Molijn et al. 2005). The detection of
genotypes will be done through direct automated sequencing of the PCR products. Specific genotypes will be assigned when sequence homology of ≥ 95% is reached. The pCR for p53 polymorphism will be done in the same material. Two pairs of primers will be used for each polymorphism, as previously described (Ojeda et al. 2003). Data will be analyzed in the program Stata v.8.0 (Stata Corp., College Station, TX). Univariate analyses will be done using t-tests for variables with normal distribution Wilcoxon (Mann-Whitney) for those with non-normal distribution. The Chi-square test or Fisher’s exact test will be used to evaluate associations between categorical variables. Variables with p-values ≤0.15 will be included in the multivariate analysis. p ≤ 0.05 will be considered statistically significant.

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RESEARCH LINE III: TOBACCO CONTROL AND PREVENTION OF NICOTINE INTOXICATION ASSOCIATED TO TOBACCO PRODUCTION

According to the International Agency for Research on Cancer (IARC), smoking is the leading cause of death by cancer in the world. Tobacco consumption is mainly associated to lung cancer, but it is also a risk factor of cancer in other locations such as esophagus, pharynx, larynx, mouth, kidney, bladder, cervix and pancreas. The risk of developing lung cancer is 20 times higher among smokers, and 15 to 30% higher among second-hand smokers (WHO, 2003). In Brazil, there are approximately 18,000 deaths and 27,270 estimated cases of lung cancer every year (BRASIL, 2007; BRASIL, 2008). Of these, approximately 90% are attributable to smoking.

Smoking prevention is the most effective way to reduce morbidity and mortality by various types of cancer, especially lung. In November 2005, Brazil ratified the Framework Convention on Tobacco Control (FCTC), the first international treaty
under the auspices of the World Health Organization, which commits signatory countries to implement a series of effective measures for tobacco control. Given the magnitude of the problem, and the commitment made by Brazil for its confrontation, it is necessary to develop a line of research on tobacco to generate knowledge on the subject and subsidize the Ministry of Health in planning and evaluating effectiveness and cost-benefit of the FCTC actions implemented in the country.

Given the context presented, aiming to meet the new challenges for tobacco control in Brazil, it is proposed the development of sub-projects for research in three fronts: (a) impact evaluation of tobacco control policies; (b) health and work conditions of tobacco workers; and (c) tobacco industry strategies to promote initiation.

Subproject III.1: **EVALUATION OF TOBACCO CONTROL POLICIES - ADVERTISEMENT AND PROMOTION OF TOBACCO PRODUCTS - A TELEPHONE SURVEY IN THE CITY OF PORTO ALEGRE**

In order to assess FCTC implementation in different levels, Brazil recently entered in the group of countries that will participate in the International Tobacco Control Policy Evaluation Project (ITC). ITC is a cohort study, with two or more waves of data collection for policy evaluation, which utilizes a standardized methodology to ensure inter-regional and international comparability. Understanding the importance of the industry advertisement and promotion strategies, an extension of the ITC, to incorporate this issue in the questionnaire, to a sub-sample of young people in Porto Alegre, is being planned.

**Objectives:** To conduct two waves of data collection of the International Tobacco Control Policy Evaluation Project (ITC), through a telephone survey in Porto Alegre.

**Experimental design:** The methodology requires phone or face-to-face interviews using a standardized questionnaire applied to a probabilistic sample of fixed telephones lines subscribers, or individuals living in selected households. Data collection will occur in two stages with an interval of one year, to evaluate the impact of policy changes during the study period. The questionnaire consists of several thematic modules. In Brazil, two ITC’s themes - health warnings on cigarette packs and smoke-free environments initiatives will be applied in 4 cities. On 3 cities - Sao Paulo, Belo Horizonte and Porto Alegre, phone interviews will be conducted in a
sample of 600 residents in each city. In Rio de Janeiro, data will be collected by telephone on a sample of 600 individuals and face-to-face on 400 individuals. Besides the activities foreseen by ITC, a module to evaluate the advertising and promotion strategies of the tobacco industry will be applied to a sub-sample of youngsters in Porto Alegre.

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**Subproject III.2: SURVEY ON ACUTE NICOTINE INTOXICATION PREVALENCE AMONG TOBACCO WORKERS AT PINHAL GRANDE – RS.**

Brazil is the leading country on tobacco leaf production and needs to define measures to protect the health of 300,000 families engaged in tobacco cultivation work. This culture is performed, under the supervision of the tobacco industry that purchases, from farmers, almost all of its production. To ensure a quality harvest, tobacco leaf requires intensive use of pesticides. The widespread use of these substances causes illness of many tobacco growers, since approximately 55% of tobacco farmers do not use protective equipment such as masks, gloves and boots. The justifications for not using include the high cost and inadequacy of equipment, considered hot for the climate. Excessive contact with pesticides can cause nausea, dizziness, headaches, allergies, kidney and liver damage, cancers, genetic alterations, inability to work and even death. Besides the risk of exposure to pesticides, contact with the tobacco leaf, moist with herbicides at harvest, leads to the absorption of nicotine through the skin, triggering a scenario of intoxication, with symptoms such as: nausea, vomiting, weakness, pain, headache, dizziness,
abdominal pain, difficulty breathing and changes in blood pressure levels. The specific intoxication by nicotine is known in the tobacco production as "green tobacco sickness" and is considered an occupational disease. The situation gets even worse because tobacco farming is, in general, an activity carried out by the whole family. Thus, comparing to other crops, dealing with tobacco can become very toxic for children and the farmer. The prevalence of this disease around us is still unknown, although the number of families involved with the tobacco cultivation is significant, especially in the southern region of the country.

**Objectives:** To estimate acute nicotine intoxication prevalence among tobacco workers of the municipality of Pinhal Grande - RS.

**Experimental design:** Population-based study in a sample of residents of Pinhal Grande - RS. Structured questionnaires will be used to collect data on health situation, acute and chronic pesticide poisoning, acute nicotine poisoning, and work characteristics on tobacco cultivation process. Urine samples for cotinine analysis will also be collected to confirm acute nicotine poisoning diagnosis.

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Subproject III.3: FACTORS ASSOCIATED TO CIGARETTE BRANDS PROMOTION AND ADVERTISEMENT STRATEGIES AT POINT-OF- PURCHASE IN THE INITIATION AND MAINTENANCE OF SMOKING

Tobacco research should generate evidence to support the four main areas of smoking prevention: initiation control, cessation stimulus, expansion of smoke-free environments, and tobacco products regulation. To prevent initiation specifically, one of the recommended strategies is a total ban on tobacco advertising. Brazil has advanced in this field, banning tobacco products advertising in all types of media, except at point-of-purchase (POP). In this context, POPs have become the main promotional vehicle of the tobacco industry. Even after banning advertisement in the major advertisement and promotion Medias, a recent study among 13 to 15 year old school children of 13 cities in Brazil, showed a high percentage of young people (36.0% to 48%) who reported seeing advertisements of tobacco in POPs "very often". This data suggests that the industry remains effective in its current promotional strategies. Thus, to review and propose new measures to control advertisement, it is essential to evaluate the strategies of the industry at the POPs.

Objectives: To describe Point-of-purchase promotion and advertisement strategies and their impact on smoking initiation and maintenance in Rio de Janeiro.

Experimental design: Population-based study on a convenience sample of 100 tobacco points-of-purchase (POP) and cigarette buyers in Rio de Janeiro. A structured questionnaire will be applied to describe POPs' advertising aspects: size of posters, most advertised brands, and use of attractive elements. A convenience sample of cigarette buyers from the selected POPs will be interviewed to collect information on the following aspects: gender, age, education, neighborhood, type of product bought, brand preferences, visual impact of packing, visual impact of health warnings, and impact of low tar cigarettes. Data will be entered, consolidated, and analyzed descriptively.

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RESEARCH LINE IV: DEVELOPMENT OF INFORMATION TECHNOLOGY ON CANCER PREVENTION AND CONTROL

Management Information System (MIS) is an integrated system to support of decision-making. It is an essential tool for management modernization. This system integrates and consolidates operational and historical data from different systems, helping institutional decision-making process providing managerial and strategic information. The system generates a series of managerial reports and graphics. Applied to health, the system is developed to convene health research considering spatial distribution of diseases, environmental hazards, and location of health services, linking them with population data. The main objective is to generate databases and development of spatial analysis methodologies and data geoprocessing in micro regions, allowing analysis of the process of population distribution in urban areas, mapping areas of epidemiological risk, allocation of public resources and location of urban equipment.

The main objective of this proposal is to promote generation and dissemination of knowledge on prevention and cancer control, specifically at cancer registries, to provide professionals, scientific community and health managers, a set of methods to promote a differentiated analysis regarding cancer actions surveillance, such as state-space time trends models, evaluation of quality of care provided by hospitals, and also evaluation of health services provided by the network care.

The proposal is developed in two central points, understood as fundamental to add quality and to promote integration of new methodologies on how to analyze, intervene and propose new strategies in the prevention and cancer control. These points are monitoring activities on cancer morbidity and mortality and evaluation of services provided to the population reflected by survival analysis studies.
Subproject IV.1: MODELLING SPATIAL EPIDEMIOLOGICAL INFORMATION AS A CANCER MORBIDITY AND MORTALITY SURVEILLANCE STRATEGY

Modeling the spatial distribution of cancer incidence and mortality can lead to situations concerning the impact of environmental and socio-economic conditions, specific to each region. The spatial variability can be interpreted, as evidence of the existence of environmental or genetic influences on cancer incidence and mortality. Recognition of the spatial pattern of cancer in a given population may lead to more elaborate studies, enabling mapping of cancer risk in this population. Experiences with the use of reprocessing data with the Population-based Cancer Registries - PBCR, qualify professionals in this field in the use of spatial data analysis tools. This experience will allow the expansion of knowledge and use of this technique in all PBCR supported by INCA.

**Objective:** To consolidate a cancer Geographical Information System - GIS at the national level, to provide indicators on this event to subsidize Brazilian health policy makers on planning, implementation and evaluation of programs on cancer prevention and control.

**Experimental design:** Incidence or mortality rates will be calculated for the smallest breakdown possible (neighborhood or district administrative region) and spatially described. Existence of spatial heterogeneity will be verified, according to the analysis of box diagram or dispersion diagram. Once detected spatial heterogeneity, existence of urban space will be tested according appropriate statistical methodology (Satscan, Moran coefficient among others). The correlation of these rates with socioeconomic and environmental indicators will be verified by statistical spatial modeling.

**Team:**
Marise Souto Rebelo (Responsible for Research line IV and co-investigator)
Marceli de Oliveira Santos (Principal investigator)
Rejane de Souza Reis (Co-investigator)
Elisângela Siqueira Costa Cabral (Co-investigator)
Julio Fernando Pinto Oliveira (Co-investigator)
Danielle Nogueira Ramos (Co-investigator)
Juliana Moreira de Oliveira Ferreira (Co-investigator)
Subproject IV.2: PROGNOSTIC FACTORS FOR MAJOR CANCERS (HOSPITAL AND POPULACIONAL BASED) TO SUBSIDIZE CANCER SURVEILLANCE ACTIVITIES

Prognostic evaluation can be subdivided into two parts: quality of care in specialized hospitals, through hospital survival rates studies, and evaluation of health services access, through population survival rates studies. Hospital survival analysis refers to a set of statistical procedures for data interpretation, in which the interest is studying development in time between disease diagnosis and the occurrence of a particular established event, which almost always is death. Information about the efficiency of treatment protocols, i.e., what happens to the patients over time, is important. Such information should integrate an information system to subsidize cancer surveillance activities. It is important to establish a routine, for Hospital-based Cancer Registries - HBCR professionals, to collect, analyze and disseminate result about patients' follow-ups. For the development of a cancer surveillance system, it is necessary to implement collection and analysis of HBCR databases, not only for knowledge of patients profile but also the quality of care offered to them. The description and monitoring of cancer incidence progression is one of the main goals of PBCR. Mortality is influenced by patients' survival, giving the possibility to calculate standardized survival rates for the population living in PBCR's coverage area. The use of these rates allows assessing the impact of quality of care provided to a population, and comparing them. If real differences are found, diagnosis and treatment actions may be directed to the sectors of the population who experience less favorable survival rates.

Objective: 1) To estimate and analyze survival rates for selected cancers attended in cancer hospitals that meet INCA’s national actions. 2) To evaluate impact of the quality of care provided to population, and compare among populations covered by PBCR.

Experimental design: For the major types of cancer selected, an active patient follow-up process will be structured. A database with variables of relevance to assess risk factors and prognosis will be developed. Overall survival and survival in five years will be calculated using Kaplan-Meyer method of analysis and significance will be tested according to log-rank statistics. Univariate analysis will be carried out by
plotting survival curves and will test for differences in categories. Multivariate analysis will be carried out according to the significance observed in the univariate model following the Cox model.

**Team:**
Marise Souto Rebelo (Responsible for Research line IV and Principal investigator)
Marceli de Oliveira Santos (Co-investigator)
Rejane de Souza Reis (Co-investigator)
Elisângela Siqueira Costa Cabral (Co-investigator)
Julio Fernando Pinto Oliveira (Co-investigator)
Danielle Nogueira Ramos (Co-investigator)
Juliana Moreira de Oliveira Ferreira (Co-investigator)

**GOALS**

1. Implementation of a multidisciplinary group, involving researchers with various expertises, ranging from basic experimental approaches and to clinical and epidemiological evaluations in order to study gene-environment interactions on the risk of carcinogenesis and/or cancer progression.
2. Transference of information to the national databank provided by the Brazilian Net of Pharmacogenetic/genomics (“REFARGEN”) regarding the frequency of genetic polymorphisms of pharmacological interest among Brazilian healthy subjects or cancer.
3. Implementation of a Brazilian Net on Pediatric Tumors, with the mission of integrating translational research for definition of diagnostic conducts on pediatric cancer in Brazil.
4. Estimation of the prevalence of HPV infection and the four genotypes covered by the quadrivalent vaccine among the general population, among patients with cervix cancer and among HIV-positive patients.
5. Identification of risk factors associated to cervical cancer cases of the study population: socio-economic, sexual behavior, reproductive history, smoking and history of other STDs.
6. Comparison of cost-effectiveness of cervical cancer screening using the Pap-test and detection of HPV.

8. Evaluation of living, health and work conditions and determination of the degree of acute nicotine intoxication poisoning in Brazilian tobacco growers.

9. Identification of advertisement and promotion strategies used by tobacco industry to promote cigarette brands.


12. Definition of a methodological model to be applied for implementation of a Geographic Information System for cancer registry.

13. Characterization of the spatial and temporal distribution of the major types of cancer in localities with Population-Based Cancer Registry - PBCR coverage.

14. Characterization of patient flow within the municipality in relation to their homes and reference centers for cancer diagnosis and treatment by geographical area.

15. Incorporation of tools and training of cancer registries professionals, state coordinators, health managers to develop population and hospital based cancer survival studies.

16. Consolidation of a model to support cancer records with at least five years of consolidated data to carry out patient active follow-up to subsidize survival studies.

**EXPECTED RESULTS**

-Generation of knowledge about the influence of gene polymorphisms as susceptibility factors for cancer development.

- With the implementation of the HPV vaccine, it is expected that the viral profile associated with cervical cancer cases in our country will modify in the medium and long term. At the same time, it is possible that the expected impact on morbidity and mortality will suffer the upsurge effect of the infection by HPV types absent in the vaccine. Monitoring the viral profile can subsidize HPV surveillance after the
implementation of the vaccine in Brazil and help future immunization policy formulation.

- The prevalence of HPV infection increases during pregnancy, but the impact of early detection of HPV infection and the determination of the infecting subtypes on the persistence of infection after delivery and its associated factors are still unknown. The results of this study will allow us to evaluate prevention policies for this population even before the clinical disease is diagnosed. It is conceivable that patients infected with HPV subtypes of so-called “high risk” must be followed more frequently than those infected by more “benign” subtypes. Another advantage of studying HPV subtypes in this population relates to the fact that soon the HPV vaccine will be largely available for general use. This vaccine is effective for some HPV subtypes of high risk, but it is not known yet whether it will be also effective to other subtypes, not represented in the vaccine formulation, which are also considered of high risk, such as subtypes 45 and 56. At last, the study of pregnant women is justified in the sense that they are more adherent to the treatment and to their clinical follow-up, and therefore this is a good opportunity to prevent loss of follow-up and more effective detection of HPV infection and its complications.

- The observation of spatial variation in cancer incidence can lead to assumptions about the impact of environmental and socio-economic conditions. With this project, INCA fulfills its mission as a reference in cancer research at the national level.

- The purpose of subprojects on tobacco research line is to subsidize the Brazilian government in the implementation of policy measures proposed by the Framework Convention for Tobacco Control, of which Brazil is a signatory. Assessment of the impact of tobacco products health warnings on will enable the development of more effective images, increasing the impact on stimulus for cessation and reduction of smoking initiation. Knowing health and work conditions of tobacco workers will turn possible to propose measures related to the work process to reduce health hazards that tobacco workers are exposed, in particular, reduce the incidence of “green tobacco sickness” (acute nicotine intoxication), even among children who, illegally, carry on activities in local agriculture. The results of the study will also allow
development of strategies to counteract industry’s promotion and sales strategies, in
general aimed at young people.

- The development of a methodological model for cancer registry in real time will allow
the organization and standardization of hospital follow-up supporting survival studies.
It will be possible to determine which factors best predict tumors prognosis and
assess quality of care in participating hospitals. At the population level, it will be
possible to build a structural model that supports cancer registries, with at least five
years of consolidated data to carry out active follow-up of cancer patients,
subsidizing survival studies.

- Capacity building: 01 Master, 04 PhDs, and 1 post-PhD.

- Publications: At least 10 scientific articles published at Qualis-A journals.

- Participation on national and international meetings and congress with publication on annals.

**TRANSFERING OF RESULTS FOR PUBLIC POLICY FORMULATION**

The results of this research group will have a direct impact on the identification
of genetic and environmental factors with potential risk for cancer development
and/or progression. These results may help the definition of public health policies
aiming at the prevention or reduction of involuntary exposure to carcinogenic agents
and may contribute for orientation of informative campaigns designed to promote
healthy habits and behavior. These results may also give scientific support for legal
decisions for restriction of smoking and for protection of workers of the tobacco
industry. Finally, the epidemiological and clinical information may help evaluation of
cost-benefit relationships for health public conducts.

Results will be delivered to the Ministry of Health to subsidize surveillance of
HPV genotypes associated with cervical cancer cases and to help development of
immunization programs. Information about prevalence of viral types of HPV in
cervical cancer will allow determining whether the vaccines currently available are
suitable for immunization against HPV types associated with cervical cancer in the country. The survey will generate data for future comparisons on: (1) prevalence of viral types associated with cervical cancer, to assess the immunization efficiency of currently available vaccines, (2) identify viral variants associated with cross immunization, (3) characterize viral variants of types with high oncogenic risk. Data will be important for definition of public health policy in the fight against cervical cancer.

Result reports will be sent to those involved in the National Tobacco Control Program at INCA, Ministry of Health, Health State Departments, and the Executive Secretariat of the National Commission for Implementation of the Framework Convention for Tobacco Control in Brazil, subsidizing planning of effective measures on tobacco control. Health promotion, and cancer and acute nicotine intoxication measures will be developed jointly with Workers Health State Coordinators.

- Observation of spatial variation in cancer incidence will allow mapping of risk of more incident tumors in the population, providing a better allocation of resources and efforts.

- Consolidation of a national model to support cancer registries with at least five years of consolidated information will enable an active follow-up of cancer patients. The result of these actions will be a diagnosis of quality of care provided by hospitals and public health network, enabling the evaluation and redirection of actions for cancer prevention and control.

PREVIOUS EXPERIENCE IN RESEARCH

Moyses Szklo
(Bolsista de Produtividade em Pesquisa do CNPq - Nível IA) – Graduated in Medicine by the Universidade Estadual do Rio de Janeiro UERJ (1963), MSc in Public Health - Johns Hopkins University (1972), and PhD in Public Health - Johns Hopkins University (1974). Editor-chef of American Journal of Epidemiology since 1988. Professor of Epidemiology and Health Polices at Universidade Federal do Rio de Janeiro. Full Professor for 33 years at Scholl of Public Health - Johns Hopkins University (EUA). Member do corpo editorial de diversas revistas científicas. Published ca. 200 scientific articles in Public Health and epidemiology.
Sérgio Koifman
(Bolsista de Produtividade em Pesquisa do CNPq - Nível IA) Graduated in Medicine at Universidade do Estado do Rio de Janeiro (1974), MSc in Social Medicine-Epidemiology, at the Universidad Nacional Autonoma de Mexico Xochimilco (1978), PhD in Preventive Medicine Preventiva at the Universidade de São Paulo (1988) and Pos-Doc. at the School of Occupational Health, McGill University, Montreal, Canada (1991-93). Coordinator of Inter-Institutional Master Program in Public Health at the Universidade Federal do Pará/ Fiocruz, Coordinator of Post-Graduation program in Public Health at the Escola Nacional de Saúde Pública (1999-2002). Works as researcher at Fundação Oswaldo Cruz, and coordinate the Post-Graduation program in Public-Health and Environment at the Escola Nacional de Saúde Pública/ Fiocruz, is Professor at Post-Graduation Program in Public Health at Universidade Federal do Acre (UFAC/Fiocruz). Develops research programs in Public Health and Epidemiology, focusing in Cancer Epidemiology and Environmental Epidemiology.

Maria do Socorro Pombo de Oliveira
(Bolsista de Produtividade em Pesquisa do CNPq - Nível 2) Graduated in Medicine by the Universidade de Pernambuco (1974), MSc in Hematology at Hopital Saint-Louis, Université de Paris VII (1979), Fellow-Research at Royal Post-Graduated Medical School-Hammersmith Hospital, Londres (1984-1988) and PhD in Cellular and Molecular Biology by the Fundação Oswaldo Cruz (1991). She is medical researcher at Instituto Nacional de Câncer, with research lines in Hematology, infant acute leukemia, molecular changes in acute leukemia and molecular epidemiology.

Gulnar Azevedo e Silva Mendonça
(Bolsista de Produtividade em Pesquisa do CNPq - Nível 2) Graduated in Medicine at Universidade do Estado do Rio de Janeiro (1978) with MSc in Coletive Health at Universidade do Estado do Rio de Janeiro (1991) and PhD in Preventive Medicine at Universidade de São Paulo (1997). She is Professor at Epidemiology Department at Instituto de Medicina Social-Universidade do Estado do Rio de Janeiro. She was coordinator of prevention and surveillance at Instituto Nacional de Câncer from October 2003 to September 2007. Develops research activities in cancer epidemiology and risk factors for non-transmissible diseases.
Marcelo Soares
(Bolsista de Produtividade em Pesquisa do CNPq - Nível 2) Prof. Marcelo Soares is appointed at the Department of Genetics from UFRJ as Professor and Researcher since 2002. Currently, he heads the Laboratório de Virologia Humana of that Department, where he develops intense research in virology, primarily in HIV, and more recently in other viruses of clinical relevance. Since 2006 Prof. Soares is also a Collaborator Researcher at the Division of Genetics from the Research Center at INCA, where he also develops part of his research activities. His intellectual production listed in the Lattes Curriculum, and the set of research projects approved and financed shows Prof. Soares’ scientific activities and the last years. Currently, Prof. Soares is Researcher 2 of Scientific Productivity from CNPq. Prof. Soares acts in two Graduate Programs, that of Genetics from UFRJ, and the one in Bioinformatics from LNCC-MCT. Prof. Soares headed the Graduate Program in Genetics from 2004 to 2008, and he has the majority of his students there. Prof. Soares is a member of the Graduate Committee of both Programs.

Elizabeth Machado
(Bolsista de Produtividade em Pesquisa do CNPq - Nível 2) Dr. Elizabeth Machado had her M.Sc. degree at UFRJ in clinical aspects of leptospirosis, and had her Ph.D. in a “sandwich” program at the Laboratory of Immunoregulation from the NIH, U.S.A. Her Ph.D. thesis was already on HIV/AIDS. After her return to Brazil, Dr. Machado developed research related to HIV pathogenesis in infected children, in collaboration with Dr. Anthony DeVico from the Institute of Human Virology, Baltimore, U.S.A., with a 5-year R01 grant from the NIH. She conducts HIV basic research at the Laboratório de Virologia Humana at UFRJ related to resistance to antiretrovirals (ARV), host restriction factors to HIV infection, and HIV pathogenesis. She also runs clinical research at UFRJ and at the Hospital dos Servidores do Esatdo involving HIV-positive teenagers and pregnant women with respect to HPV, tuberculosis, and side effects of ARV. She is also currently involved in a research in dengue at FIOCRUZ. Dr. Machado is also part of a clinical research group from NIAID for observational studies in pregnant women and their children exposed to HIV (NISDI Longitudinal Study in Latin American Countries, LILAC) – Niaid – NIH. She also took part on the clinical trial ATN 024i: A Randomized, Open-Label Trial of Three Hepatitis B
Cibele Rodrigues Bonvicino
(Bolsista de Produtividade em Pesquisa do CNPq - Nível 2) PhD in Genetics at Universidade Federal do Rio de Janeiro (1994). Researcher at Instituto Nacional de Câncer and Fundação Oswaldo Cruz (FIOCRUZ). The main research fields are: genetic diversity, phylogeny and molecular evolution; genetics of hereditary cancer (Retinoblastoma); genetic diversity of HBV. Adviser of the following Pos-Graduation courses: Pós-Graduação em Genética at UFRJ, Pos-Graduation in Oncology at Instituto Nacional de Câncer, Pos Graduação em Bioquímica e Biologia Molecular at FIOCRUZ.

Liz Maria de Almeida
PhD on Sciences by the Universidade do Estado de São Paulo (2002). Currently working as Head of Epidemiology Division of the National Cancer Institute of Brazil (INCA). Large experience on cancer behavioral risk factors surveys and co-investigator on the study: "Prevalence of human papillomavirus DNA in a community in the city of Rio de Janeiro" - partial funding: GlaxoSmithKline

Valeska Carvalho Figueiredo
Graduated in Medicine at the Universidade do Estado do Rio de Janeiro (1986), MSc (1997) and PhD Doutorado (2007) in Public Health-Epidemiology at the Universidade do Estado do Rio de Janeiro. She is researcher at the Epidemiology Division and Surveillance at Instituto Nacional de Câncer, developing and participating in research programs about tobacco and risk factors in transmissible diseases. She is co-investigator in collaborative projects between the Istituto Nacional de Cancer and the Institute for Global Tobacco Control at the Johns Hopkins: Epidemiology & Intervention Research for Tobacco Control. International and Health Research & Capacity Building (The Study in Brazil), Secondhand Smoke Exposure in Non-
smoking Women and Children (The Study in Brazil); and Determinants of Salivary Cotinine Levels in Smokers from Different National and Ethnic Groups (The Study in Brazil).

**Cristina de Abreu Perez**
She is graduated in Psychology by the Universidade Gama Filho (1993). Supervises the Cancer Control Program of the Instituto Nacional do Câncer. She has specialized in Collective Health.

**Cristiane Ferreira Vianna**
Graduated in Law, works at National Tobacco Control Program (CQCT) and at the Vice-Secretária Executiva da Comissão Nacional para Implementação da CQCT were she is Substitute Coordinator of the Brazilian Intergovernmental Commission Vice for Tobacco Control-MERCOSUL. Worked in police for tobacco control in Brazil since 1999.

**Ana Lucia de Souza Mendonça**
Has a degree in Nutrition from the Universidade do Estado do Rio de Janeiro (1985) and Master's in Public Health from the University of California - Berkeley (1990). Currently she is a senior analyst in the Cancer Program of the Instituto Nacional do Câncer.

**Luís Felipe Martins**
Statistics has a Master in Public Health at the Universidade Estadual do Rio de Janeiro. His master's thesis was in "Factors associated with the non Papanicolaou examination. - cross population based study in two Brazilian capitals." He participated in various surveys conducted by the Division of epidemiology of CONPREV / INCA.

**Mirian Carvalho de Souza**
Statistics, Master in Public Health at the Escola Nacional de Saúde Pública da FIOCRUZ. She participated in various surveys conducted by the Division of epidemiology of CONPREV / INCA.
Paulo Antonio Silvestre de Faria
He has experience in the field of medicine, with emphasis on Pathological Anatomy, including Surgical Pathology, Cytopathology and Autopsies. In the Surgical Pathology field has special interest in Oncology Pediatric Pathology and Uropatologia. Also in Pathological Anatomy has experience in Management Services of Pathology and in Pathology Information Systems.

Lucília Maria Gama Zardo
Graduated in Medicine by the Faculty of Medicine of Petropolis in 1978 and specialized in Cytopathology. Has post-graduate degree in Management Systems and Health Services by the Escola Nacional de Saúde Pública (Fundação Oswaldo Cruz) and MSc at the Instituto Nacional de Câncer. Was Secretary of the Brazilian Society of Cytopathology. Their professional activities and publications are focused mainly on the following themes: prevention and control of cervical cancer, citopatologia, HPV, breast cancer and cancer of the uterine cervix. Currently, the head of laboratory activities of the Integrated Technology Section in Cytopathology.

Cláudio Pompeiano Noronha
Graduated in Medicine by the Universidade do Estado do Rio Janeiro (1979), residency in Preventive and Social Medicine (1983), specializing in Epidemiology (1984) and Master in Public Health by the National School of Health Pública by the Fundação Oswaldo Cruz (1993). Today is a medical doctor in public health - Municipal Health Secretariat of Rio de Janeiro. Is the head of the Coordination for the Prevention and Monitoring the National Cancer Institute. He has experience in the field of Public Health, working in the following themes: information systems in health, the evaluation of performance indicators in health, prevention, control and surveillance of cancer, studies of incidence, mortality and survival in câncer.

Maria do Carmo Esteves da Costa
Graduated in Medicine at Universidade Federal do Rio de Janeiro (1980), and especialization in Public Health at Universidade Federal do Rio de Janeiro (1982), specialisation in Planning and Administration of Public Hospitals by the Fundação Oswaldo Cruz (1986), and Master in Public Health by the Universidade Estadual do
Rio de Janeiro (1997). He is currently Physician at the Instituto Nacional de Cancer. Between 1990 and 1996 was responsible for follow up examinations of patients with citopatológicos changed in Rio de Janeiro. Since 2002, was member of the Division of Early Detection of Coordination on Prevention and Monitoring at the Instituto Nacional de Câncer. It was co-investigator of the study: "Prevalence of human papillomavirus DNA in a community in the city of Rio de Janeiro."

**Silvana Rubano Barretto Turci**

Graduates in Pharmacy and Pharmaceutical Sciences Biochemistry at Faculdade Oswaldo Cruz Sao Paulo (1984) and Master in Public Health at the Fundação Oswaldo Cruz (1994). Currently occupies the post of senior tecnologista at the Escola Nacional de Saúde Pública National (ENSP / Fiocruz). Developis technical collaboration with the |Instituto nacional de Câncer at the Coordination for Monitoring of Cancer Prevention. She has experience in the field of Public Health, with emphasis on Environmental and Occupational Toxicology, acting mainly in the following subjects: treatment of smoking, primary prevention, prevention of cancer, occupational cancer and tobacco control

**Rosane Vianna Jorge**


**Miguel Ângelo Martins Moreira –**

PhD in Genetics at Universidade Federal do Rio de Janeiro (1996). Researcher at Instituto Nacional de Câncer. The main research fields are: Hereditary Breast and
Ovary Cancer, Gene Mapping, genome Sequencing, Molecular Evolution and Phylogenetics, drug resistance. Adviser in the following Pos-Graduation courses: Pos-Graduation in Genetics at Universidade Federal do Rio de Janeiro.

**Marcelo Alex de Carvalho**

Has a degree in pharmacy from the Universidade Federal Fluminense (1995), a master's degree in biochemistry from the Universidade Federal do Rio de Janeiro (1998), a Ph.D. degree in Biological Sciences (Biophysics) from the Universidade Federal do Rio de Janeiro (2004) and post - doctorate from the H. Lee Moffitt Cancer Center & Research Institute (2006). He is currently professor of the Centro Federal de Educação Tecnológica de Química and researcher of the Division of Pharmacology of INCA. He has experience in biochemistry, with emphasis on Cellular and Molecular Biology, working mainly in the study of molecular models of BRCA1 and breast cancer.

**Beatriz de Camargo**

Professsor at the Universidade de Medicina de Sao Paulo, develops activities of teaching in the post-graduation (lato senso and strictu senso) with extensive experience in clinical research in the field of pediatric oncology. Through the "Coordenação de Estudos Cooperativos Brasileiros" executed projects of epidemiological character in pediatric cancer. Currently developing a line of research at INCA in the Program of Hematology-Oncology Pediatric-CPq, together with CONPREV, regarding records of cancer incidence and mortality of children and youth. Active participant of the International Society of Pediatric (SIOP) and reference in studies of tumor, neuroblastoma and Wilms.

**Rinaldo Wellerson Pereira**

Graduated in Veterinary at the Universidade Federal de Viçosa (1995), Master in Biochemistry and Immunology at the Univesidade Federal de Minas Gerais (1997), Ph.D. in Biochemistry and Immunology at the Universidade Federal de Minas Gerais (2002) developed in part at the Stanford Genome and Technology Center - Stanford University (2000-2001). He is currently a Doctor, professor at the Universidade Católica de Brasilia where he coordinates the genetics courses for graduation and also minister the disciplines of Basic Genetics, Human Genetics, Genetics Applied to
Medicine and Forensic Genetics. He is the supervisor of Master’s and Ph.D. from the Graduate Program in Genomic Sciences and Biotechnology and in the Program of Fitness. His research projects focus on the application of genetic variability for understanding complex phenotypes and the development of applications for Forensic Genetics.

**Gutemberg de Almeida Filho**
Prof. Gutemberg de Almeida Filho had his M.Sc. in Medicine (Gynecology) at the Universidade Federal do Rio de Janeiro (1992), Ph.D. in Medicine (Gynecology) at the Universidade Federal do Rio de Janeiro (1998) e medical residence at INAMPS (1979). Currently he is Associate Professor at UFRJ, Clinician at UFRJ, member of the Editorial Board of *Femina*, of the Brazilian Journal of Ob/Gyn and of the Brazilian Journal of Gynetoscopy. He has experience in Medicine, primarily in the fields of vulvar neoplasias, intra-epithelial neoplasias and HPV.

**Tomaz Pinheiro da Costa**
Prof. Tomaz Pinheiro da Costa had his undergraduate in Medicine at PUC of Paraná (1974) and M.Sc. in Medicine (Infectious Diseases) at UFRJ (1980). Currently, he is Associate Professor at UFRJ. He has experience in Medicine, with emphasis in mother-and-child health. He works primarily in the followinf fields: prematurity, prevention and intrauterine infections.

**Cristina Hoffer**
Dr. Cristina Hoffer works on HIV in pregnant women since 2003, with several articles in the field. She is the principal investigator in some related projects, such as the evaluation of infection by *Listeria monocytogenes* among HIV-pregnant females.

**Marise Souto Rebelo –**
With graduation in Medicine from the Federal University of the State of Rio de Janeiro (1982), Master in Public Health by the Oswaldo Cruz Foundation (1996) and Ph.D. in Clinical Medicine (Clinical Research) by the Federal University of Rio de Janeiro (2004). Today is manager of the Division of Information Coordination of Prevention and Surveillance of the National Institute of Cancer. Has experience in the field of Public Health, with emphasis on epidemiology, acting mainly in the
following areas: cancer, epidemiology, records of cancer, cancer incidence and mortality. It was responsible for the technical specification for development of specific application to RHC - Regional Cancer Hospital and also the technical specification of the application to national integration of RHC.

Marceli de Oliveira Santos
With graduation in Statistics for the National School of Statistical Sciences (1991), Master in Public Health by the Oswaldo Cruz Foundation (1997). Today is Analyst Program for the Control of Cancer of the National Institute of Cancer. Has experience in the area of Probability and Statistics, with emphasis on Statistics. Acting mainly on the following themes: surveillance, public health, analysis of data and studies on cancer prognosis. Participated with the improvement and development of application specific to the basis of Cancer Registries Population-RCBP. It is responsible for coordinating the RCBP at central level (training, supervision, analysis and dissemination of information). Run also geo-analysis of studies on cancer.

Rejane de Souza Reis
It has a graduate degree in Biological Sciences Full by the Faculty of the City (2000), post-graduate in Course To Recorders of Cancer by the National Institute of Cancer (1997), refinement in the Customer Excellence by the National Institute of Cancer (1996), post-graduate Ongoing In the winter of Cancer Epidemiology by the Oswaldo Cruz Foundation (2003), post-graduate in Update On Course To Epidemiology Service by the Oswaldo Cruz Foundation (2003), post-graduate in Update on Cancer Epidemiology by the National Institute of Cancer (2003) and post-graduate in Update Course In Molecular Epidemiology of the Oswaldo Cruz Foundation (2004). Master in Public Health by the Institute for Studies on Public health - IESC / UFRJ (2007). Today is analyst for Cancer Control Programmes of the National Cancer Institute.

Ana Maria Ramalho
Head of the Division Attention Oncológica of CONPREV / INCA, responsible for the national programme for the control of cancer of the cervix.

Tereza Maria Piccinini Feitosa
Works in the Division of Attention Oncológica, in the national programme to control cancer of the cervix.

REFERENCES

Birch Jm. Arch Dis Child 80: 1 (1999)
Downloaded On March 7th (2008)
Garbe, Eigentler. Melanoma Res 17:117 (20070
Gonçalves, Barbosa. Atep Caxambu – Mg (2006)
IARC Monographs Vol. 90 (2007)
Kupper, Muller. Applied Regression Analysis And Other Multivariable Methods.
Pombo De Oliveira Et Al, Cancer Epid Biomarkers And Prevention 15: 2336 (2006)
Munóz et al., Vaccine 24s:S3/1 (2006)
CRONOGRAM

1st Year

First semester:
Review literature, selection and recruitment of human resources, training the team for various activities (approaches to recruitment of individuals, conducting interviews and collecting data, use of computational tools), acquisition of material consumption and for cartography, acquisition and of data processing equipments for molecular analysis, establishment of methodologies for molecular analysis, purchase of animals and establishment of experimental models for toxicological evaluation.

Second semester: Recruitment of participants, collecting data, collecting biological samples, conducting analyses of molecular and cellular, clinical monitoring of patients, creation of the database, analysis of consistency, purification and geocoding of data, training of external partners (Regional Offices).

2nd Year

First semester: Cleaning and analysis of clinical data and molecular.

Second semester: Analysis of correlation between genetic and environmental variables and clinical outcomes for risk of development and progression of cancer, Cost-effectiveness.

3rd Year

Carrying out experiments for assessment of gene expression and of mRNA stability associated to genetic polymorphisms; Improvement of demographic analyses, continuation (of follow-up analysis) for correlation between genetic and environmental variables and clinical outcomes for cancer progression; analysis of consistency and data evaluation(final stage); Relationship between databases; data evaluation; Preparation of articles for publication

Years 4 and 5: evaluation of strategies and priorities for analysis. Spatial statistical data analysis; correlation studies with socioeconomic and environmental indicators; Identification of patient health-assistance flow; analysis of the indicators for
assessing the quality of health-care. Survival Analysis, final data evaluation for publication.

FINANCED PROJECTS IN THE LAST 5-YEARS

Sergio Koifman
- Qualidade de vida subseqüente ao tratamento do câncer de colo de útero (CNPq e FAPERJ)
- Exposição a pesticidas organoclorados e efeitos reprodutivos em Cidade dos Meninos, Duque de Caxias, RJ (CNPq)
- Polimorfismos genéticos (CYP17 e MTHFR), exposições ambientais e câncer de mama em mulheres jovens no Rio de Janeiro (CNPq)
- Avaliação epidemiológica e caracterização radiológica ambiental na população residente dos municípios de Monte Alegre, Prainha e Alenquer, PA (CNPq)
- Exposições ambientais e câncer de tireóide: estudo caso-controle de base populacional em Goiânia, GO (CNPq)
- Estudo Latino-Americano de Sobrevida de Câncer (CNPq)
- Exposição a campos eletromagnéticos e leucemias na infância: análise exploratória de sua associação no município de São Paulo (CNPq)
- Efeitos na saúde decorrentes da exposição a substâncias químicas em pilotos agrícolas (CNPq).

Maria do Socorro Pombo de Oliveira
- Estudo multidisciplinar e imunomolecular das Leucemias Agudas

Marcelo Alves Soares:
- “Reativação e Manutenção de Seqüenciador Automático de DNA para Projetos de Seqüenciamento do Departamento de Genética da UFRJ”. Projeto FAPERJ PROGRAMA Apoio à manutenção de equipamentos Multiusuários – 05/2008. Value: R$ 44.600,00
- “Citometria de fluxo em estudos de interação hospedeiro/vírus e função de proteínas celulares e compostos exógenos na ativação celular e câncer”. Projeto FAPERJ PROGRAMA APOIO A GRUPOS EMERGENTES DE PESQUISA NO ESTADO DO RIO DE JANEIRO – 08/2008. Value: R$ 194.400,00
- “Incidência de polimorfismos nos genes humanos TRIM5alfa e APOBEC3G/3F em crianças HIV-positivas correlacionados com diferentes padrões de evolução da Aids”. Projeto CNPq Bolsa de Produtividade em Pesquisa 2007
- “Incidência de polimorfismos nos genes humanos TRIM5alfa e APOBEC3G/3F em crianças HIV-positivas correlacionados com diferentes padrões de evolução da Aids”. Projeto CNPq Universal 2007. Value: R$40.000,00
- “Caracterização de uma nova classe de mutações de resistência a anti-retrovirais no domínio da RNAse H do vírus da imunodeficiência humana do tipo 1 (HIV-1)”. Edital FAPERJ Cientistas do Nosso Estado 2006. Value: R$ 48.000,00

“11º Workshop Internacional em Epidemiologia Molecular e Evolução Viral”. Editais de Apoio a Organização de Eventos Internacionais de 2005: Programa Nacional de
DST e Aids – MS – Value: R$ 95.500,00; CAPES – Value: R$ 30.000,00; FAPERJ – Value: R$ 12.000,00; CNPq – Value: R$ 10.000,00
- “Transmissibilidade materno-infantil do HIV-1 dos subtipos B e C na Região Sul do Brasil: fatores preditivos e momento da transmissão vertical”. Edital CNPq 36/2004 - Cooperação Técnica CNPq/MS/DECIT. Value: R$ R$ 19.000,00
- “Caracterização do polimorfismo genético no gene da protease do HIV-1 de subtipos B e C e seu impacto na susceptibilidade aos inibidores de protease em pacientes em tratamento anti-retroviral”. Edital Primeiros Projetos FAPERJ/CNPq 2003. Value: R$ 27.600,00
- “Estudo de determinantes moleculares de susceptibilidade de primatas do novo mundo a infecção por lentivirus”. Edital CNPq Universal 2003. Value: R$ 20.000,00

Elizabeth S. Machado:
- “Investigation of the role of Integrin alfa4beta7 in HIV transmission”. Intramural NIH Grant 2008. Value: US$ 60,000.00 (Colaboradora)
- “Fatores associados à ocorrência de dengue grave: da assistência e ambiente à imunologia e genética”. Edital FAPERJ Apoio ao Estudo de Doenças Negligenciadas e Reemergentes – 2008 (Colaboradora)

Gulnar Azevedo e Silva
- CNPq – Produtividade em Pesquisa “Fatores de risco e prognósticos para câncer”
- FAPERJ Modalidade APQ1 “Sobrevida e fatores de risco para câncer de próstata em pacientes assistido em Serviço Especializado no Rio de Janeiro
- CNPq – Edital Universal “ Fatores associados ao risco de progressão para o câncer do colo do útero e lesões precursoras em mulheres residentes em municípios da Baixada Fluminense”.
- Empresa GSK – Prevalência de HPV e fatores associados em mulheres de comunidade do Rio de Janeiro.

Cibele Rodrigues Bonvicino

Liz Maria de Almeida
- “Estudo dos fatores que influenciam o seguimento das mulheres que realizam o exame preventivo para o câncer do colo do útero na rede SUS no município do Rio de Janeiro” – FAPERJ R$ 43.000,00.

Valeska Carvalho Figueiredo
- Secondhand Smoke Exposure in Non-smoking Women and Children. The Study in Brazil. Financiado pelo Institute for Global Tobacco Control e pelo Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, 2006. R$ 10.000,00


**Silvana Rubano Turci**

- Condições de vida, saúde e trabalho dos fumicultores do Brasil. Inquérito Domiciliar sobre as condições de saúde e trabalho da população de Paraíso do Sul. Financiado pelo Fundo Nacional de Saúde. 2006-2008. R$ 300.000,00

**Tânia Maria Cavalcante**

- Promoção de Ambientes Livres de Tabaco. Bloomberg Global Initiative to Reduce Tobacco Use Foundation 2008. R$ 908.000,00

**Cristiane Ferreira Vianna**

- Beliefs, Values and attitudes of Brazilian lawmakers towards the Framework Convention on Tobacco Control. International Development Research Centre - Research for International Tobacco Control, 2004. R$ 10.000,00

**Rosane Vianna Jorge**

Miguel Ângelo Martins Moreira
- Identificação de Alterações e Metilação no Promotor do Gene BRCA1 em Pacientes com Câncer de Mama e/ou Ovário Hereditários. FAPERJ. Value: R$ 12.000,00 (2005).
- Desenvolvimento de Resistência ao STI571 em Leucemia Mielóide Crônica. FAPERJ. Value: 19.300,00 (2003).

Marcelo Alex de Carvalho
- APQ-1 (FAPERJ - 2006: Pesquisador Responsável)

Cristina Hoffer:

Luiz Claudio Thuler
- Instituto Avon/2003 - Rastreamento para o câncer de mama por meio do exame clínico: projeto piloto no Estado do Mato Grosso do Sul

Marise Souto Rebelo
- INCA/ Fiocruz: Acesso à assistência oncológica – mapeamento de fluxos origem-destino. Linha Temática: Avaliação de modelos de atenção em pacientes com câncer de mama e colo de útero.
Research line: Cancer surveillance and risk factors
-Cepesq/INCA: Estudo de sobrevida para tumores selecionados em pacientes atendidos no Instituto Nacional de Câncer - Rio de Janeiro

Research line: Cancer Prognosis
-UERJ: Estudo Pró-Saúde
-ELSA: Estudo Longitudinal da Saúde do Adulto – MS
-CENEPI/MS: Projeto Centro Colaborador INCA/ CENEPI

NATIONAL AND INTERNATION INTERACTION

Moyses Szklo
Johns Hopkins University

Sergio Koifman
Interações Locais: Grupo de Pesquisa em Oncologia Ginecológica – Instituto Nacional do Câncer

Maria do Socorro Pombo de Oliveira
Interações Locais: Alexandre Apa2, Jozina Aquino1, Alejandro Mauricio Arancibia12, Flávia Nogueira Araújo1, Rosania Maria Basegio16, Silvia R. Brandalise3, Lilian Burlemaqui1, Eni Guimarães Carvalho4, José Carlos Córdoba5, Virginia M. Coser6, Imaruí Costa, Maria Lydia D’Andrea14, Tereza Cristina Cardoso Fonseca17, Mônica Lankszner15, Maria Lucia de Marino Lee9, Luis Fernando Lopes10, Carmen M. Mendonça10, Núbia Mendonça1, Flávia Nogueira1, Waldir Pereira7, Vitória P. Pinheiro5, Elisângela Ribeiro13, Terezinha J M Salles9.


Interações Internacionais:
Anthony Ford, Ph.D e Mel Greaves, Ph.D- Institut of Cancer Research-UK através de intercâmbios com formação de recursos humanos e desenvolvimento de projetos em leucemias;
Ralph Marchleck, Ph.D e Clauss Mayer, Ph.D- Diagnostikzentrum für Akute Leukämie (DCAL) Frankfurt- Alemanha, colaboradores com finalização das análises de identificação de parceiros do gene MLL através de clonagem;
Giovanni Casagniga, Ph.D e Andréa Biondi M.D., Ph.D- Fundazioni Tettamanti: através de intercâmbios com formação de recursos humanos na abordagem de SNP arrays nos projetos de leucemias agudas linfoblasticas;
Joseph Wielmels, Ph.D - Associate Professor Laboratory for Molecular Epidemiology UCSF: através de intercâmbios com formação de recursos humanos com elaboração e desenvolvimento de projetos referentes a fatores etiopatológicos das neoplasias pediátricas;
Patrícia Buffler, Ph.D- Professor of Epidemiology and Dean Emerita Kenneth and Marjorie Kaiser Endowed Chair University of California, Berkeley School of Public Health - Division of Epidemiology: coordenadora do Childhood Leukemia International Consortium e colaboradora nos desenhos de estudos epidemiológicos referentes a epidemiologia-molecular.

Gulnar Azevedo e Silva
Instituto de Biologia da UERJ, Instituto Ludwig de Pesquisa em Câncer, Departamento de Medicina Preventia-USP, IARC
Marcelo Alves Soares
Departamento de Genética; Laboratório de Virologia Humana – Inst. Biologia – UFRJ.

Elizabeth Stankiewicz Machado
**Interações Locais**: Programa de Assistência Integral à Gestante soropositiva (Hospital Universitário Clementino Fraga Filho); Hospital dos Servidores do Estado–RJ;
**Interações Internacionais**: Dr. Anthony DeVico (Institute of Human Virology, Baltimore, USA); NISDI Longitudinal Study in Latin American Countries (LILAC) – Niaid – NIH.

Liz Maria de Almeida
WHO/CDC – Global Tobacco Surveillance System
OPAS - Brasília

Tania Cavalcante
Secretaria Executiva da Comissão Inter-ministerial para Implementação da Convenção-Quadro para o Controle do Tabaco (FCTC).
Representação do Ministério da Saúde na Comissão Inter-governamental para Controle do Tabaco no Mercosul a Países Associados.
Coordenação da Rede Ibero Americana do Controle do Tabaco.
Coordenação da Rede Por um Mundo sem Tabaco.

Valeska Carvalho Figueiredo
Institute for Global Tobacco Control – Johns Hopkins Bloomberg School of Public Health
Organização Panamericana da Saúde
“International Tobacco Control Policy Evaluation Project”, Universidade de Waterloo, Canadá
Cristina de Abreu Perez
“International Tobacco Control Policy Evaluation Project”, Universidade de Waterloo, Canadá
Rede Ibero Americana do Controle do Tabaco

Cristiane Vianna
Secretaria Executiva da Comissão Inter-ministerial para Implementação da Convenção-Quadro para o Controle do Tabaco (FCTC).
“International Tobacco Control Policy Evaluation Project”, Universidade de Waterloo, Canadá
Rede Ibero Americana do Controle do Tabaco

Rosane Vianna Jorge
Local and Institutional Interactions: Programa de Farmacologia – INCA; Programa de Pesquisa em Farmacologia Celular e Molecular – Instituto de Ciências Biomédicas/UFRJ; Rede Nacional de Farmacogenética/Farmacogenômica; Guilherme Suarez Kurtz – INCA; Edson Rondinelli - IBCCF/UFRJ; Patrícia Torres Bozza – FIOCRUZ; Carlos Gil Ferreira – INCA

Gutemberg Leão de Almeida Filho
Serviço de Ginecologia da UFRJ

POST-GRADUANTION ACTIVITIES

Prof. Moyses Szklo
Programa de Pós-graduação Saúde Pública (IESC)

Prof. Sergio Koifman
Post-Graduantion programs-Saúde Pública e Meio Ambiente da Escola Nacional de Saúde Pública – Fiocruz
Profª. Maria do Socorro Pombo de Oliveira
Post-Graduantion programs-em Oncologia (INCA)

Profª Gulnar Azevedo e Silva:
Programa de Pós-graduação em Oncologia (INCA)
Pós-graduação em Saúde Coletiva do Instituto de Medicina Social (UERJ)

Prof. Marcelo Soares
Post-Graduantion programs- Genética (UFRJ)
Post-Graduantion programs- Modelagem Matemática e Computacional, Ênfase em Bioinformática (LNCC-MCT)

Profª Elizabeth Machado
Post-Graduantion programs- Doenças Infecciosas e Parasitárias (UFRJ)
Programa de Pós Graduação em Genética (UFRJ)

Profª Cibele Rodrigues Bonvicino
Post-Graduantion programs- Genética (UFRJ)
Post-Graduantion programs- Bioquímica e Biologia Molecular (FIOCRUZ)

Profª Liz Maria de Almeida
Post-Graduantion programs- Oncologia (INCA)

Valeska Carvalho Figueiredo
Post-Graduantion programs- Oncologia (INCA)

Mirian Carvalho de Souza
Post-Graduantion programs- Oncologia (INCA)

Luís Felipe Leite Martins
Post-Graduantion programs- Oncologia (INCA)

Prof. Rosane Vianna Jorge
Post-Graduantion programs- Oncologia (INCA)
Post-Graduantion programs- Farmacologia e Química Medicinal (UFRJ)

**Prof. Miguel Ângelo Martins Moreira**
Post-Graduantion programs- Oncologia (INCA)
Post-Graduantion programs- Genética (UFRJ)

**Prof. Marcelo Alex de Carvalho**
Post-Graduantion programs- Biofísica (UFRJ)
Post-Graduantion programs- Oncologia (INCA)

**Profª Beatriz de Camargo, M.D., Ph.D**
Post-Graduantion programs- Ciências Fundação Antonio Prudente-São Paulo
Post-Graduantion programs- Ciências em Oncologia da Universidade de São Paulo

**Prof. Rinaldo Wellerson Pereira, DSc**
Post-Graduantion programs- Ciências Genômicas e Biotecnologia - Universidade Católica de Brasília – UCB

**Prof. Luiz Claudio Santos Thuler:**
Pós Graduação em Neurologia - UNIRIO
Pós Graduação em Oncologia - INCA

**INSTITUTIONAL FACILITIES.**

**INCA: Institutional infrastructure:**
National Institute of Câncer, Brazil (INCA): PCR machines (6), horizontal and vertical electrophoresis systems, PCR preparation hoods, clinical centrifuges (5), CO-2 incubators, Biosafety hood class II-A2 (4), microcentrifuges (3), refrigerated microcentrifuge, heat block, speed-vac, Reichter-Jung cryostat, orbital shakers, air-heated shakers, refrigerators, freezers (4), -80ºC freezers (4), refrigerated centrifuge (2), direct and inverted microscopes (contrast phase and fluorescence), dissection microscope, fluorimeter, Milli-Q water purification system, computers, laser and inkjet printers.
Institutional equipments and facilities: Animal facility with pathogen free animals and inter-room isolation for infection control. Cell and animal manipulation laboratory with safety hoods and clinical centrifuge, several mouse strains available. FACScalibur flow cytometer with two lasers and sorter. Beta counter, MEGA-BACE and 377 ABI DNA sequencers, Real-Time PCR system, electronic-transmission microscope, ultracentrifuge, microarray system, tissue microarray, laser capture microscope, Nanodrop spectrophotometer, BL-II safety room with 2 class II-B2 safety cabinets, air-lock and restricted access. We are acquiring a confocal imaging system with multiphoton system (expected in 05/2009), an in vivo bioluminescence IVIS system (expected in 02/2009) and a new BL-II+ laboratory and mouse embryo bank laboratory, both placed in the animal facility (expected in 10/2009)

**ENSP/Fiocruz:** Collaboration in the activities for implementation and data analysis.

**Laboratório de Virologia Humana (LVH) da UFRJ:** The Laboratório de Virologia Humana (LVH) from UFRJ possesses all necessary infrastructure to the processing and ulterior molecular analyses of HPV clinical samples from the proposed sub-project. The laboratory contains a BL2-A separate facility, with two laminar flow chambers class 2A, a fluorescence microscope, an ultrafreezer at -80°C, a top bench refrigerated centrifuge, CO₂ incubators and 2 liquid nitrogen containers. This environment is adequate for processing HIV- and HPV-positive samples. In the molecular biology rooms, the lab contains 4 thermocyclers and an automated DNA sequencer (in a separate room). The lab also has available another, more robust DNA sequencer for multi-user purposes. The lab contains all small equipment necessary for molecular biology techniques, such as electrophoresis chambers and fonts, micropipettes and common reagents and kits.

For the clinical and laboratorial follow-up of patients, the Institute of Puericulture from UFRJ disposes of all infrastructure and logistics necessary to perform those tasks. In fact, patients are already being followed-up according to the regular routine of the service. Likewise, antenatal and complementary laboratory exams, such as ultrasound, CD4 T-cell counts and HIV viral load, are readily available to patients and will be provided by the service.
With respect to human resources, it is worth mentioning that all the time dedicated by the researchers, technicians and graduate student at the lab, and of clinicians, nurses, medical residents and social assistants to the project are to be considered as provided by the Institutions involved.
THEME 5:
KNOWLEDGE TRANSFER FOR THE GOVERNMENT AND THE SOCIETY
ADVANCEMENTS IN EDUCATION AND KNOWLEDGE MANAGEMENT’S ON CANCER

The Science and Technology Institute on Cancer intends to perform activities that lead to an advance on current initiatives in the institutions here associated and which may acquire consolidation or magnification through this interaction. The different technical areas in the lato sensu education and communication’s field will be able to fortify with this project, generating impact for health services and for the society.

Sub-project 1: Specialization Course for Nursing on Clinical Research in semi-presential Oncology Area

The clinical research on cancer lacks specialized infrastructure and personnel. For two years, INCA has been graduating professionals in nursing clinical research, with a larger focus on local and locoregional.

By using communication, information and telemedicine devices technologies within RUTE Net, our proposal aims at expanding the Specialization Course for Nursing in Clinical Research in Oncology, adapting it to the semi-distance model, where only the practical module would take place at INCA, thus favoring the access of professionals from other states and making the initiative dynamic which responds to a clear demand from the clinical research centers in Oncology.

Objectives
1. Identify the setup capacity of 100% of the partner institutions as users of practices for the semi-distance course in nursing research in DL (distance learning).
2. Define strategies to articulate practice/theory in the initiative design.
3. Develop a semi-distance course with the support of qualified tutors for the activities.
Goals
Quantitative
1. Annually offer the semi-distance Specialization Course for Nursing in Clinical Research to all members of the Institute involved in the assays subgroups from 2009 on.
2. Expand the vacancy offer in 50% for the course and offer it to other institutions within the country from 2010 on.

Sub-project 2: Oncological Care in RUTE Network

The full use of University Network of Telemedicine (RUTE) by institutional partners will extend the motivating potential of the research network. The possibility of high resolution images use, the sharing of clinical and scientific sessions by means of information and communication technologies use may confer greater connectivity and integration to the participant education and research institutions. INCA has videoconferencing devices and basic infrastructure organization to occupy the place of articulator pole in research on cancer field in Brazil.

Objectives
1. Identify the connectivity degree and potential of 100% of the partner institutions
2. Set up the biannual calendar of events shared by communication and information technologies, so that it can verify:
   (i) Synergy and complementarity to the qualification and HR development for research initiatives
   (ii) Facilitate the council communication and decisions in virtual environment
   (iii) Foster the culture of building virtual communities

Goals
Qualitative
1. Establish a regular communication channel among research groups, providing the interface among institutions through the RUTE net.
Sub-project 3: Competences Mining for Space-Temporal Analysis of Research on Cancer in Brazil

Much information contained in documents or static spreadsheet, when properly analyzed and processed by automated techniques, allow generating knowledge that will guide and counsel decision-making by different characters involved in a determined theme. Texts Mining technique is a process used to extract standards or knowledge, interesting and non-trivial, from textual documents (TAN, 1999 - http://ww.ewastrategist.com/papers/text_mining_kdad99.pdf), treating the information in order to submit the user some type of useful and new knowledge, avoiding a handcrafted and prolonged search. Oncological search, although possibly having its groups identified in CNPq’s Lattes platform, does not allow to the user a systematized and objective analysis of data presented there. In the multidisciplinary approach to cancer pursuing, organizing the researchers in nets, in which expertises and technological infrastructure complementariness are fundamental, the implantation of a research on cancer platform using texts and spreadsheets mining has proved to be quite strategic.

Objectives

1. To value and measure individual competences, of a research group, section, department, organization or cooperation on cancer in Brazil network, as well as their specializations and geographical distribution.

2. To evaluate strong and weak points, threats or opportunities in technical-scientific field on cancer.

3. To aid strategic planning of research institutions and funding agencies, generating data for decision-making in research financing, interactions among researchers groups, networks formation, creation of new research on cancer areas, with optimization of financial resources.

4. To aid homogenization strategies of labor market and scientific production in Brazil.
Goals

Quantitative

1. Create an automatic or semi-automatic identification instrument of scientific competences on cancer through the mining from the scientific production indicators, making it available for the scientific community and administrators.

Reference


Sub-project 4: Communication project for the Science and Technology on Cancer Institute

To implement a Communication on Cancer Program inside Science and Technology Institute with the intention of demystifying the disease that still carries a lot of stigma and prejudice. Communication on health is a way of democratizing science and technology information produced in the country, contributing for discussing some of our society problems. Thus, scientific dissemination field can configure in an excellent instrument for circulating information, being within scientific community itself or to laic public, and to strengthen national research.

Objectives

1. Expand the communication among research groups members of the project and the interaction among other cancer research groups.
2. Expand the interface with the media that work in Healthcare and with the society, increasing the access to the learning about cancer.

Goals

Quantitative

1. To create an electronic communication vehicle among members of C&T Institute on Cancer and other research on cancer groups in Brazil.

2. To create an Internet environment for dissemination, which routinely provide journalists with information on researches in progress, approximating researchers to the media and to the society through communication actions.
Experience in the area of Teaching and Knowledge Management

Eliana Claudia de Otero Ribeiro, graduated in Medicine at the Federal University of Rio de Janeiro (1975), got her Master’s degree in Public Health from Harvard University (1981) and her PhD in Collective Health from Rio de Janeiro State University (2003). She is currently the coordinator for teaching and scientific diffusion at the National Cancer Institute (INCA) and an Adjunct Professor at the Federal University of Rio de Janeiro. She has experience in the area of Collective Health, with emphasis in Healthcare Human Resources, acting mainly in the following themes: medical education, human resources formation in healthcare, teacher formation and permanent education in the area of health.

Antonio Augusto Gonçalves, graduated in Civil Engineering at the Federal University of Juiz de Fora (1985), got his Master’s degree in Production Engineering at the Federal University of Rio de Janeiro (1990) and his PhD in Production Engineering at the same university (2004). He is currently an Adjunct Professor at UNESA and a Systems Manager at INCA. He has experience in the area of Administration, with emphasis in Technology Information, acting mainly in the following themes: restrictions theory, computational simulation and information systems. His focus is to investigate the entrepreneurial practices used for the transformation of knowledge into technology, and its direct application in products and processes innovation. Areas: know-how generation, know-how transfer, technology management (development and acquisition), innovation process management, capture and protection of the organizational know-how, organizational learning.

Claudia Jurberg, graduated in Social Communication at the Catholic University of Rio de Janeiro (1987) and got her PhD in Education, Management and Diffusion in Biosciences from the Federal University of Rio de Janeiro (2000). She is currently a technologist at Oswaldo Cruz Foundation and a journalist at the Federal University of Rio de Janeiro, where she coordinates the Oncobiology Program Diffusion Center. She is also a corresponding journalist for the World Health Organization Bulletin and for the Intellectual Property Watch website. She is a collaborator in the area of press assistance for the National Academy of Medicine and also for the Federation of Societies for Experimental Biology. She has experience in the area of Communication, with emphasis in scientific journalism, intellectual property, public health and biosciences.
Valdelice Oliveira Santos, nurse at INCA, professor at the Specialization Course for Nursing in Clinical Research in Oncology
## BUDGET

### 1- Equipments

<table>
<thead>
<tr>
<th>EQUIPMENT</th>
<th>TOTAL VALUE (R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Camera MegaView 3, high resolution for transmission electronic microscopy, with CCD camera and an adapter for transmission electron microscope (01 Unit)</td>
<td>57.600,00</td>
</tr>
<tr>
<td>2- Digital high resolution camera, Axiocam mrc, 5 color resolution: 646 x 484/861 x 645/1292 x 968/2584 x 1936 pixels, 5 megapixels, 2/3 &quot; ccd, spectro: 400 710nm, with software of image acquisition included, peltier refrigeration, connection firewire/ieee1394 with socket of 6 rod (400 mbits/s), for fluorescence microscope (01 unit)</td>
<td>16.248,00</td>
</tr>
<tr>
<td>3- Computer 625 W, 64 Bits, Intel Processor Duo Core E6850 (3Ghz 4, Windows Vista, 2 GB of memory DDR2, graphical Plate NVIDIA NVS 290, 256 Mb, SATA-1, Hard disk of 320 Gb SATA 3.0 Gb/s, with NCO 2 16Mb Data Burst Cachê and monitor DELL 19’’, E198AP, analoogical plain Screen, with accessory, to be adapted next to the camera MegaView 3 for electron microscope (01 unit)</td>
<td>4.382,00</td>
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<tr>
<td>4- Transference chamber half-dries (01 unit)</td>
<td>6.000,00</td>
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<tr>
<td>5- Camera of high digital sensitivity for microscope</td>
<td>32.000,00</td>
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<tr>
<td>6- Optic Reader for Real Time PCR in adaptable thermal cycler model PTC200 - Bio-Rad</td>
<td>30.000,00</td>
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<td>7- Termomixer ependorf for 1,5 ml tubes</td>
<td>9.000,00</td>
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<tr>
<td>8- Two (02) Flow Cytometers FACscalibur, with two lasers, 4 colors, 6 parameters for fluorescence analysis</td>
<td>400.000,00</td>
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<tr>
<td>9- Real Time PCR- for DNA quantification, capacity for 96 tubes of 0,2µL or 96 wells plates</td>
<td>55.190,00</td>
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<tr>
<td>10- Thermalcylers for plates of 96 wells with temperature gradient (02 unit)</td>
<td>48.000,00</td>
</tr>
<tr>
<td>11- Vertical eleetroforese System - BioRad (Mini Protein) (01 unit)</td>
<td>5.300,00</td>
</tr>
<tr>
<td>12- Transfer System Half-Dry, BioRad (01 unit)</td>
<td>4.800,00</td>
</tr>
<tr>
<td>13 – Tissue homogenizer (01 unit)</td>
<td>1.580,00</td>
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<td>14-Spectphotometers Nanodrop, for small volumes (01 unit)</td>
<td>16.000,00</td>
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<td>15 - Agilent Analyzer Analyser (01 unit)</td>
<td>40.000,00</td>
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<tr>
<td>16- Speed-Vac, with trap for organic solvents (01 unit)</td>
<td>20.000,00</td>
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<tr>
<td>17- Ultracentrifuge (01 unit)</td>
<td>120.000,00</td>
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<tr>
<td>18 - Knife to maker</td>
<td>12.000,00</td>
</tr>
<tr>
<td>19 - Elisa Plate Reader (01 unit)</td>
<td>45.000,00</td>
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<tr>
<td>20- Two (02) Regulating ones of diving (1º and 2º periods of training)</td>
<td>3.000,00</td>
</tr>
<tr>
<td>21- Two (02) Consoles for diving with manometer and magnetic compass</td>
<td>1.600,00</td>
</tr>
<tr>
<td>22 - Two (02) Computers for diving</td>
<td>2.600,00</td>
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<tr>
<td>23- One (01) Handle for data transferf and program for compilation of the diving description</td>
<td>500.00</td>
</tr>
<tr>
<td>24- One (01) digital Camera and box for submarine use</td>
<td>2.000,00</td>
</tr>
<tr>
<td>25- One (01) Field Microscope (01 unit)</td>
<td>2.000,00</td>
</tr>
<tr>
<td>26- One (01) Ultrafreezer -80C (01 unit)</td>
<td>60.000,00</td>
</tr>
<tr>
<td>27 - Colposcope (01 unit)</td>
<td>15.000,00</td>
</tr>
<tr>
<td>28 - PALM-Top computers (06 units)</td>
<td>7.800,00</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1.017.600,00</strong></td>
</tr>
</tbody>
</table>

**Item 01 the 04** - The requested item aim to equip the laboratory of the Cellular Biology Division at Instituto Nacional de Câncer, accessories for electron microscope
and fluorescence microscope, to increase sample processing capability. Multi-users equipments.

**Item 05** - The requested item will be used as an accessory for fluorescence microscope, placed in the Immunopharmacology Laboratory at Physiology Depment - FIOCRUZ. Fisiologia and Farmacodinâmica. Multi-user equipment.

**Item 06** - Component for Real Time PCR adaptable to PCT-200 thermocyclers model (Bio-Rad), equipment allocated at Division of Cellular Biology of Instituto Nacional de Câncer, destined to increase quantitative analyses for gene expression (RNA). Multi-user equipment.

**Item 07** - To increase sample processing capacity. Item will be placed at the Division of Clinical Research of Instituto Nacional de Cancer.

**Item 08** - The cytometers will equip the Thymus Research Laboratory (at FIOCRUZ) and he Immunoreumathology Laboratory at Institute of Biomedical Research (Pontifícia Universidade Católica do Rio Grande do Sul) with the aim to increase sample processing capability. Multi-user equipment.

**Item 09** - Equipment for increase the sample processing capability for polymorphism characterization, will be allocated at Experimental Medicine Division of Instituto Nacional de Cancer.

**Item 10** - Equipment required for increase the sample processing capability of Genetics Division and Experimental Medicine Division of Laboratories at Instituto Nacional de Cancer. Multi-user equipment.

**Item 11 and 12** - Equipment required for increase sample processing capability for proteins gels. The equipment will be allocated at the Pharmacology Division Laboratory of Instituto Nacional de Cancer.

**Item 13 and 14** - Item required for sample processing for microarray analyses. The item will be allocated at in the Experimental Medicine Division of Instituto Nacional de Cancer.

**Item 15** - Item to improve samples processing capability. Required for sample processing for microarray analyses

**Item 16** - Required for samples processing for proteomic experiments, to be allocated at Bone Marrow Transplantation Center at Instituto Nacional de Cancer.

**Item 17 the 19** - Equipments required for sample processing at the Urogenital Research Unit of the Universidade Estadual de Rio de Janeiro.
Item 20 the 25- Equipments required for sample collection that will be allocated at the Museu Nacional (Universidade Federal do Rio de Janeiro).

Item 26- Equipment required for sample storage at the Laboratory of Organic Chemistry of Natural Products at Universidade de São Paulo -São Carlos.

Item 27- Equipment required for epidemiological data collection to be used in selected Hospitals for HPV studies and will be allocated at Pediatric Hospital of the Universidade Federal do Rio de Janeiro.

2- CONSUMABLES, DAILY, TICKETS, AND SCHOOLARSHIPS

<table>
<thead>
<tr>
<th>ITEM</th>
<th>TOTAL VALUE (R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumables</td>
<td>3.775.541,00</td>
</tr>
<tr>
<td>Daily Income</td>
<td>147.322,00</td>
</tr>
<tr>
<td>Tickets</td>
<td>213.250,00</td>
</tr>
<tr>
<td>Research Scholarships</td>
<td>846.287,16</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>4.982.400,16</strong></td>
</tr>
</tbody>
</table>

These funds will be distributed for different research themes accordingly the criteria described below. This fund allocation was established by the investigators involved in the present project.

<table>
<thead>
<tr>
<th>Theme 1: Tumor Cell Biology:</th>
<th>R$ 815,849, 68</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theme 2: Molecular Markers and Cancer</td>
<td>R$ 909,089, 65</td>
</tr>
<tr>
<td>Theme 3: Clinical Studies on Oncology: Base for Development and Technological Incorporation in the Economical Industrial Complex of Health</td>
<td>R$ 330,224, 87</td>
</tr>
<tr>
<td>Theme 4: Cancer Epidemiology in Brazil</td>
<td>R$ 534,834, 79</td>
</tr>
</tbody>
</table>

The Theme 5 (knowledge transfer for the government and the society: Advancements in education and knowledge management’s on cance) will be contemplated with scholarships. The value of R$ 1.546.113,85, corresponding to 1/3 of the total requested (excluding the scholarships), will be reserved to destination based on decisions of the Managing Committee.
INSTITUTIONAL AGREEMENTS
DECLARAÇÃO

Pela presente eu, MARISA MARIA DREYER BREITENBACH, Coordenadora de Pesquisa do Instituto Nacional de Câncer, instituição que abriga alguns grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: “Instituto de Ciência e Tecnologia para o Controle do Câncer”, declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital nº 15/2008 - MCT/CNPq/FNDCT/CAPES/FAPEMIG/ FAPERJ/FAFESP-INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLÓGIA, a instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o descumprimento dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

Nome: Marisa Maria Dreyer Breitenbach
Instituição: Instituto Nacional de Câncer
Cargo ou função: Coordenadora de Pesquisa
Nº do CPF: 223.262.864-72
Nº RG: 52.32717-6
Ôrgão Expedidor: CRM/RJ
Data de Expedição: 06/06/1979

Rio de Janeiro, 18 de setembro de 2008.
DECLARAÇÃO

Pela presente eu, Paulo Ermani Gadelha Vieira, Vice-Presidente da FIOCRUZ, instituição que abriga um dos grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: “Instituto de Ciência e Tecnologia para o Controle do Câncer”, declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital nº 15/2008 - MCT/CNPq/FNDCT/CAPES/FAPEMIG/ FAPEI/FAPESP- INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLOGIA, a instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o desempenho dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

Nome: Paulo Ermani Gadelha Vieira
Instituição: FIOCRUZ
Cargo ou função: Vice-Presidente
Nº do CPF: 422.312.887-04
Nº RG: 5222430-4
Órgão Expedidor: CRM
Data de Expedição: 18/05/1977

Local e data: Rio de Janeiro, 16 de setembro de 2008.
DECLARAÇÃO

Pela presente, eu, Irene Garay, Vice-Diretora do Instituto de Biologia da Universidade Federal do Rio de Janeiro, instituição que abriga um dos grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: “Instituto Ciência e Tecnologia para o Controle do Câncer”, declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital nº 15/2008 – MCT/CNPq/FNDCT/CAPES/FAPEMIG/FAPERJ/FAPESP–INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLOGIA, a instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente que o descumprimento dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

Rio de Janeiro, 18 de setembro de 2008

[Assinatura]

Professora Irene Garay
Instituto de Biologia da Universidade Federal do Rio de Janeiro
Vice-Diretora
DECLARAÇÃO

Pela presente eu, Mário Alberto Cardoso da Silva Neto, Vice-Diretor do Instituto de Bioquímica Médica da Universidade Federal do Rio de Janeiro, instituição que abriga um dos grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: "Instituto de Ciência e Tecnologia para o Controle do Câncer", declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital nº 15/2008 - MCT/CNPq/FNDCT/CAPES/FAPEMIG/ FAPERJ/FAPESP- INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLOGIA, a instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o descumprimento dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

Nome: Professor Mário Alberto Cardoso da Silva Neto
Instituição: Instituto de Bioquímica Médica da Universidade Federal do Rio de Janeiro
Cargo ou função: Vice-Diretor
Nº do CPF: 000 735 037 62
Nº RG: 06 54 53 05-02
Órgão Expedidor: Detran
Data de Expedição: 26.07.04

Local e data: Rio de Janeiro, 16 de setembro de 2008.
DECLARAÇÃO

Pela presente eu, Cid Manso, diretor do Instituto de Medicina Social da Universidade do Estado do Rio de Janeiro, instituição que abriga um dos grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: "Instituto de Ciência e Tecnologia para o Controle do Câncer", declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital n° 15/2008 - MCT/CNPq/FNDCT/CPES/FAPEMIG/ FAPERJ/FAPESP/ INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLOGIA, a instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o descumprimento dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

Nome: Cid Manso  
Instituição: Instituto de Medicina Social  
Cargo ou função: Diretor  
Nº do CPF: 363.085.607-15  
Nº RG: 0460403-9  
Órgão Expedidor: IEF  
Data de Expedição: 11/06/1992

Local e data: Rio de Janeiro, 16 de setembro de 2008.
DECLARAÇÃO

Pela presente eu, Silvana Teresa Lacerda Jales, Diretora do Laboratório de Tecnologia Farmacêutica da Universidade Federal da Paraíba, instituição que abriga um dos grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: "Instituto de Ciência e Tecnologia para o Controle do Câncer", declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital nº 15/2008-MCT/CNPq/FNDC/FAPEMIG/FAPERJ/FAPEG- INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLOGIA, a instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o descumprimento dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

[Assinatura]

Nome: Silvana Teresa Lacerda Jales
Instituição: Laboratório de Tecnologia Farmacêutica da Universidade Federal da Paraíba, Campus I, Caixa Postal 5609, CEP: 58051-970
Cargo ou função: Diretora
Nº do CPF: 977396007-25
Nº RG: 09185288-9 IFP-RJ
Órgão Expedidor: IFP-RJ
Data de Expedição: 07 de junho de 1989

Local e data: Rio de Janeiro, 17 de setembro de 2008.
DECLARAÇÃO

Pela presente, eu, Luiz R. Nunes, Pro-Rector de Pesquisa, Pós-Graduação e Extensão da Universidade de Mogi das Cruzes, Instituição que abriga um dos grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: Instituto de Ciência e Tecnologia para o Controle do Câncer, declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital nº 15/2008 - INCT/CNPQ/FINEST/FAPEMIG/FAPEM/FAPEP/INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLOGIA, a Instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o desempenho dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

Nome: Luiz R. Nunes
Instituição: Universidade de Mogi das Cruzes
Cargo ou função: Pro-Reitor de Pesquisa, Pós-Graduação e Extensão
Nº do CPF: 1481357148-60
Nº RG: 15.260.999
Orgão Expedidor: SSP/SP
Data de Expedição: 03/11/1980

Local e data: Rio de Janeiro, 16 de setembro de 2008.
DECLARAÇÃO

Pela presente eu, Roberto Gomes de Souza Berinck, Professor Associado de
Instituto de Química de São Carlos – USP, instituição que abriga um dos grupos incluídos
na proposta do Instituto Nacional de Ciência e Tecnologia “Instituto de Ciência e
Tecnologia para o Controle do Câncer”, declaro que durante a vigência do contrato que
tem por base o mesmo, de acordo com o Edital nº 15/2008 -
MCT/CNPq/FAPESP/FAPEMIG/ FAPERJ/ FAPESP/ INSTITUTO
NACIONAL DE CIÊNCIA E TECNOLOGIA, a instituição oferecerá todo o suporte
necesário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades
relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o descumprimento dos termos desta declaração
poderá prejudicar o andamento de futuras solicitações apresentadas.

Nome: Roberto Gomes de Souza Berinck
Instituição: Instituto de Química de São Carlos – USP
Cargo ou função: Professor Associado

Nº do CPF: 051.821.718-38
Nº RG: 14.480.952-5
Orgão Expedidor: SSP/SP
Data de Expedição: 12/10/2005

Locais e data: São Carlos, 16 de setembro de 2008.
DECLARAÇÃO

Pela presente eu, Carlos Alexandre Sanchez Ferreira, Diretor da Faculdade de Biociências da PUCRS, instituição que abriga um dos grupos incluídos na proposta de Institutos Nacionais de Ciência e Tecnologia: "Instituto de Ciência e Tecnologia para o Controle do Câncer", declaro que durante a vigência do contrato que tem por base o mesmo, de acordo com o Edital nº 15/2008 - MCT/CNPq/FNDCT/CAPES/FAPEMIG/FAPERJ/FAPESP- INSTITUTOS NACIONAIS DE CIÊNCIA E TECNOLOGIA, a instituição oferecerá todo o suporte necessário ao desenvolvimento do projeto de forma que sejam atendidas as necessidades relevantes para o cumprimento das atividades descritas no projeto.

Declaro que estou ciente de que o descumprimento dos termos desta declaração poderá prejudicar o andamento de futuras solicitações apresentadas.

Nome: Carlos Alexandre Sanchez Ferreira
Instituição: Pontifícia Universidade Católica do Rio Grande do Sul
Cargo ou função: Diretor da Faculdade de Biociências

Nº do CPF: 60736330097
Nº RG: 9030229505
Orgão Expedidor: SSP/RS
Data de Expedição: 15/03/1990

Local e data: Porto Alegre, 16 de setembro de 2008.