

# CTC

Centro de Terapia Celular

Center for Cell-Based Therapy

**CENTER FOR RESEARCH ON CELL-BASED THERAPY**

**2008**

**Ribeirão Preto  
University of São Paulo**



**Hemocentro de Ribeirão Preto**



## 1. Summary of the Proposal for the Center

The simplest form of cell-based therapy is the transfusion of cells obtained by processing blood collected from donors, and it represents one of the most extensively used therapeutic approach of medicine. Bone marrow transplantation is another form of cell-based therapy in which foreign cells are not only transfused but they engraft and proliferate in the new host. Variants of this method of treatment are rapidly gaining wide acceptance. These include the use of stem cells and hematopoietic stem cells obtained from the umbilical cord. Our knowledge of the conceptual and practical issues related to the more recently developed forms of cell-therapy is incomplete; for instance, the properties of stem cells obtained from the bone marrow, the peripheral blood and the cord blood are different, but the full extend of the differences as well as the advantages of each source have not been fully evaluated. In addition, the novel techniques of cell collection, culture and modification of cell's genetic material makes it possible to manipulate cell populations or the cells themselves to increase their therapeutic potential.

The previous paragraph was extracted from the original proposal to FAPESP for the creation of the Center for Cell-Based Therapy (CTC). As it will become clearer in the following sections, CTC investigators increased substantially their focus on basic and clinical studies using stem cells and the collaboration among investigators has been intensified and allowed the branching of our activities by incorporating new methodologies in which CTC investigators acquired expertise. Importantly, the results obtained on the first phase of the project were the basis for choosing subprojects considered as with greater potential (e.g. the analysis mesenchymal stem cells). As a result a significant scientific contribution to the field of cell therapy was made by the CTC. Articles were published in prestigious journals such as *Science*, *JAMA*, *Stem Cells* and *Blood*. Important awards were conferred to the studies developed, and significant additional support was obtained from National (FINEP and CNPq mainly) and International (Vitae Foundation) agencies. Moreover, the activities of the CTC had an important impact in the community, as evident by the participation of over 1,200 teachers and students from public middle schools in Ribeirão Preto and nearby towns in our educational project. It is also worthwhile to point out that the book "Células-tronco: a Nova Fronteira da Medicina. [Stem-cells: the new frontiers of medicine]" written by two of the CTC investigators was quoted four times by Supreme Court Judge in his analysis of the bill concerning the use of embryonic stem cells for research purposes in Brazil. This book is part of the diffusion program and has received the Jaboti Award of Literature in the category of Life and Health Sciences.

In the original proposal of the research project, we have presented a Table describing the main fields of research and their respective subprojects. In the Table below we updated the original Table by adding the published articles associated with each subproject. We divided the Table in proposals and results related to the first four years and those developed thereafter. Practically, all initial research subprojects were concluded successfully and led to new related subprojects focused on the more relevant results obtained. In some projects, we have already successfully conducted research from the bench to the bedside.

## Research Program

<b>Subprojects developed in the first 4 years</b> (numbers in red refer to the subprojects presented in the original proposal)		<b>Published Articles</b>	<b>Subprojects developed between 4th – 8th year</b>	<b>Published Articles</b>
<b>Characterization of the disease and of the patient: identification of features that are related to the neoplastic process or that may interfere with the cell-therapy</b>			<b>Characterization of the disease and of the patient: identification of features that are related to the neoplastic process</b>	
Morphology of cells, flow cytometry, molecular biology	1,2,4,5	1-8	Morphology of cells, flow cytometry, molecular biology	9-29
Cancer genome anatomy	3	30-37	Cancer genome anatomy	17,38-40
Bio-informatics	3,7,8	30-37	Bio-informatics	38,41-43
Functional roles of tumor associated antigens	2,8	35	Functional roles of tumor associated antigens	41,44-48
<b>Infectious diseases that may be transmitted or may be treated by cell therapy</b>			<b>Infectious diseases that may be transmitted by cell therapy</b>	
HIV	7	49-51	Retroviruses	49,52-55
<b>Identification, isolation and characterization of cells for therapy</b>			<b>Identification, isolation, characterization and manipulation of cells for therapy</b>	
Collection, identification and culture	2,5,6,7	1-3,8	Genomic and functional characterization of hematopoietic stem cells	11,13,41
Purging	5	1	Genomic and functional characterization of mesenchymal stem cells	56-60
Functional analysis: the proteome	7,8	8	Isolation and manipulation of mesenchymal stem cells	56-59,61
<b>Manipulation of cells that may increase the efficiency of cell-therapy</b>			<b>Clinical trials</b>	
Neoplasia	5,7,8	35,44,45	Type 1 diabetes mellitus	62,63
Infectious disease	7	8	Other auto-immune diseases	64-67
<b>The patient submitted to cell-therapy</b>			Neoplastic diseases	
Immunologic mechanisms	7,8	1,2,68,69	Hematopoietic stem cell transplantation	7,70-72
Hematopoietic stem cell autograft	5	1,2,68,69		
Cell transfusion	6	73-76		
<b>Experimental animal models</b>			<b>Experimental animal models</b>	
Pathogenesis of the diseases	9,10	69,77	Models of acute promyelocytic leukemia	78-85
Immunologic and/or inflammatory reaction	8,10	69	Dkc1 mutants	86,87
Cell therapy	8		Models for tissue repair	

Science teaching in most public middle and high schools in the region was at considerable disadvantage when compared with the quality of teaching and research at the USP campus; the main reason was the lack of access of school teachers and students to practical training and contact with researchers who are carrying out state-of-art and competitive research, and the lack of opportunity for school teachers to improve and update their training. To overcome these obstacles, the CTC ***Diffusion Program*** was aimed in improving science teaching in public middle and high schools. The strategies originally proposed were:

- Theoretical and laboratory courses on selected subjects for middle and high school teachers and students;
- Development of introductory research projects for middle and high school students, and presentation of the results in mini-congresses;
- Setting up standardized education modules to help teachers to maximize the use of laboratory and educational resources available in the public education system;
- Organizing mini-congresses, exhibits and competitions to disseminate the approach and the results of the projects;
- Organization and publication of printed material reporting results of introductory research projects, the innovative teaching experiences and the House of Science experience;
- Collaboration with high school teachers for the development of educational resources
- Development of projects using molecular modeling to instruct high school students to think about molecules in three dimensions and the different aspects of genomics, modern biotechnology, the mechanisms of diseases and the ethical aspects of modern scientific development

Beside the educational activities the diffusion program of the CTC also aimed to bring to the community the latest scientific achievements in the field of cell therapy, molecular basis of neoplastic diseases and different aspects of genomics and proteomics. In addition, our proposal included offering post-graduate training to health and laboratory professionals.

Regarding Innovation, our original proposal was to increase the collaboration between the industry and the scientific community and to collaborate with the governmental sector in order to create better public policies and services for the community. We have proposed to develop products generated using biotechnology; to create a micro and small business incubator, to help the State of São Paulo government in the implementation of a public Policy for Blood and Blood Components, to establish quality control programs for hemotherapy; to develop a blood irradiator based on telemetry and to establish a umbilical cord bank.

## 2. Summary of the Main Achievements

### 2.a) Research

The CTC was originally based on the broad concept of using cells for therapy, under different conditions and from several sources. Our research has been focused in five areas considered essential for the understanding of the cellular mechanisms involved in regulation of stem cells activity, establishment of methods for stem cells isolation and *ex vivo* manipulation, and in the development of treatment strategies based on the use of stem cells to treat autoimmune diseases. For clarity, the main results will be presented divided in these five topics: *i. Characteristics and manipulation of the cells used for therapy, ii. Characteristics of the recipients, affected with neoplastic diseases, iii. Relationship of the host with parasites transmitted by cell therapy, iv. Transgenic animals as experimental models, v. Clinical trials using stem cells to treat autoimmune diseases.*

i) *Characteristics and manipulation of the cells used for therapy.* We have published eight articles<sup>11,13,41,57-61</sup> about the genetic and proteomic characterization of hematopoietic (HSC) and mesenchymal stem cells (MSC), as well as describing methods of isolating them from different tissues. MSCs are multipotential non hematopoietic progenitor cells capable of differentiating into multiple lineages of the mesenchyme. MSCs have emerged as a promising therapeutic modality for tissue regeneration and repair. Articles 59 and 60 have special relevance for being one of first descriptions of the gene expression profile of MSCs, and were important to the identification of pathways involved in the maintenance of these cell pool or associated with the differentiation in osteoblasts, muscle cells or adipose tissue. Moreover, we were the first to demonstrate that mesenchymal stem cells isolated from bone marrow (BM) or umbilical cord blood (UCB) despite the fact of sharing morphological features, immunophenotypic markers and similar differentiation ability, have specific genetic profiles. Using the SAGE (serial analysis of gene expression) method, we generated specific libraries from BM and UCB MSCs<sup>59</sup>. The two libraries share almost all of the first thousand most expressed transcripts, some of which were validated by RT-PCR, including the genes VIM, LGALS1, SPARC, COL1A1, COL1A2, TPT1, TAGLN, TAGLN2, ANXA2 and MMP2. Nevertheless, a set of genes related to anti-microbial activity, to osteoblast differentiation and adherence to the matrix, and to osteogenesis was expressed at higher levels in BM-MSC, whereas higher expression in UC-MSC was observed for genes that participate in pathways related to matrix remodeling via metalloproteinases and angiogenesis<sup>59</sup>.

We have also compared HSCs from different origins, since differences in engraftment, recover of the thymic function, and in the incidence of the graft-versus-host disease have been reported when UCB transplants were compared with bone marrow (BM)<sup>41</sup>. To understand the molecular mechanisms causing these intrinsic differences, we analyzed the differentially expressed genes between BM and UCB hematopoietic stem and progenitor cells. The overrepresentation of nuclear factor-kappaB (NF-kappaB) pathway components and targets was found to be a major characteristic of UCB HSPCs. Additional promoter analysis of 41 UCB-overrepresented genes revealed a significantly higher number of NF-kappaB cis-regulatory elements (present in 22 genes)

than would be expected by chance. Our results point to an important role of the NF-kappaB pathway on the molecular and functional differences observed between BM and UCB HSPCs<sup>41</sup>.

Experimental evidence shows that the vessel wall contains multipotential stem cells that may share properties with mesenchymal stem cells. For instance, pericytes, which were originally defined by their morphology and close contact to endothelial cells, can differentiate into other cell types, including osteoblasts, chondrocytes, adipocytes, fibroblasts, myofibroblasts, and smooth muscle cells. We have compared MSC from many sources with retinal pericytes using morphological, functional, and gene expression analysis, in order to test the hypothesis that these cells, as defined by culture methods, are similar and universally distributed in the body. Our results showed that retinal pericytes obtained by two different approaches and fibroblasts derived from four different sources showed morphologic appearance and immunophenotype profiles similar to MSC. Moreover, pericytes and three of four fibroblast cultures could be induced to differentiate. The gene-expression profile of retinal pericytes was also very similar to the one related to MSC. Our SAGE clustering results showed a consistent grouping of MSC, pericytes, and hepatic stellate cells, independent of the set of tags. Our study was the first to present experimental evidence that human MSC and pericytes, as operationally defined by culture methods, are similar cells located in the wall of the vasculature, where they function as sources of cells for repair and tissue maintenance<sup>61</sup>.

Recently, we have compared the expression of multidrug resistance proteins (MDR) in stem cells from normal bone marrow with the more primitive cells detected in acute myelogenous leukemia (AML)<sup>18</sup>. The latter have recently been called Leukemic Stem Cells (LSCs) by analogy with the normal hematopoietic stem cells (HSCs), since they retain similar properties of self-renewal, high proliferative capacity and predominant quiescent cell cycle status. We have analyzed the expression of P-glycoprotein, MDR-related protein 1, breast cancer resistance protein, and lung-resistance protein expression in different subsets of leukemic cells and in HSCs. P-gp was highly expressed by the most immature CD34+CD38-CD123+ and also by the more mature CD34-leukemic cells. In addition, BCRP was preferentially expressed by LSCs in comparison to the more committed subpopulations. Considering the importance of the MDR drug efflux pumps in resistance to chemotherapy our study suggest that future therapeutic strategies should be aimed in overcoming LSC intrinsic resistance to genotoxic stimuli<sup>18</sup>.

ii) *Characteristics of the recipients, affected with neoplastic diseases.*

We have published 38 articles describing: cytogenetic abnormalities, immunophenotypic features, analysis of microRNA expression profile and genetic polymorphisms in patients affected with hematological and non hematological malignancies<sup>1-10,16-30,35-40,42-48</sup>. Of particular relevance are the studies about Cancer/Testis Antigens (CT) because they may be the basis of therapeutic approaches to malignant disease and may also be used to quantitate minimal residual disease. The most extensively studied families are MAGE, GAGE, BAGE, LAGE, NY-ESO1 and PRAME, which are expressed exclusively in cancer, testis and trophoblasts. We focused our efforts on the analysis of the PRAME (Preferentially Expressed Antigen in Melanoma) gene,

located on chromosome 22 (22q11.22), for we have detected it in approximately 90% of the patients with chronic lymphocytic leukemia (CLL) and mantle cell lymphoma, but in none of the normal controls<sup>35,44</sup>. We have also determined subcellular localization the PRAME protein in these abnormal lymphocytes. Moreover, in another study we demonstrated that PRAME is one of the most frequently expressed CT antigen in other cancers as well; for instance, CT antigens are expressed in 67% in squamous cell carcinomas of the head and neck<sup>47</sup>. Recently, we have extended our research on CT antigens to multiple myeloma<sup>45</sup>. The frequencies at which CT antigen were found to be expressed in MM patients were MAGEC1/CT7 77%, LAGE-1 49%, MAGEA3/6 41%, MAGEA2 36%, GAGE family 33%, NY-ESO-1 33%, BAGE-1 28%, MAGEA1 26%, PRAME 23%, SSX-1 26%, MAGEA12 20.5%, MAGEA4 0%, and MAGEA10 0%. Cox's regression model showed that GAGE family expression and having >6 CT antigens expressed were independent prognostic factors in this disease<sup>45</sup>.

We also have analyzed the role of adhesion and inflammatory molecules in different malignancies, based on the fact that the inflammatory microenvironment of tumors is characterized by the presence of cytokines and growth factor's network both in the supporting stroma and in tumor areas, and these molecules may contribute to tumoral growth and progression<sup>21,25,48,54,69,78</sup>. In the study by Matos et al.<sup>25</sup> the expression of 10 adhesion molecules was analyzed on peripheral blood tumor cells of 17 patients with chronic lymphocytic leukemia, 17 with mantle-cell lymphoma, and 13 with nodal or splenic marginal B-cell lymphoma, all in the leukemic phase. Compared to lymphocytic lymphoma, a lower expression of CD11a and CD49d in chronic lymphocytic leukemia was detected suggesting that these adhesion molecules are probably responsible for the different compartment the disease infiltrates. In the study by Oliveira et al<sup>48</sup>, we studied gene polymorphisms of the TNF-a, TNF-b, IL-6, IL-10, PECAM-1, and MPO in 80 osteosarcoma patients and 160 control individuals. We detected that the presence of the variant genotype (GG) of the +252A>G TNF-b polymorphism, which leads to higher level of cytokine production, could be a facilitator mechanism in tumor progression leading to a poor event-free survival. Finally, we also analyzed the association between the polymorphisms at exon 4 (G241R) and exon 6 (E469K) of ICAM-1 and exon 3 (L125V) of PECAM-1 genes with development of the Differentiation Syndrome (DS) in patients with Acute Promyelocytic Leukemia (APL)<sup>21</sup>. APL treatment is based on the use of all trans-retinoic acid (ATRA), which induces terminal granulocytic differentiation of blasts and clinical remission. ATRA is generally well-tolerated but may be associated with a potentially lethal side effect, referred to as RA or differentiation syndrome (DS). We detected an association between development of DS and the AA genotype at Codon 469 of ICAM-1 (odds ratio of 3.5; 95% confidence interval: 1.2–10.2). There was no significant association between ICAM-1 G241R or PECAM-1 L125V polymorphisms and DS<sup>21</sup>.

### iii) *Relationship of the host with parasites transmitted by cell therapy*

Seven articles have been published by our group characterizing viruses that may be transmitted during cell therapy<sup>49-55</sup>. The main focus is the serological, genetic and epidemiological analyses of retroviruses affecting Brazilians, and specially donors from the state of São Paulo. In the study by

Kashima et al.<sup>53</sup> the molecular and epidemiological characterization of 128 human T cell lymphotropic virus type 1 (HTLV-1) isolates from Brazilian patients with different clinical manifestations of the infection were reported. The results suggest that there is a molecular signature among the Brazilian isolates of HTLV-1.

iv) *Transgenic animals as experimental models*

We have published eleven articles using genetically modified mice as models. We have focused our research in the study of the molecular basis of leukemogenesis using the hCG-PML/RAR $\alpha$  transgenic model, and in the analysis of the role of ribogenesis in hematopoiesis using mice mutant for the *Dyskeratosis congenita 1* (Dkc1) gene<sup>77-81,83-88</sup>. In the former model, we aim to determine how the deregulation of signaling pathways lead to acute promyelocytic leukemia (APL), whereas in the second we aim to determine the mechanisms by which the impairment of ribogenesis leads to hematopoietic stem cell failure.

Acute promyelocytic leukemia is associated with chromosomal translocations involving the locus of the *Retinoic Acid Receptor  $\alpha$*  (RAR $\alpha$ ) on chromosome 17. In 95% of the cases, the t(15;17) is detected causing the formation of the PML/RAR $\alpha$  hybrid gene. Transgenic mice (TM) expressing PML/RAR $\alpha$  or the PLZF/RAR $\alpha$  under the control of human cathepsin G were originally developed by Pier Paolo Pandolfi's group and the first studies of our group were done with his collaboration. The PML/RAR $\alpha$  TM develop a form of leukemia that mimics the hematological findings of human APL. Leukemia is diagnosed after a long latency (approximately 12 months) during which no hematological abnormality is detected in peripheral blood (preleukemic phase). Our first aim was determine whether the variants of the hybrid genes associated with APL, namely the PLZF/RAR $\alpha$  and the NPM/RAR $\alpha$  would also cause leukemia and in the affirmative case, whether the phenotype would be the same. This is a relevant question because these oncoproteins only differ in their variable N-terminal domain (the so called X moiety). For that we have generated the hCG-NPM/RAR $\alpha$  and obtained the hCG-PML/RAR $\alpha$  from Pandolfi's group. In the three models the fusion gene is expressed under the control of a human cathepsin G (hCG) minigene, and all of them developed a form of leukemia after a long latency phase. The leukemic cells displayed distinct cytomorphological features. hCG-NPM/RAR $\alpha$  leukemic cells resembled monoblasts. This phenotype contrasts with what was observed in the hCG-PML/RAR $\alpha$  TM model in which the leukemic phase was characterized by the proliferation of promyelocytic blasts. Similarly, hCGPLZF/ RAR $\alpha$  TM displayed a different phenotype where terminally differentiated myeloid cells predominated. Importantly, the NPM/RAR $\alpha$  oncoprotein was found to localize in the nucleolus, unlike PML//RAR $\alpha$  and PLZF/RAR $\alpha$ , thus possibly interfering with the normal function of NPM. Similarly to what was observed in human APL patients, we found that NPM/RAR $\alpha$  and PML/RAR $\alpha$ , but not PLZF/RAR $\alpha$  leukemia, was responsive to all-trans retinoic acid (ATRA) or As<sub>2</sub>O<sub>3</sub> treatments. Taken together, our results underscore the critical relevance of the X moiety in dictating the biology of the disease and the activity of the APL fusion oncoprotein<sup>77,81,83-85,88</sup>.



Our studies regarding the *Dkc1m* mice were done in collaboration with Dr Davide Ruggero presently at University of San Francisco, USA. These studies were the first to prove that impairment of ribosome biogenesis may affect normal and aberrant hematopoiesis. The *DKC1* gene encodes a pseudouridine synthase that modifies ribosomal RNA (rRNA). *DKC1* is mutated in people with X-linked dyskeratosis congenita (X-DC), a disease characterized by bone marrow failure, skin abnormalities, and increased susceptibility to cancer. *DKC1* mutant mice develop a disease that resembles human X-DC. We discovered a specific defect in IRES (internal ribosome entry site)-dependent translation in *Dkc1(m)* mice and in cells from X-DC patients. This defect results in impaired translation of messenger RNAs containing IRES elements, including those encoding the tumor suppressor p27(Kip1) and the antiapoptotic factors Bcl-xL and XIAP (X-linked Inhibitor of Apoptosis Protein). Moreover, *Dkc1(m)* ribosomes were unable to direct translation from IRES elements present in viral messenger RNAs. These findings reveal a potential mechanism by which defective ribosome activity leads to disease and cancer<sup>86,87</sup>.

v. *Clinical trials using stem cells to treat autoimmune diseases.*

We chose type 1 diabetes mellitus (DM) as a model of autoimmune disease to test the effect of cell therapy. This choice was made based on the incidence and severity of the disease, but also because previously trials have evaluated the role of immunointervention in preventing residual beta cell loss by using immunosuppressive agents. These therapies were shown to induce a slower decline or some improvement in C-peptide levels when compared with placebo groups. However, almost all patients required exogenous insulin use. To determine the safety and metabolic effects of high-dose immunosuppression followed by autologous nonmyeloablative hematopoietic stem cell transplantation (AHST) in newly diagnosed type 1 DM. We conducted a prospective phase 1/2 study of 15 patients with type 1 DM (aged 14-31 years) diagnosed within the previous 6 weeks by clinical findings and hyperglycemia and confirmed with positive antibodies against glutamic acid decarboxylase. Patients with previous diabetic ketoacidosis were excluded after the first patient with diabetic ketoacidosis failed to benefit from AHST. Hematopoietic stem cells were mobilized with cyclophosphamide (2.0 g/m<sup>2</sup>) and granulocyte colony-stimulating factor (10 microg/kg per day) and then collected from peripheral blood by leukapheresis and cryopreserved. The cells were injected intravenously after conditioning with cyclophosphamide (200 mg/kg) and rabbit antithymocyte globulin (4.5 mg/kg). During a 7- to 36-month follow-up (mean 18.8), 14 patients became insulin-free (1 for 35 months, 4 for at least 21 months, 7 for at least 6 months; and 2 with late response were insulin-free for 1 and 5 months, respectively). Among those, 1 patient resumed insulin use 1 year after AHST. At 6 months after AHST, mean total area under the C-peptide response curve was significantly greater than the pretreatment values, and at 12 and 24 months it did not change. Anti-glutamic acid decarboxylase antibody levels decreased after 6 months and stabilized at 12 and 24 months. Serum levels of hemoglobin A(1c) were maintained at less than 7% in 13 of 14 patients. The only acute severe adverse effect was culture-negative bilateral pneumonia in 1 patient and late endocrine dysfunction (hypothyroidism or hypogonadism) in 2 others. There was no mortality. We concluded that high-dose immunosuppression and AHST were performed with acceptable toxicity in

a small number of patients with newly diagnosed type 1 DM. With AHST, beta cell function was increased in all but 1 patient and induced prolonged insulin independence in the majority of the patients<sup>62,63</sup>.

#### *Awards to the center*

- **Young Researcher Award for 2005.** Two students of the Center were awarded prizes in the annual national contest promoted by the CNPq (National Council for Science and Technology), among 181 candidates: Rodrigo A Panepucci won one of the three prizes in the Graduate category, with the work that was part of his PhD thesis, and Fabio M Nascimento won one of the three prizes for undergraduates with a work on the distribution of the PRAME antigen in normal cells.

Panepucci RA – ***Molecular bases of biology of the stem-cells from the hematopoietic system.*** Supervisor: MA Zago

Nascimento FP – ***Analysis of the PRAME antigen expression in normal lymphocytes.*** Supervisor: Eduardo M. Rego

- **2<sup>nd</sup> Latin America Novartis Scientific Award for 2007.** Novartis has sponsored this award in order to encourage the scientific development in Latin America. The criteria of the award is strictly scientific and is made by an International Board of Researchers. The award is given in three areas: Hematology, Oncology and Endocrinology. Two students of the Center were awarded the two prizes in Hematology, among 150 candidates: Lorena Lobo Figueiredo Pontes and Antônio Roberto Lucena de Araújo, with studies that were part of her PhD and his MSc thesis, respectively.

Figueiredo-Pontes, Lorena L. – ***Halofunginone inhibits TGF- $\beta$ /VEGF signaling in Acute Promyelocytic Leukemia.*** Supervisor: Eduardo M. Rego

Araújo, Antônio R.L. – ***Analysis of gene expression pattern of Tap73 and  $\Delta$ Np73 isoforms in samples from patients with Acute Myelogenous Leukemia.*** Supervisor: Eduardo M. Rego

- **Jaboti Award of Literature in 2007**– The book edited by M.A. Zago and D.T. Covas on the subject of stem cells has won this award for the category Life Sciences and Health Sciences. The Jaboti award is the most important award of literature in Brazil, and is given by the Câmara Brasileira do Livro since 1959.

## 2.b) Innovation

The innovation activities have five components:

- The Small Business Incubator (Supera-Hemocentro);
- Technological projects aimed to develop diagnostic procedures and new therapies for hematological diseases
- To develop quality control programs for hemotherapy
- To develop a blood irradiator based on telemetry
- To establish a umbilical cord bank.

The Incubator has started its activities in 2002 and now is housing four small enterprises. Until now, two companies have graduated. The Incubator is part of the initiative intended to setup a Technological Park in the university campus of Ribeirão Preto dedicated to R&D in biotechnology.

The main technological project is focused in the development and production of recombinant factor VIII to be used in the treatment of type A hemophiliacs. We have engineered 14 factor VIII recombinant molecules in retro and lentivirus vectors and have generated 41 transgenic cell lines expressing more than 100 IU/mL of FVIII. Two transgenic cell lines are higher factor VIII producer (more than 500 IU/mL of FVIII) and were chosen for industrial process escalation. This new project phase is funded by FINEP an Plantarium (a private company) and the general objective is the GMP production of rFVIII in quantities sufficient for the pre-clinical and clinical studies. The project budgeted is US\$2.5 million. The process, the developed vectors and the transgenic cell lines are being patented.

The other innovation activities, including the development of equipments for blood irradiation and for non-invasive iron quantification, have been completed and related paper was published<sup>76,89-91</sup>.

The Regional Blood Center developed and installed a quality control program (ISO 9002) certified in 10/29/1999. The institute responsible for the certification process had had no previous experience in certifying a blood center, so that the process had to be developed by our Center. In September, 2003 the Regional Blood Center was evaluated and preliminarily certified by the AABB (American Association of Blood Banks). The resulting experience of both processes is available to all public blood centers of the State of S. Paulo through the Blood Coordination of Sao Paulo State Health Secretary.

The development of the umbilical cord bank is at its final phase. A new laboratory for that end has been built and personnel have been trained. This program (BrasilCord) involves three blood centers that will operate in collaboration (Ribeirão Preto, Campinas and Albert Einstein in S. Paulo) and had funds donated by the Albert Einstein Institute.

## 2.c) Diffusion

The CTC created and coordinated several activities focused on the improvement of science teaching in public schools. These activities have the participation of teachers and students of public elementary and middle schools, and are held in two facilities: the House of Science (Casa das Ciências), and Museum and Laboratory for Science Teaching (MuLEC), which in 2005, has received a financial support of US\$ 100.000 from the prestigious Vitae Foundation for the project **“Consolidation of the Museum and Laboratory for Science Teaching”**. The following activities were developed:

a. **Science Exhibitions** – Every Thursday the MuLEC is open to public visitation. The general population and students groups are guided by graduated students through the exposition that includes topics related to cell biology, botanic, genomics, zoology and others.

b. **Science on Tuesdays** – Since January of 2006, we setup a special program to bring small groups of students to meet with senior investigators or to take post graduated students of the CTC to nearby towns with the purpose to discuss topics such as diabetes, stem cells, embryology, virus diseases, genetics, evolution and Mendel’s Laws, botany, and other scientific topics. Three schools should be pointed out: “EE Dom Romeu Alberti” from Ribeirão Preto, from which ex-students are still participating of our activities for more than 5 years; “EMEF Técnica de Química” and “EMEF Roberto Brayan” from Luis Antonio (60 km far from Ribeirão Preto), which have been participating once a week for three years. In the case of the latter two schools, the municipality has agreed to provide transport for the students and teachers. Together, More than 1200 students have been involved in these activities.

c. **Tutoring Young Scientists** is a program designed to enroll University graduated students in the orientation of small groups of students from the public schools. The aim is the development of scientific initiation of talented students, narrowing the distance between research and the middle school. From 2004 onwards, the program “Iniciação Científica Junior” has brought middle school students to CTC laboratories, where they have developed small projects and discussed several aspects of life sciences. In 2006, the program *“Adopt a scientist”* has been developed to allow a small number of talented students to develop longer and more ambitious projects. Thus far, more than 250 students from public schools have been involved in all programs.

d. **Educational Publications** – The Center produced various kinds of printed materials destined to the public schools, including: the Science Newsletter with a circulation of 4,000 issues; and a series of brief publications called *“Folhetim”* that are intended to build up the memory of the projects developed in the House of Science. This material is available on line in the Center’s Educational site, and last year we have develop PIPOC - *“Ponto de informação pesquisa e organização em ciências”*, that has centralized and organized all material produced by the CTC since its beginning.

e. The Center offered an **online certified course** using the COL platform developed by the University of São Paulo since 2006. The course was called *“A aula: um exercício de investigação”* [Teaching class as a research exercise] and was opened for the participation of 20 teachers from public schools.

In 2007, a group of 15 students has produced a theatrical play “Agonia de uma Célula” (“A cell’s agony”) , which was written in 2001 by two previous students of the program. The play was presented in schools of Brasília, Paranoá and Taguatinga, and at the National Museum in Brasilia during the “IV National Week of Science and Technology”.

Book on Stem Cells. Two investigators of the center published a book on the subject of stem cells, comprising the basic aspects of stem cells, the medical applications and a part on ethical and legal issues, distributed into 15 chapters and written by 27 collaborators: Zago MA & Covas DT – Células-tronco: a Nova Fronteira da Medicina. [Stem-cells: the new frontiers of medicine] Editora Atheneu, Rio de Janeiro, 2006, 245 pages. ISBN 85-7379-809-2

### **3. Summary of the Academic Proposal for the Next Three Years**

#### **3.a) Research**

As the project developed the interaction between investigators increased and the efforts became more focused. The proposal for the next three years is largely based on our published results, and will be divided in the same four topics used in 2.a).

i. *Characteristics and manipulation of the cells used for therapy.*

Continuing on our studies about the role of NF $\kappa$ B in the differentiation of hematopoietic stem cells (HSCs), we now propose to analyze the effect of specific inhibitors and activators of the NF $\kappa$ B pathways. We chose as model the analysis of T-cell lymphopoiesis using HSCs stimulated with OP9-DL1. We will analyze the changes in the immunophenotypic and gene expression pattern as they differentiate, as well as we will focus on the regulatory effects of the transcription factors RUNX1, GATA3 and USF1.

Regarding the manipulation of stem cells, we intend to determine if the forced expression of lineage-associated or multilineage-associated transcription factors in mesenchymal stem cells (MSCs) lead to preferential differentiation in a specific tissue or quiescence. We will use retrovirus systems to transduce MSCs with Nanog, Oct3/4, Sox2,  $\beta$ -catenin, Klf4, c-myc, Tbx, Tcf and Esrrb and analyze the change in the morphology, expression of markers and genes upon differentiating stimuli.

Our group was the first to demonstrate the existence of mesenchymal stem cells (MSCs) on vessels walls. These cells were detected in the umbilical cord and saphena vein and presented a gene expression profile similar to MSCs of the bone marrow. We now propose to search and characterize MSCs in different adult tissues, such as arteries, muscle, adipose tissue, thymus and lungs. This study is important to understand whether all MSCs have the same phenotype and functional capabilities and, in some cases, the data to be obtained may provide new insights in methods for increasing the availability to MSCs to therapeutic purposes.

The isolation and characterization of MSCs in vessels raised the question whether these cells are similar to pericytes. Our preliminary results demonstrated that morphologic and immunophenotypic features as well as the

potential of differentiation of MSCs and pericytes from retinal vessels are similar. We now propose to extend this study by using SAGE for the analysis of gene expression and to determine the potential use of pericytes for repairing of different tissues.

To evolve from the studies aiming to isolate and characterize the different stem cells into more “functional” assays, we propose the development of co-culture *in vitro* assays and transplantation models using NOD/SCID mice. In the former, MSCs or pericytes will be cultured over a layer of Human Umbilical Vein Endothelial Cells (HUVECs) and the differentiation on muscle, chondrocytes and adipose tissue will be evaluated. The morphologic and immunophenotypic analysis will be associated with the analysis of gene expression by Real-Time PCR. In the *in vivo* assays, we propose to analyze the immunological properties of MSCs by performing competitive transplantation assays using melanoma cell lines. In these assays, we intend to determine whether MSCs may enhance the graft versus tumour effect. We also propose to evaluate the effect of endothelial progenitors in a murine model of ischemia.

The immunomodulatory effects of MSCs will also be analyzed in the graft versus host (GVH) context. We plan to characterize the changes in the gene expression profile of human T lymphocytes induced to proliferate in presence or absence of MSCs. We opted for the microarray technique to perform this comparative analyses.

ii. *Characteristics of the recipients, affected with neoplastic diseases.*

We have previously demonstrated that Cancer Testis (CTs) antigens are aberrantly expressed in several malignancies (hematologic and non-hematologic) and that they are good candidates for immunotherapy. We propose now to study the expression of PRAME in a particular subset of lymphoproliferative disorder that although rare, represent a challenge to hematologist due its dismaying prognosis, the NK cell neoplasias. In addition, we will analyze whether PRAME abnormal expression in hematological malignancies reflects the fact that the transformation has occurred during early stages of differentiation or it is caused by the expression of any oncogenic product. We propose to analyze the interaction between the oncogenic protein PML/RAR $\alpha$  and the PRAME protein. For that we will use a model the expression of PML/RAR $\alpha$  is conditional and analyze the transcription, subcellular localization and degradation of PRAME.

Our previous analysis of the gene expression profile in Chronic Lymphocytic Leukemia (CLL) revealed that the TOSO and CD72 genes are expressed at higher levels in CLL cells compared to their normal counterparts. We now propose to study the pattern of expression of these genes in CLL patients Zap70<sup>+</sup> versus Zap70<sup>-</sup> and to determine whether these two subsets present distinct activities as determined by *in vitro* assays of B-cell function. At the clinical point of view, CLL patients whose cells express the Zap70 marker have a poorer outcome and the presence of Zap70 correlates with the unmutated of IgVH gene. Therefore, a better characterization of Zap70<sup>+</sup> may indicate potential therapeutic targets.

Several groups including ours have demonstrated that the PML/RAR $\alpha$  oncoprotein represses the transcription of genes involved in myeloid

differentiation. The most studied mechanism is the formation of repressing complexes that ultimately cause histone deacetylation and chromatin remodeling. Nevertheless, the recent demonstration that DNA methyl transferases are part of the complex suggested that DNA methylation may play a role in leukemogenesis. We intend to search for genes that are hypermethylated in acute promyelocytic leukemia (APL) cells using a commercial array. We will analyze the methylation status of these genes in blasts from patients with APL at diagnosis and in remission. Moreover, we also analyze candidate genes in myeloid progenitors from hCG-PML/RAR $\alpha$  transgenic mice.

We propose to compare the microRNAs profile in subsets of patients with acute lymphocytic leukemia (ALL). Several groups have demonstrated that microRNAs have an important role in the differentiation of lymphocytes and that lymphoid malignancies have a distinct "signature" (microRNAs profile). Nevertheless, the clinical relevance of these differences and the mechanisms by which microRNAs contribute to lymphopoiesis is not known. We have collected and preserved leukemic cells from over 80 patients with T or T/NK ALL. Our clinical data suggest that these two subgroups have distinct response to treatment, despite their immunophenotypic similarities. Our aim is to compare the microRNAs profile in these two ALL subsets.

iii. *Relationship of the host with parasites transmitted by cell therapy*

Following up with the previous studies analyzing host factors associated with retrovirose, we now propose to evaluate the role of CD4+ and CD8+ lymphocytes in the infection by HTLV-1. We will determine the gene profile of CD4+ and CD8+ cells from healthy and HTLV-1 infected individuals using as methodology microarrays and/or SAGE. Additionally, the profile of expression of microRNAs (miRNAs) in this same population will be analyzed. We intend to identify possible targets of miRNAs in HTLV-1 regulatory region. In addition, we will analyze single nucleotide polymorphisms (SNPs) in the following genes: perforin, granzyme B, DC-SIGN (CD209) and HLA-G in order to test if they may be associated with retroviral infection and activity.

iv. *Transgenic animals as experimental models*

The more primitive cells in acute leukemia have recently been called leukemic stem cells (LSCs) by analogy with the normal hematopoietic stem cells (HSCs), since they retain similar properties of self-renewal, high proliferative capacity, and predominant quiescent cell cycle status. LSCs are more resistant to proapoptotic stimuli than HSCs and differentiated leukemic blasts and, experimental data suggest that current therapies are unable to eradicate LSCs thus leading to disease relapse. Nevertheless, the mechanisms controlling LSCs quiescence are poorly understood. We propose to compare gene expression profile of LSCs obtained from hCG-PML/RAR $\alpha$  transgenic mice (TM) with their CD117<sup>+</sup> bone marrow (BM) counterparts. We will also analyze the change of these gene expression profiles upon total body irradiation.

We have recently analyzed the antileukemic effect of the small molecule halofunginone on acute promyelocytic leukemia (APL) cell lines. Halofunginone induced block of the cell cycle and apoptosis in retinoic acid sensitive and

resistant cells. Moreover, there was a significant decrease in the production of the pro-angiogenesis molecules VEGF and angiogenin. We now propose to analyze the *in vivo* effect of halofunginone using a murine leukemic transplatation model. NOD/SCID mice will be transplanted with leukemic cells obtained from hCG-PML/RAR $\alpha$  TM and treated with halofunginone. We will determine the microvascular density in bone marrow, the concentration of VEGF on plasma, as well to test with this molecule can induce morphologic and molecular remission of the disease.

v. *Clinical trials using stem cells to treat autoimmune diseases.*

Based on our results of the treatment with AHST of recently diagnosed type 1 diabetes mellitus, we now plan to extent the treatment to patients with DM with longer periods after the diagnosis. The National Ethics Committee (CONEP) has already analyzed and approved the protocol and we will soon start enrolling patients. In addition, we will start a multicentric international study to evaluate the benefit and toxicity of AHST for the treatment of multiple sclerosis. This protocol has been approved by CONEP. A third clinical protocol aims to study of stem cell infusion for the treatment of degenerative retinopathies. This last protocol is under analysis by CONEP.

### **3.b) Innovation**

We successfully generated transgenic cell lines that produce FVIII at concentrations higher than 100 UI/ml. We propose to take these cell lineages and scale up production until industrial amounts. In the next 3 years, we aim to reach a production of rFVIII in quantities sufficient for the pre-clinical and clinical studies. For this new phase, we set up a collaboration with a private company called Plantarium and have received fund from FINEP.

We aim also to conclude the development of the umbilical cord blood bank. As mentioned the infrastructure is ready and personnel have been trained. We will now systematize the routine of collection at several hospitals and clinics and test the information technology developed for the management of the system.

### **3.c) Diffusion**

We will maintain the activities at the MuLEC and the House of Science, offering to new middle and high students and teachers the opportunity to benefit from the infrastructure. We will particularly expand the program “*Adopt a scientist*” and “*Scientific Initiation*” by including teachers from middle and high schools. Audiovisual material is being prepared with this aim. Over a hundred conferences and interviews proffered during CTC seminars and related activities have been taped and together if written reviews about the themes will constitute the core for these activities for the teachers. We also plan to expand the activities at the MuLEC by creating scientific “*expeditions*” (daily trips) with students in the USP Campus under the supervision of teachers and post graduated students to teach basic concepts of environmental care.



#### **4. Proposed budget for the next three years**

Below is the FAPESP form with the description of the requested budget for the next 3 years. We included support from other sources that have already granted or that are an estimative of our Institutions to invest in the project (USP and FUNDHERP)



FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

Nome do Interessado: Marco Antônio Zago

PROCESSO: 1998/14247-6

### PROGRAMA CEPID FAPESP

ORÇAMENTO CONSOLIDADO POR RUBRICA E POR FONTES DE FINANCIAMENTO (FAPESP e outras fontes tais como universidades, institutos, outras agências)

item	Descrição (somente 1 linha para cada item)	FAPESP		OUTRAS FONTES			TOTAL	
		VALOR R\$	VALOR US\$	ESPECIFIQUE	VALOR R\$	VALOR US\$	VALOR R\$	VALOR US\$
1	MATERIAL PERMANENTE NACIONAL (MPN)	R\$ 600.000,00		FINEP, Plantarium, FUNDHERP, Fundação Vitae	R\$ 700.000,00	US\$ 2.500.000,00	R\$ 1.300.000,00	US\$ 2.500.000,00
2	MATERIAL PERMANENTE IMPORTADO (MPI)		US\$ 900.000,00					US\$ 900.000,00
3	MATERIAL DE CONSUMO NACIONAL (MCN)	R\$ 1.500.000,00		FINEP, FUNDHERP, CNPq	R\$ 800.000,00		R\$ 2.300.000,00	
4	MATERIAL DE CONSUMO IMPORTADO (MCI)		US\$ 1.200.000,00	FINEP, CNPq		US\$ 200.000,00		US\$ 1.400.000,00
5	SERVIÇO DE TERCEIROS NO PAÍS (STB)	R\$ 600.000,00		International Consortium on Acute Promyelocytic Leukemia		US\$ 40.000,00	R\$ 600.000,00	US\$ 40.000,00
6	SERVIÇO DE TERCEIROS NO EXTERIOR (STE)		US\$ 90.000,00					US\$ 90.000,00
7	DESPESAS DE TRANSPORTE (DET)	R\$ 10.500,00					R\$ 10.500,00	
8	DESPESAS COM DIARIAS (DIP)	R\$ 15.000,00					R\$ 15.000,00	
9	RECURSOS HUMANOS			FUNDHERP, USP	R\$ 1.800.000,00		R\$ 1.800.000,00	
9a	RECURSOS PARA BOLSAS:							
	9ai- BOLSAS DE INICIAÇÃO CIENTÍFICA (IC)	R\$ 40.780,00					R\$ 40.780,00	
	9aii- BOLSAS DE MESTRADO (MS)							
	9aiii- BOLSAS DE DOUTORADO / DOUTORADO DIRETO (DR/DD)							
	9aiv- BOLSAS DE POS DOUTORADO (PD)	R\$ 2.594.304,00					R\$ 2.594.304,00	
	9av- BOLSAS DE TREINAMENTO TÉCNICO (TT)	R\$ 54.064,80					R\$ 54.064,80	
9b	COMPLEMENTAÇÃO SALARIAL PARA PESQUISADORES							
9c	CONTRATAÇÃO TEMPORÁRIA E OUTROS							
10	OUTROS (ESPECIFICAR)							
	<b>TOTAL</b>	<b>R\$ 5.414.648,80</b>	<b>US\$ 2.199.000,00</b>		<b>R\$ 3.300.000,00</b>	<b>US\$ 2.749.000,00</b>	<b>R\$ 8.714.648,80</b>	<b>US\$ 4.930.000,00</b>

FAPESP, MARÇO DE 2008

## List of items that cost more than R\$ 100.000,00

### **A. Magnetic cell sorter AUTOMACS, Miltenyi Biotech GMBH – Ref : 201-01, Euro\$ 27.875,00 – 1 Unit (alinea: MPI)**

*Justification:* this system is used for the isolation of specific cell types previously labeled with antibody-revested magnetic beads. The cells after sorting will be used in transplants in animal models, for the analysis of gene expression and/or proteomics. At present, we perform our sorting experiments using a flow cytometer with sorter in the laboratory of Prof. Flávio Meireles in Pirassununga (about 150 km far from Ribeirão Preto) or using another magnetic device called VarioMACS, which works well for cells labeled with a single marker. The travelling affects the viability of the cells and the success of the engraftment. Moreover, the number of experiments involving cell sorting is large and justifies the acquisition of this system. It should be pointed out that this is not a flow cytometer and it is a much cheaper alternative to the acquisition of one.

### **B. 7500 Real Time PCR System with notebook, Applied Biosystems – Ref: 4351104, US\$ 42.500,00 – 1 Unit (alinea: MPI)**

*Justification:* Analysis of gene expression. This is probably the most used system for all investigators of CTC. We have currently one system that is overbooked, with an average waiting time of a month to perform one experiment. By acquiring this second Real Time PCR System to be allocated on a different site of the previous one, we hope to increase the agility of the experiments.

### **C. MACSquant analyzer , Miltenyi Biotech GMBH – Ref : 201-01, Euro\$ 86.500,00 – 1 Unit (alinea: MPI)**

*Justification:* Analysis of cells labeled with DNA and RNA dyes as well as surface markers. This system is equipped with a violet laser that allows the analysis of cells labeled with Hoechst dyes and Pacific Dyes, together with a blue laser that allows to co-analyze propidium iodide (PI) staining and a red laser to analyze APC or PE-Cy7 staining. The configuration of this system is ideal for the analysis of stem cells (quiescent cells) and it is a cheaper alternative to an upgrade of our system of 4-colour flow cytometer.

### **D. Ultracentrifuge, Biotron KR – Ref: Ultra 5.0, R\$ 156.089,00 – 1 Unit (alinea: MPN)**

*Justification:* Equipment necessary for retrovirus transduction in experiments generating transgenic cells as well as for the isolation of subcellular fractions for functional and structural analysis.

#### 4.a) Request for fellowship support



FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

Nome do Interessado: Marco Antônio Zago

PROCESSO: 1998/14247-6

#### PROGRAMA CEPID - FAPESP ORÇAMENTO CONSOLIDADO - BOLSAS

item	título do plano de trabalho	modalidade da bolsa	número de meses	valor mensal	valor total
1	Functional Analysis of the Interaction between PRAME and PML/RAR $\alpha$ Oncoprotein	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
2	In vivo and in vitro characterization of pericytes	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
3	Expression profile of T-lymphocytes induced to proliferate in presence or absence of mesenchymal stem cells	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
4	MicroRNAs expression in hematological malignancies	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
5	Proteomics of stem cell differentiation	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
6	Analysis of protein modification upon apoptosis induced by different agents	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
7	In vivo model for the analysis of tissue repair by mesenchymal stem cells	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
8	Genomic hybridization (CGH) and Spectral Karyotyping for the analysis of lymphoproliferative disorders	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
9	Immunological effects of autologous hematopoietic transplantation in the treatment of autoimmune diseases	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
10	Biochemical and functional characterization of recombinant factor VIII	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
11	Evaluation of galectin 3 in tumor progression	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
12	Analysis of the antileukemic effect of Halofunginone in vivo	Bolsa de Pós-doutorado no País	48	R\$ 4.504,00	R\$ 216.192,00
13	Biological effects of autologous hematopoietic transplantation in the treatment of autoimmune diseases	Bolsa de Iniciação Científica	24	R\$ 424,80	R\$ 10.195,20
14	Analysis of serum levels of angiogenin, VEGF and angiopoietin in patients with acute promyelocytic leukemia	Bolsa de Iniciação Científica	24	R\$ 424,80	R\$ 10.195,20
15	MicroRNAs expression in hematological malignancies	Bolsa de Iniciação Científica	24	R\$ 424,80	R\$ 10.195,20
16	Analysis of the antileukemic effect of Halofunginone in vivo	Bolsa de Iniciação Científica	24	R\$ 424,80	R\$ 10.195,20
17	Genomic hybridization (CGH) and Spectral Karyotyping for the analysis of lymphoproliferative disorders	Bolsa de Treinamento Técnico Nível III	24	R\$ 750,90	R\$ 18.021,60
18	Analysis of hematopoiesis in transgenic models of acute promyelocytic leukemia	Bolsa de Treinamento Técnico Nível III	24	R\$ 750,90	R\$ 18.021,60
19	In vivo model for the analysis of tissue repair by mesenchymal stem cells	Bolsa de Treinamento Técnico Nível III	24	R\$ 750,90	R\$ 18.021,60
				<b>TOTAL</b>	<b>R\$ 2.689.149,60</b>

## Summary of the requested fellowships

### 1) *Functional analysis of the interaction between PRAME and PML/RAR $\alpha$ oncoprotein*

- *Post-doctoral fellowship*
- *Supervisor: Eduardo M. Rego*
- *Summary:* Retinoic acid (RA) induces proliferation arrest, differentiation, and apoptosis, and defects in retinoic acid receptor (RAR) signaling have been implicated in cancer. The human tumor antigen PRAME is overexpressed in a variety of cancers, but its function has remained unclear. Recently, experimental data revealed that PRAME acts as a dominant repressor of RAR signaling. PRAME binds to RAR in the presence of RA, preventing ligand-induced receptor activation and target gene transcription through recruitment of Polycomb proteins. We aim to determine whether PRAME abnormal expression in hematological malignancies reflects the fact that the transformation has occurred during early stages of differentiation or it is caused by the expression of any oncogenic product. We propose to analyze the interaction between the oncogenic protein PML/RAR $\alpha$  and the PRAME protein and to determine if promoter regions containing Retinoic Acid Receptor Responsive Elements are modulated by PRAME. For that we will use a model the expression of PML/RAR $\alpha$  is conditional and analyze the transcription, subcellular localization and degradation of PRAME.

### 2) *In vivo and in vitro characterization of pericytes*

- *Post-doctoral fellowship*
- *Supervisor: Dimas T. Covas*
- *Summary:* Our preliminary results demonstrated that morphologic and immunophenotypic features as well as the potential of differentiation of MSCs and pericytes from retinal vessels are similar. We now propose to extend this study by using SAGE for the analysis of gene expression and to determine the potential use of pericytes for repairing of different tissues.

### 3) *Gene expression profile of T-lymphocytes induced to proliferate in the presence or absence of mesenchymal stem cells*

- *Post-doctoral fellowship*
- *Supervisor: Dimas T. Covas*

*Summary:* The immunomodulatory effects of MSCs will be analyzed in the graft versus host (GVH) context. We plan to characterize the changes in the gene expression profile of human T lymphocytes induced to proliferate in presence or absence of MSCs. We opted for the microarray technique to perform this comparative analyses.

#### 4) *MicroRNAs expression in hematological malignancies*

- *Post-doctoral fellowship (1) and Scientific initiation (1)*
- *Supervisor: Marco A. Zago*
- *Summary:* We propose to compare the microRNAs profile in subsets of patients with acute lymphocytic leukemia (ALL). Several groups have demonstrated that microRNAs have an important role in the differentiation of lymphocytes and that lymphoid malignancies have a distinct “signature” (microRNAs profile). Nevertheless, the clinical relevance of these differences and the mechanisms by which microRNAs contribute to lymphopoiesis is not known. We have collected and preserved leukemic cells from over 80 patients with T or T/NK ALL. Our clinical data suggest that these two subgroups have distinct response to treatment, despite their immunophenotypic similarities. Our aim is to compare the microRNAs profile in these two ALL subsets.

#### 5) *Proteomics of stem cell differentiation*

- *Post-doctoral fellowship (1) and Scientific initiation (1)*
- *Supervisor: Lewis J Greene*
- *Summary:* we intend to determine if the forced expression of lineage-associated or multilineage-associated transcription factors in mesenchymal stem cells (MSCs) or hematopoietic stem cells (HSCs) lead to preferential differentiation in a specific tissue or quiescence. We will use retrovirus systems to transduce stem cells with Nanog, Oct3/4, Sox2,  $\beta$ -catenin, Klf4, c-myc, Tbx, Tcf and Esrrb or classical cytokines that lead to the preferential differentiation into a lineage. The change in the proteomics of the cells will be analyzed at different points of their differentiation towards one lineage or the other.

#### 6) *Comparative genomic hybridization (CGH) and Spectral Karyotyping for the analysis of lymphoproliferative diseases*

- *Post-doctoral fellowship (1) and Technical training III (1) fellowship*
- *Supervisor: Roberto Passetto Falcão*
- *Summary:* We aim to establish a method for obtaining proliferating B lymphocytes from patients with chronic lymphocytic leukemia (CLL) and to determine the frequency of cryptic cytogenetic abnormalities in this disease. We will test if these terminal mature B-cells respond to the stimulus with DSP30/IL-2. We opted to use the spectral karyotyping (SKY) and Fluorescence *in situ* hybridization (FISH) to perform the analysis.

7) *Immological effects of autologous hematopoietic transplantation in the treatment of autoimmune disorders*

- *Post-doctoral fellowship (1) and Scientific initiation (1) fellowship*
- *Supervisor: Julio César Voltarelli*
- *Summary: The results obtained in the clinical trial of AHST for the treatment indicate that the treatment can interrupt or slow down the process of immunologic aggression to pancreatic  $\beta$ -cells. We aim to characterize the changes in the T-cell repertoire after the transplant and to correlate it with the clinical outcome.*

8) *Biochemical and functional characterization of recombinant factor VIII*

- *Post-doctoral fellowship (1)*
- *Supervisor: Dimas T Covas*
- *Summary: We successfully generated transgenic cell lines that produce FVIII at concentrations higher than 100 UI/ml. We now propose to characterize the structure and the function of these recombinant molecules in pre-clinical studies.*

9) *Evaluation of galectin 3 in tumour progression VIII*

- *Post-doctoral fellowship (1)*
- *Supervisor: Roger Chammas*
- *Summary: Beta1-6 branching of N-linked oligosaccharides has been correlated with the progression of different cancers. The leucoagglutinins of Phaseolus vulgaris (L-PHA) have been used to study this pattern of glycosylation whose biological significance is incompletely understood. The animal lectin, galectin-3, also binds to structures recognized by L-PHA. We have recently developed a probe containing the human galectin-3 was fused to bacterial alkaline phosphatase (gal3/AP). We aim to analyze the pattern of expression of galectin 3 in different cell types using as well as to evaluate tumor progression in knock out mice for galectin 3.*

10) *Analysis of the antileukemic effect of Halofunginone in vivo*

- *Post-doctoral fellowship (1) and scientific initiation (1)*
- *Supervisor: Eduardo M. Rego*
- *Summary: We have recently analyzed the antileukemic effect of the small molecule halofunginone on acute promyelocytic leukemia (APL) cell lines. Halofunginone induced block of the cell cycle and apoptosis in retinoic acid sensitive and resistant cells. Moreover, there was a significant decrease in the production of the pro-angiogenesis molecules VEGF and angiogenin. We now propose to analyze the *in vivo* effect of halofunginone using a murine leukemic transplantation model. NOD/SCID mice will be transplanted with leukemic cells obtained from hCG-PML/RAR $\alpha$  TM and treated with halofunginone. We will determine the microvascular density in bone marrow, the concentration of VEGF on*

plasma, as well to test with this molecule can induce morphologic and molecular remission of the disease.

11) *Determination of serum levels of angiogenin, VEGF and angiopoetin in patients with acute myeloid leukemia*

- *Scientific initiation (1)*
- *Supervisor: Eduardo M. Rego*
- *Summary:* The data obtained in transgenic mice PML/RAR $\alpha$  indicate that the PML/RAR $\alpha$  oncoprotein interfere with the expression of pro-angiogenic molecules, mainly the angiogenin, leading to increased angiogenesis. In order to determine if this is a specific finding in acute promyelocytic leukemia or if it reflects a common process during leukemogenesis, we will compare the serum levels of angiogenin, VEGF and angiopoetin in patients with acute myeloid leukemia

12) *Analysis of hematopoiesis in transgenic models of acute promyelocytic leukemia*

- *Technical training III (1)*
- *Supervisor: Eduardo M. Rego*
- *Summary:* The more primitive cells in acute leukemia have recently been called leukemic stem cells (LSCs) by analogy with the normal hematopoietic stem cells (HSCs), since they retain similar properties of self-renewal, high proliferative capacity, and predominant quiescent cell cycle status. LSCs are more resistant to proapoptotic stimuli than HSCs and differentiated leukemic blasts and, experimental data suggest that current therapies are unable to eradicate LSCs thus leading to disease relapse. Nevertheless, the mechanisms controlling LSCs quiescence are poorly understood. We propose to compare gene expression profile of LSCs obtained from hCG-PML/RAR $\alpha$  transgenic mice (TM) with their CD117<sup>+</sup> bone marrow (BM) counterparts. We will also analyze the change of these gene expression profiles upon total body irradiation.

13) *In vivo model for the analysis of tissue repair by mesenchymal stem cells*

- *Technical training III (1)*
- *Supervisor: Marco A. Zago*
- *Summary:* To evolve from the studies aiming to isolate and characterize the different stem cells into more “functional” assays, we propose the development of transplantation models using NOD/SCID mice. MSCs will be cultured over a layer of Human Umbilical Vein Endothelial Cells (HUVECs) and transplanted in NOD/SCID in which ischemic lesions have previously been induced surgically.



## **5) Research Team**

### **Marco Antonio Zago**

Professor of Clinical Medicine, Coordinator of the CTC Center. *Main research interests:* genome, gene expression, stem cell differentiation, gene abnormalities in neoplasias.

### **Roberto Passetto Falcão**

Professor of Clinical Medicine. Deputy coordinator of the CTC Center. *Main research interests:* flow cytometry, lymphoid cell differentiation, hematopoietic neoplasias

### **Dimas Tadeu Covas**

Associate Professor of Medicine, Technology Transfer Coordinator. *Main research interests:* stem cell research, transfusion medicine, HIV, HTLV-II

### **Lewis Joel Greene**

Professor of Biochemistry. *Main research interests:* protein chemistry, proteomics, dendritic cell differentiation.

### **Roger Chammas**

Associate Professor of Medicine. *Main research interests:* oncology, cell biology, glycobiology.

### **Julio Cesar Voltarelli**

Associate Professor of Medicine. *Main research interests:* applied human immunology, bone marrow transplantation.

### **Wilson Araujo Silva Jr**

Assistant Professor of Genetics. *Main research interests:* genome, gene expression, population genetics, bioinformatics.

### **Eduardo Magalhães Rego**

Associate Professor of Medicine. *Main research interests:* leukemia, leukemogenesis, animal model of human diseases.

### **Marisa Barbieri**

Former High School Biology Teacher and Former Associated Professor of Biology. Coordinator of Education and Dissemination.

All researchers belong to the faculty of the Medical School of Ribeirão Preto, University of S. Paulo, except for R. Chammas, who is from the USP Medical School in S. Paulo, and M. Barbieri, who retired from the USP Faculty of Sciences in Ribeirão Preto.

### **International Collaboration**

Many of the researchers of the center have scientific links with foreign researchers, forged outside the context of the CEPID. There are, however, two collaborations that are more constant and were established in the framework of the center. There are frequent exchanges of visits and shared research activity:

**Vanderson Rocha:** Clinical coordinator of the EuroCord Project, Hôpital Saint-Louis, Paris

**Pier Paolo Pandolfi:** Professor of Genetics, Sloan-Kettering Cancer Institute, N. York

**Davide Ruggero:** Associate Professor Fox Chase Cancer Center, Philadelphia

**Rodrigo Callado** and **Neal S. Young:** Hematology Branch, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, USA

## 5.a) Executive Committee Members

Name	Institution	Position/Responsibility
Marco Antonio Zago	FMRP/USP	Coordinator of the Center of Cell-Based Therapy.
Dimas Tadeu Covas	FMRP/USP	Coordinator of Technology Transfer.
Marisa Ramos Barbieri	FUNDHERP	Coordinator of Education and Dissemination.

### **Marco Antonio Zago - FMRP/USP**

Subproject Coordinator:

- Functional genomics of B-cell malignancies: the gene expression profiles of chronic lymphocytic leukemias and mantle cell lymphomas
- The impact of gene polymorphisms on the response to treatment with cell therapy.
- The impact of gene polymorphisms on the susceptibility to hematological diseases
- The functional genomics of cells used for cell therapy: the gene expression profiles of human mesenchymal stem cells obtained from different sites
- The functional genomics of cells used for cell therapy: the comparison of the gene expression profiles of human CD34+ cells obtained from bone marrow, umbilical cord and peripheral blood
- The early gene expression changes in the hematopoiesis: the erythroid and granulocytic-monocytic pathways

### **Dimas Tadeu Covas - FMRP/USP**

Subproject Coordinator:

- Generation, characterization and in vitro manipulations of mesenchymal stem cells aiming at their use for cell therapy
- The impact of gene polymorphisms on the response to HIV and HTLV infection.
- The functional genomics of cells used for cell therapy: the gene expression profiles of human mesenchymal stem cells obtained from different sites
- The functional genomics of cells used for cell therapy: the comparison of the gene expression profiles of human CD34+ cells obtained from bone marrow, the umbilical cord and peripheral blood
- Cloning and expression of recombinant human coagulation factor VIII in mammalian cells using retrovirus as a vector.
- Brazil Cord Blood Bank.
- Development of an animal model for the study of mesenchymal stem cell differentiation in vivo.
- Assessment and treatment of iron overload in  $\beta$  thalassemia homozygous patients.

### **Eduardo Magalhães Rego - FMRP/USP**

Subproject Coordinator:

- Animal model of dyskeratosis congenita
- Analysis of the molecular basis of leukemogenesis in the transgenic model of acute promyelocytic leukemia
- Analysis of leukemic cells adhesion and tethering upon ATRA Histone Deacetylases inhibitors,  $AS_2O_3$  and G-CSF treatment in acute promyelocytic leukemia
- Analysis of the effect of vitamin E isomers in acute promyelocytic leukemia
- Study of the effect of Histone Deacetylase Inhibitors on gene transcription in acute promyelocytic leukemia cells.
- Analysis of PRAME antigen expression in normal lymphoid cells
- Study of the pathogenesis of disseminated intravascular coagulation in the transgenic model of acute promyelocytic leukemia.

### **Roberto Passetto Falcão - FMRP/USP**

Subproject Coordinator:

- The expression of adhesion molecules in the leukemic phase of non-Hodgkin's lymphomas
- Analysis of blasts adhesion and tethering upon histone deacetylase inhibitors and G-CSF treatment in acute promyelocytic leukemia
- Analysis of the expression of glucocorticoid receptors in non-Hodgkin's lymphomas
- Development of a method of cytotoxicity of leukemic cells based on the association of peroxidase with 3-indol-acetic acid

### **Lewis Joel Greene - FMRP/USP**

Subproject Coordinator:

- Evaluation of gene expression during differentiation and maturation of cord blood CD34-derived dendritic cells using proteomic analysis.
- Proteome modification during the early stages of melanoma malignization.
- Proteomic analysis of human metastatic cells treated with antitumoral drugs.
- Proteomic and genomic analysis of target genes modulated by miRNA-155 in non Hodgkin lymphoma cells.
- Proteomic analysis of dendritic differentiation induced by different stimuli of CD34+ or monocytes obtain from umbilical cord vein, peripheral
- Identification of ligands of galectin-3
- Proteomic analysis of osteogenic differentiation of mesenchymal stem cells Stro-1+
- Comparison of the proteomic profile of mesenchymal stem cells obtained from bone marrow and umbilical cord vein
- Analysis of the post translational changes of proteins differentially expressed by Melan-A, Tm1 and Tm5 cell lines.

**Júlio César Voltarelli - FMRP/USP**

Subproject Coordinator:

- Treatment of immunological diseases by high dose chemotherapy and autologous bone marrow transplantation.
- Treatment of late onset type II diabetes mellitus by bone marrow transplantation
- Development of animal models for testing cell therapy for lung disorders.

**Roger Chammas - FM/USP**

Subproject Coordinator:

- The use of pulsed autologous dendritic cells for the treatment of melanoma
- Gene expression profile during melanoma progression
- Analysis of cell membrane molecules changes during melanoma progression
- Gangliosides in hematological malignancies and normal lymphoid cells
- Genes associated with melanoma progression and development of chemoresistance
- Analysis of cell membrane molecules changes during melanoma progression and development of chemoresistance
- Gangliosides and dendritic cell function
- Tumor cell interaction with microenvironmental cells

## **Senior Investigators**

### **Wilson Araújo da Silva Jr - FMRP/USP**

Subproject Coordinator:

- Initiative to validate of the human transcriptome
- The bioinformatics high throughput approaches to analyzing the gene expression of human tissues
- The comparison of gene expression in human neoplasias, with especial emphasis in interleukines, adhesion molecules and angiogenesis.
- Analysis of the sequences generated by the Human Genome of Cancer project.
- Clinical Genomics Project – Bioinformatics Laboratory.
- Genome data mining.
- Gene expression of microRNAs in hematopoietic stem cells

### **Aparecida Maria Fontes - FUNDHERP**

Subproject Coordinator:

- Cancer vaccine for chronic myeloid leukemia.
- Cloning and expression of recombinant human coagulation factor VIII in mammalian cells
- Cloning and expression of recombinant human coagulation factor IX in mammalian cells
- Isolation and characterization of murine mesenchymal cells
- Gene modification of stem cells

### **Marisa Ramos Barbieri - FUNDHERP**

Subproject Coordinator:

- The cells, the genome and you.

### **Vanderson Rocha - EUROCORD**

Subproject Coordinator:

- Facilitating cord blood cells for engraftment: importance of specific lymphocyte subpopulations.
- Expansion of cord blood mononuclear cells in coculture with autologous human umbilical vein endothelial cells (HUVEC).
- The impact of gene polymorphisms on the response to treatment with cell therapy.
- The impact of gene polymorphisms on the susceptibility to hematological diseases

**Belinda Pinto Simões - FMRP/USP**

Subproject Coordinator:

- Haploidentical bone marrow transplantation
- Strategies to reduce or avoid graft-versus-host disease
- Facilitating cord blood cells for engraftment: importance of specific lymphocyte subpopulations.
- The impact of gene polymorphisms on the susceptibility and evolution of GVHD

**José César Rosa - FMRP/USP**

Subproject Coordinator:

- Proteome modification during the differentiation of dendritic cells from CD34+ cells of human umbilical cord, during the early stages of melanoma malignization, and of human metastatic cells treated with antitumoral drugs

**Evamberto Garcia de Góes - FUNDHERP - FAPESP**

Subproject Coordinator:

- Use of Telecobalt therapy for the prevention of graft versus host disease associated with transfusion: dosimetry and quality control of irradiated blood
- Effects of diagnostic X-ray dose on peripheral blood mononuclear cells

## Associate Investigators

### Simone Kashima Hadad - FUNDHERP

Subproject

- Genetic characterization of host factors and role of microRNA expression in HTLV-1 infection.

### Rodrigo Alexandre Panepucci – FUNDHERP - FAPESP

Subproject

- Molecular basis of T lymphocyte differentiation from hematopoietic stem cells

### Greice A Molfetta – FUNDHERP - FAPESP

Subproject

- Changes of gene expression in the early differentiation of CD34+ along the erythroid and the granulocytic-monocytic pathways

### Rita de Cássia Viu Carrara - FUNDHERP - FAPESP

Subproject

- Identification of genes differentially expressed in CD34+ Bcr/Abl+ cells of patients with chronic myeloid leukemia

### Clarice Izumi - FMRP/USP

Subproject

- Proteome modification during the differentiation of dendritic cells from CD34+ cells of human umbilical cord, during the early stages of melanoma malignization, and of human metastatic cells treated with antitumoral drugs

### Paulo Peitl Júnior - FUNDHERP - FAPESP

Subproject

- Changes of gene expression in human cells treated *in vitro* with antitumoral drugs

## Post Doctoral Fellows

Name	Institution	Adviser
Paulo Peitl Jr	FMRPUSP – FAPESP	Marco Antonio Zago
Rodrigo Proto-Siqueira	FMRPUSP – FAPESP	Marco Antonio Zago
Rodrigo Alexandre Panepucci	FMRPUSP – FAPESP	Marco Antonio Zago
Fábio Morato de Oliveira	FMRPUSP – FAPESP	Roberto Passeto Falcão
Idalete da Silva	FMRPUSP – FAPESP	Lewis J. Greene
Kelen Cristina Malmegrin Farias	FMRPUSP – FAPESP	Julio Cesar Voltarelli
Bárbara Amelia A Santana	FMRPUSP – FAPESP	Eduardo Magalhaes Rego
Alexandre Krause	FMRPUSP – FAPESP	Eduardo Magalhaes Rego
Simone Kashima Haddad	FMRPUSP – FAPESP	Dimas Tadeu Covas
Rita de Cássia Viu Carrara	FMRPUSP – FAPESP	Dimas Tadeu Covas
Kamilla Swiech	FMRPUSP – FAPESP	Dimas Tadeu Covas



## Graduate Students (PhD and MSc) active

Name	Institution	Adviser
Evandra Strazza Rodrigues	FMRPUSP FUNDHERP	Aparecida Maria Fontes
Danilo Candido de Almeida	FUNDHERP	Aparecida Maria Fontes
Bruno Catrim Trindade	FMRP-USP	Simone Kashima Haddad
Larissa D N Figueredo	FCFRP-USP	Simone Kashima Haddad
Tathiane Maistro Malta	FCFRP-USP	Simone Kashima Haddad
Rochele Azevedo	FMRPUSP	Dimas Tadeu Covas
Fernanda Bernadelli Garcia	UFMG	Dimas Tadeu Covas
Andriele Castilho Fernandes	FUNDHERP	Dimas Tadeu Covas
Fernanda Bernadelli Garcia	FUNDHERP	Dimas Tadeu Covas
Karen Lima de Prata	FUNDHERP	Dimas Tadeu Covas
Simone Kashima Haddad	FMRP-USP	Dimas Tadeu Covas
Ana Cristina Silva Pinto	FMRPUSP	Dimas Tadeu Covas
Bruno Marcos Verbeno Azevedo	FMRPUSP	Dimas Tadeu Covas
Ana Valéria Gouveia Andrade	FMRPUSP	Dimas Tadeu Covas
Lucila Habib B de Oliveira	FMRPUSP	Dimas Tadeu Covas
Jorge Luis Curado Siufi	FMRPUSP	Dimas Tadeu Covas
Ana Paula C Nunes Cunha Cozac	FMRPUSP	Dimas Tadeu Covas
Maria Fernanda Castro Amarante	FMRPUSP	Dimas Tadeu Covas
Flora Cristina Lobo Penteado	FCF/ARARAQUARA	Dimas Tadeu Covas
Rodrigo Haddad	FMRPUSP	Dimas Tadeu Covas
Antonio Roberto Lucena Araújo	FMRPUSP – FAPESP	Eduardo Magalhães Rego
Rafael H Jácomo	FMRPUSP – FAPESP	Eduardo Magalhães Rego
Luciana O. Oliveira	FMRPUSP	Eduardo Magalhães Rego
Mirela de B. Tamarozzi	FMRPUSP – FAPESP	Eduardo Magalhães Rego
Priscila Santos Scheucher	FMRPUSP – FAPESP	Eduardo Magalhães Rego
Maria Carolinia Tostes Pintão	FMRPUSP	Eduardo Magalhães Rego
Hamilton Luis G. Teixeira	FMRPUSP	Eduardo Magalhães Rego
Guilherme Augusto Silva Santos	FMRPUSP	Eduardo Magalhães Rego
Lorena Lobo e Figueiredo	FMRPUSP – FAPESP	Eduardo Magalhães Rego
Rodrigo Abreu e Lima	FMRPUSP – FAPESP	Eduardo Magalhães Rego
Vivian Toussef Khouri	FMRP - USP	Julio César Voltarelli
Alessandra de Paula Souza	FMRPUSP - FAPESP	Julio César Voltarelli
Marcia Pinho	FMRP - USP	Julio César Voltarelli
Danielle F Godoi	FMRP - USP	Julio César Voltarelli
Elisa Vendramini Nogueira	FMRP - USP	Julio César Voltarelli
Carolina Calari de Oliveira	FMRP - USP	Julio César Voltarelli
Gabriela Trentin Sorteguaga	FMRP - USP	Julio César Voltarelli
Juliana Navarro Ueda	FMRP - USP	Julio César Voltarelli
Maria Carolina O Rodrigues	FMRP - USP	Julio César Voltarelli
Carolina Hassibe Thomé	CNPq	Lewis J. Greene
Germano Aguiar Ferreira	FMRPUSP – FAPESP	Lewis J. Greene
Glauce Gaspar Gomes	FMRPUSP – CAPES	Lewis J. Greene
Ana Cristina D'Ávilla de Castro	UNIFESP – FAPESP	Lewis J. Greene
Gisele Guiçardi Tomazella	UNIFESP – FAPESP	Lewis J. Greene
Lucas Oliveira de Sousa	FAPESP	Lewis J. Greene
Ana Elisa Ramão	FUNDHERP	Lewis J. Greene
Alana Maria Cerqueira Catalan	FAPESP	Lewis J. Greene
Marcela Gimenes	FAPESP	Lewis J. Greene
Vanessa Cristina de Oliveira Sousa	CNPq	Lewis J. Greene
Helen Cristina Miranda	FMRPUSP – FAPESP	Lewis J. Greene
Nívea Maria Rocha Macedo	FMRPUSP – CAPES	Lewis J. Greene/José C. Rosa
Francisco Careta	FMRPUSP – FAPESP	Marco Antonio Zago
Felipe Saldanha de Araujo	FMRPUSP – FAPESP	Marco Antonio Zago
Lucila Habib B. Oliveira	FMRPUSP – FAPESP	Marco Antonio Zago
Manuela Ramos Barbieri	FMRPUSP – FAPESP	Marco Antonio Zago
Dalila Zanete	FMRPUSP – FAPESP	Marco Antonio Zago

André Marinato	FMRPUSP	Roberto Passetto Falcão
Daniel Mazza	FMRPUSP – FAPESP	Roberto Passetto Falcão
Leandro Felipe Dalmazzo	FMRPUSP	Roberto Passetto Falcão
Tharcisio Citrangulo Tortelli Jr	USP	Roger Chammas
Guilherme Francisco	Fund A Prudente - FAPESP	Roger Chammas
Fabio Luiz Navarro Marques	USP	Roger Chammas
Patrícia Leusa Nunes da Costa	Fund A Prudente - FAPESP	Roger Chammas
Lara Zimmermann	FAPESP	Roger Chammas
Renata de Freitas Saito	FAPESP	Roger Chammas
Luciana Nogueira Sousa Andrade	USP – FAPESP	Roger Chammas
Verônica Rodrigues Teixeira	USP – FAPESP	Roger Chammas

## Ongoing Projects: Dissertations (MSc), Theses (PhD) and Postdoctorals

### Master of Science (MSc)

- Manuela Ramos Barbieri. **Padrão de expressão celular da proteína S100 calcium binding protein A7 (psoriasin)**. Faculdade de Medicina de Ribeirão Preto. Orientador: Prof. Dr. Marco Antonio Zago
- Germano Aguiar Ferreira. **Identificação e caracterização de algumas proteínas com modificações pós-traducionais em células dendríticas humanas**. Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Prof. Dr. Lewis Joel Greene
- Lucas Oliveira de Sousa. **Análise de 10 proteínas diferencialmente expressas de células-tronco hematopoéticas (HSCs) e células-tronco leucêmicas (LSCs) relacionadas a apoptose**. Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Prof. Dr. Lewis Joel Greene
- Danilo Candido de Almeida. **Análise da atividade da telomerase e comprimento do telômero em células-tronco mesênquimais humanas isoladas de tecidos fetais e adultos**. Faculdade de Medicina de Ribeirão Preto – USP. Orientadora: Dra. Aparecida Maria Fontes
- Larissa D N Figueredo - **Papel dos microRNAs humanos na infecção pelo HTLV-1**. Faculdade de Medicina de Ribeirão Preto. Orientadora: Simone Kashima Haddad
- Tathiane Maistro Malta - **Análise do Perfil de Expressão Gênica em Linfócitos T CD8+ isolados de pacientes infectados pelo HTLV-1**. Faculdade de Medicina de Ribeirão Preto. Orientadora: Simone Kashima Haddad
- Bruno Marcos Verbeno Azavedo. **Avaliação do potencial vasculogênico de células progenitoras endoteliais**. Faculdade de Medicina de Ribeirão Preto USP. Orientador: Prof. Dr. Dimas Tadeu Covas.
- Lucila Habib B Oliveira. **Análise da expressão gênica das células AC 133+ isoladas a partir do sangue de cordão umbilical**. Faculdade de Medicina de Ribeirão Preto USP. Orientador: Prof. Dr. Dimas Tadeu Covas.
- Ana Valéria Gouvêa de Andrade. **Análise da Expressão do SDF-1 e do CXCR4 em células progenitoras mesenquimais fetais e avaliação do efeito das 3 isoformas do TGF-Beta na expressão destes genes**. Faculdade de Medicina de Ribeirão Preto USP. Orientador: Prof. Dr. Dimas Tadeu Covas.

- Carolina Caliarí Oliveira. **Potencial terapêutico das células tronco mesenquimais na regeneração de feridas ocasionadas por queimaduras em ratos.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Julio Cesar Voltarelli
- Gabriela Trentin Scortegaqua. **Análise da expressão de genes relacionados às células Treguladoras, Th17, Th1 e Th2 em pacientes com doenças auto-imunes submetidos ao transplante autólogo de células tronco hematopoéticas.** Faculdade de Medicina de Ribeirão Preto-USP, Fundação de Amparo à Pesquisa do Estado de São Paulo. Orientador: Julio Cesar Voltarelli
- Juliana Navarro Ueda Yaochite. **Infusão de células mesenquimais estromais como tratamento de doenças diabete auto-imune experimental.** Faculdade de Medicina de Ribeirão Preto-USP, Fundação de Amparo à Pesquisa do Estado de São Paulo. Orientador: Julio Cesar Voltarelli
- Lara Zimmermann. **Impacto da hipóxia na expressão gênica, alterações pós-traducionais e atividade pró-migratória de galectina-3.** Fundação Antônio Prudente, Fundação de Amparo à Pesquisa do Estado de São Paulo. Orientador: Roger Chammas
- Renata de Freitas Saito. **Quimiossensibilização e resposta de proteínas mal-enoveladas.** Fundação Antônio Prudente, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. Orientador: Roger Chammas
- Tharcisio Citrangulo Tortelli Jr. **Proibitina e seu papel na quimiorresistência de melanomas humanos.** Universidade de São Paulo. Orientador: Roger Chammas

## Doctorate (PhD)

- Francisco de Paula Careta. **Diferenças de atividades proliferativa e apoptótica entre células do sangue periférico e da medula óssea de pacientes com Leucemia Linfóide Crônica.** Orientador: Prof. Dr. Marco Antonio Zago
- Felipe Saldanha de Araujo. **A Imunomodulação dos Linfócitos T CD4 e CD8 Mediada pelas Células Tronco Mesenquimais.** Faculdade de Medicina de Ribeirão Preto USP. Orientador: Prof. Dr. Marco Antonio Zago.
- Dalila Zanete. **Influência da metilação e da acetilação na expressão gênica de células progenitoras hematopoéticas.** Orientador: Prof. Dr. Marco Antonio Zago
- Gisele Guiçardi Tomazella. **Identificação dos ligantes de galectina-3 a partir de células dendríticas e seus monócitos precursores e neutrófilos humanos.** Universidade Federal de São Paulo - Escola Paulista de Medicina. Orientador: Prof. Dr. Lewis Joel Greene
- Ana Cristina D'Ávila de Castro. **Análise funcional e perfil proteômico . Efeito de diferentes estímulos sobre a diferenciação das células dendríticas plasmocitóides a partir de células CD34+ de sangue de cordão umbilical humano.** Universidade Federal de São Paulo-Escola Paulista de Medicina. Orientador: Prof. Dr. Lewis Joel Greene
- Hélen Cristina de Miranda. **Comparação da expressão gênica de células tronco mesenquimais obtidas de veia de cordão umbilical e medula óssea** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Prof. Dr. Lewis Joel Greene

- Alana Maria Cerqueira de Catalán. **Identificação de genes alvos do miR-155, por transcriptoma e na análise proteômica em células de linfoma humano.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Prof. Dr. Lewis Joel Greene
- Glauce Gaspar Gomes. **Modificação na expressão de proteínas e mRNA das células tronco mesenquimais STRO-1+ durante a diferenciação osteogênica e senescência.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Prof. Dr. Lewis Joel Greene
- Carolina Hassibe Thomé. **Avaliação dos mecanismos de ação de um alquilfosfolípídeo em células de leucemia mielóide aguda.** Universidade Federal de São Paulo-Escola Paulista de Medicina. Orientador: Prof. Dr. Lewis Joel Greene
- Andrielle de Castilho Fernandes. **Transplante de células hepáticas estreladas produtoras de fator IX da coagulação sanguínea humana em camundongos NOD/scid.** Faculdade de Medicina de Ribeirão Preto – USP. Orientador: Dr. Dimas Tadeu Covas
- Fernanda Bernadelli Garcia. **Análise da distribuição populacional de polimorfismos: Gene da Granzima B (exons 2, 3 e 5) e Região Promotora do Gene da Perforina.** Faculdade de Medicina de Ribeirão Preto – USP. Orientador: Dr. Dimas Tadeu Covas
- Bruno Catrim Trindade. **Papel de Leucotrienos na infecção por HTLV-1.** Faculdade de Medicina de Ribeirão Preto – USP. Orientador: Dr. Dimas Tadeu Covas
- Karen de Lima Prata. **Isolamento, clonagem e avaliação da expressão gênica diferencial de células-tronco com fenótipo "side population" do sangue de cordão umbilical e placentário humano.** Faculdade de Medicina de Ribeirão Preto USP. Orientador: Dr. Dimas Tadeu Covas
- Jorge Luiz C. Siufi. **Avaliação do potencial terapêutico das células tronco mesenquimais no tratamento da GVHD experimental.** Faculdade de Medicina de Ribeirão Preto USP. Orientador: Dr. Dimas Tadeu Covas
- Maria Fernanda C. Amarante. **Isolamento, caracterização, clonagem e expressão dos genes de fatores de crescimento celular a partir de um banco de cDNA de fígado fetal.** Faculdade de Medicina de Ribeirão Preto USP. Orientador: Dr. Dimas Tadeu Covas
- Ana Paula Costa Nunes da Cozac. **Estudo do potencial antigênico relativo dos antígenos de grupos sanguíneos menores em pacientes em esquema de transfusão da região de Ribeirão Preto.** Faculdade de Medicina de Ribeirão Preto USP. Orientador: Dr. Dimas Tadeu Covas
- Flora C Lobo Penteadó. **Clonagem e Expressão das Glicoproteínas do Envelope do Retrovírus HTLV em Sistemas Procaríoto e Eucarioto.** Faculdade de Medicina de Ribeirão Preto USP. Orientador: Dr. Dimas Tadeu Covas
- Vivian Youssef Khouri. **Uso do laser terapêutico para tratamento de mucosite e GVHD orais: estudo randomizado controlado.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Julio César Voltarelli
- Alessandra de Paula Souza. **Influência do transplante de medula óssea autóloga na expressão gênica diferencial no sangue periférico de pacientes com esclerose múltipla.** Faculdade de Medicina de Ribeirão Preto-USP, Fundação de Amparo à Pesquisa do Estado de São Paulo. Orientador: Julio César Voltarelli

- Maria Carolina Oliveira Rodrigues. **Transplante de células tronco mesenquimais no diabetes melito do tipo 1- Estudo clínico e experimental.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Julio César Voltarelli
- Márcia Pinho. **Associação entre doença periodontal e artrite reumatóide.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Julio César Voltarelli
- Dannielle F. Godoi. **Modelo experimental de transplante de células tronco hematopoéticas em doença inflamatória intestinal.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Julio César Voltarelli
- Elisa Vendramini Nogueira. **Transferência de atopia cutânea e pulmonar no transplante alogênico de medula óssea.** Faculdade de Medicina de Ribeirão Preto-USP. Orientador: Julio César Voltarelli
- Rafael H Jacomo. **Estudo do papel da anexina II na ativação da coagulação na leucemia promielocítica aguda.** Faculdade de Medicina de Ribeirão Preto - USP. Orientador: Eduardo Magalhães Rego
- Luciana C Oliveira Oliveira. **Estudo do papel das células progenitoras do endotélio na gênese e progressão do mieloma múltiplo.** Faculdade de Medicina de Ribeirão Preto - USP. Orientador: Eduardo Magalhães Rego
- Mirela de Barros Tamarozzi. **Análise da fisiopatologia da lesão pulmonar aguda relacionada a transfusão (TRALI) em um modelo murino.** Faculdade de Medicina de Ribeirão Preto - USP, Fundação de Amparo à Pesquisa do Estado de São Paulo. Orientador: Eduardo Magalhães Rego
- Priscila Santos Scheucher. **Estudo do efeito da propólis e do seu derivativo CAPE na leucemia mielóide aguda.** Faculdade de Medicina de Ribeirão Preto - USP. Orientador: Eduardo Magalhães Rego
- Maria Carolina Tostes Pintão. **Análise da ativação da cascata de coagulação no modelo transgênico hCG-PML/RARalfa.** Faculdade de Medicina de Ribeirão Preto - USP. Orientador: Eduardo Magalhães Rego
- Hamilton Luiz Gimenes Teixeira. **Análise da influência do C/EBPalfa na expressão do microRNA223 em células da leucemia promielocítica aguda.** Faculdade de Medicina de Ribeirão Preto - USP, Conselho Nacional de Desenvolvimento Científico e Tecnológico. Orientador: Eduardo Magalhães Rego
- Guilherme Augusto Silva dos Santos. **Estudo da atividade antileucêmica da rapamicina no modelo de xenotransplante de leucemia mielóide aguda.** Faculdade de Medicina de Ribeirão Preto - USP. Orientador: Eduardo Magalhães Rego
- Lorena Lobo Figueiredo. **Análise da interferência do TGFbeta na leucemia promielocítica aguda induzida pelo gene de fusão PML-RARalfa.** Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Fundação de Amparo à Pesquisa do Estado de São Paulo. Orientador: Eduardo Magalhães Rego
- Patrícia Luisa Nunes da Costa. **Fatores microambientais no desenvolvimento do melanoma: implicações terapêuticas.** Fundação Antônio Prudente, Fundação de Amparo à Pesquisa do Estado de São Paulo. (Orientador). Roger Chammas

## Postdoctoral

- Barbara Amélia Santana Lemos. **Estudo dos mecanismos epigenéticos reguladores da expressão do gene C/EBP $\alpha$  na leucemia promielolítica aguda.** Supervisor: Prof. Dr. Eduardo Magalhães Rego
- Alexandre Krause. **O papel da disquerina na diferenciação das células hematopoéticas.** Supervisor: Prof. Dr. Eduardo Magalhães Rego
- Fábio Morato de Oliveira. **Ferramentas de citogenômica aplicadas à investigação da instabilidade cromossômica na leucemia linfocítica crônica (LLC)**". Supervisor: Prof. Dr. Roberto Passeto Falcão
- Idalete da Silva. **Estudo do efeito de diferentes estímulos na maturação funcional e perfil proteômico de células dendríticas preparadas a partir de monócitos de sangue periférico humano.** Faculdade de Medicina de Ribeirão Preto-USP. Supervisor: Prof. Dr. Lewis Joel Greene
- Simone Kashima Haddad. **Perfil quantitativo da expressão de microRNAs em populações de linfócitos T CD8+ de pacientes infectados pelo HTLV-1.** Supervisor: Prof. Dr Dimas Tadeu Covas
- Kamilla Swiech. **Desenvolvimento de um Bioprocesso de Produção do Fator VIII em Biorreator Utilizando Células Humanas em Suspensão: Escala Laboratorial.** Supervisor: Prof. Dr Dimas Tadeu Covas
- Kelen Cristina Malmegrim de Farias. **Características biológicas e genéticas de células tronco hematopoéticas e mesenquimais de pacientes submetidos a transplante autólogo de células tronco hematopoéticas para doenças auto-imunes.** Supervisor: Prof. Dr. Julio César Voltarelli

## **5.b) International Advisory Board**

We selected the following Advisory Committee:

- Bob Löwenberg, Professor of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands
- Graça Almeida-Porada, Department of Animal Biotechnology, University of Nevada, Reno, USA
- Manoel Barral Netto, Professor Titular da Universidade Federal da Bahia e do Instituto Oswaldo Cruz, Salvador (BA)
- Rafael Linden, Professor Titular do Instituto de Biofísica da Universidade Federal do Rio de Janeiro (RJ)

The four researchers, two foreigners and two Brazilians, cover most of the aspects included in the Center's research program, especially: stem cell isolation and culture, animal models to study stem cell properties, stem cell differentiation, immunology of diseases and of transplantation, the molecular basis of cancer, diagnostic and therapeutic approaches to hematological malignancies.

We organized two visits for the members of the committee:

1-2 December, 2006: Manoel Barral-Netto and Graça Almeida-Porada

20-21 March, 2007: Bob Löwenberg and Rafael Linden

The schedule of the first visit and the written report is appended (it is written in Portuguese because Prof. Graça, although working in the USA for 15 years is from Portuguese origin). Additionally, we have asked the two Brazilian researchers (Prof. Linden and Prof. Barral) to evaluate and send written report about the research proposal and curriculum of candidates to post-doctoral fellowship candidates. Our personal evaluation of the first committee visit (December 1 and 2): Very productive. It was a good opportunity to expose ideas and practical proposals to experienced researchers who felt at easy to discuss and analyze in depth our activities. Prof. Graça works actively in subjects very similar to those in which we focus at the moment; she came to Ribeirão Preto one day earlier, so she spent one day visiting the laboratories and discussing on-going research with many of the senior investigators. Their report is appended. However, there was one comment, supported by both visitors, which should be transmitted to FAPESP. When asked if they thought that the investment of FAPESP was worth in view of the center's output, they answered that considering the characteristics of the CEPID project, it should be measured by international standards, by which 7 principal investigators would receive 1.7-3.5 million dollars annually (each senior investigator would receive grants in the order of 250-500 thousand dollars annually).

## 6 ) Publication List

### 6.a) Articles in International Journals

1. Castro FA, Palma PV, Morais FR, Voltarelli JC. Immunological effects of donor lymphocyte infusion in patients with chronic myelogenous leukemia relapsing after bone marrow transplantation. *Braz J Med Biol Res.* 2004;37:201-206. Epub 2004 Jan 2030.
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## 6.b) Articles in Conference Proceedings

1. Coutinho MA, Orellana MD, Silva ARL, Palma PVB, Carrara RCV, Fontes AM, and Covas DT. "Estabelecimento de cultura primária de HIV-1 em linhagens celulares". XVIII Congresso Nacional do Colégio Brasileiro de Hematologia – HEMATO 2001. Fortaleza/CE, setembro/2001.
2. Orellana MD, Silva ARL, Kashima S, Fontes AM and Covas DT. "Estabelecimento de cultura primária de HIV-1 em linhagens celulares". XVIII Congresso Nacional do Colégio Brasileiro de Hematologia – HEMATO 2001. Fortaleza/CE, setembro/2001.
3. Pereira SR, Gomes GG, Siufi JLC, Silva ARL, Orellana MD, Fontes AM, Faça VM, Covas DT, Greene LJ "Isolamento de células precursoras, monócitos e extração de proteínas para análise proteômica da diferenciação de células dendríticas". XVIII Congresso Nacional do Colégio Brasileiro de Hematologia – HEMATO 2001. Fortaleza/CE, setembro/2001.
4. Soussumi LMT, Biase RR, Valente WV, Fontes AM, Covas DT. "Sorologia de triagem para doença de Chagas em doadores de sangue da região norte e noroeste do estado de São Paulo". XVIII Congresso Nacional do Colégio Brasileiro de Hematologia – HEMATO 2001 Fortaleza/CE. Setembro/2001.
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6. Kuramoto ACK, Biagi PM, Palma PVB, Orellana MD, Fontes AM, Covas DT "Cloning of human immunodeficiency virus type 1 p17 matrix protein in mammalian expression vector". 25<sup>o</sup> Congresso Brasileiro de Hematologia e Hemoterapia e do 1<sup>o</sup> Congresso Brasileiro de Hematologia Pediátrica. Salvador - Bahia, maio 2002.
7. TTV genotypes among Brazilian blood donors
8. Apresentação oral no 8th European Workshop on Virus Evolution and Molecular Epidemiology – Leuven – Bélgica no período de 04 a 11 de setembro de 2002.
9. HTLV-I subtypes in the southeast region of Brazil.
10. Kashima S, Hachiya EM, Takayanagui OM, Alcântara LCJ, Galvão-Castro B, Pombo de Oliveira MS, Covas DT.
11. Apresentação em forma de poster no VII Simpósio Internacional sobre HTLV – Belém – período de 18 a 21 de agosto de 2002
12. In vitro infection of cell lineages with the Human T-cell Lymphotropic virus type I . Orellana MD, Silva ARL, Kashima S, Hachiya EM, Takayanagui OM, Covas DT.
13. Apresentação oral no VII Simpósio Internacional sobre HTLV – Belém – período de 18 a 21 de agosto de 2002
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20. *Aplicação de Ferramentas Computacionais para Análise de Genoma*
21. *Palestra ministrada na disciplina de pós-graduação RBQ-5788-Tópicos em Bioinformática, no Departamento de Bioquímica e Imunologia da Faculdade de Medicina de Ribeirão Preto – USP, em 30 de outubro de 2001.*
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### *6.c) Presentation in Conferences*

Zago MA – Stem cells from bone marrow and from other tissues: are they alike? International Symposium on Biological Cardiac Repair: a Critical Appraisal. Instituto do Coração (InCor), São Paulo, 18/09/2006

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Covas DT - Seminário sobre o tema “Células-Tronco”, realizado na Faculdade de Medicina de Ribeirão Preto, em 11 de maio de 2006.

Covas DT - Palestra proferida durante o II Seminário sobre Rotas Tecnológicas da Biotecnologia: Oportunidades de Investimentos e Inovações, realizado em Ribeirão Preto de 01 a 02 de junho de 2006.

Covas DT - Palestra sobre o tema “Aplicações Práticas das Pesquisas com Células-Tronco”, durante a XV Jornada do Departamento de Medicina da Santa Casa de São Paulo “Avanços no Diagnóstico e Tratamento em Clínica Médica”, em São Paulo no dia 11 de agosto de 2006.

Covas DT - Conferência da Mesa Redonda Inovação e Tecnologia, sobre o tema “Em Busca do Desenvolvimento de Tecnologia Nacional para Produção de Concentrado de Fatores VIII e IX de Origem Recombinante”, durante o I Simpósio de Hemostasia e Trombose, em Uberaba, de 21 a 23 de agosto de 2006.

Covas DT - Conferência sobre o tema “Terapia Celular” durante a Jornada Brasileira de Hemoterapia, realizada em São Paulo no dia 14 de outubro de 2006.

Chammas R - Melanomas, no ciclo de conferências do COPEA-UFRJ, em 18 de maio de 2006.

Chammas R - The double face of cell death within tumor microenvironments, no "6th International Cell Death Symposium", em 5 de junho de 2006, em Angra dos Reis, Rio de Janeiro.

Chammas R - Polimorfismos de genes de reparo de DNA e risco de melanoma maligno, no "I Simpósio Internacional de Pesquisa em Câncer: integrando pesquisa básica, clínica e epidemiológica", em 1 de dezembro de 2006, em São Paulo.

Falcao RP - NK and T-Cell Lymphomas, I Tutorial de Neoplasias Malignas: Foco em Neoplasias Linfóides, Escola Brasileira de Hematologia, Angra dos Reis, 6 /5/ 2006.

Voltarelli JC - Brazilian experience with BEAM for hematopoietic stem cell transplantation for multiple sclerosis. MIST trial meeting, Salt Lake City, UT, USA, 2/April/2005.

Voltarelli JC - Hematopoietic stem cell transplantation for early onset diabetes mellitus. Diabetes Research Institute, University of Miami, FL, USA, 21/April/2006.

Voltarelli JC - Hematopoietic stem cell transplantation for diabetes mellitus, Genzyme meeting, San Francisco, CA, 8/June/2006.

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## 6.d) Book chapters

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## 7. Other support

Supported by:	Project Title	Coordinator	Period	Identification	Total Amount (R\$)
FINEP	CLONAGEM E EXPRESSÃO DOS FATORES VIII E IX DE COAGULAÇÃO SANGÜÍNEA HUMANA EM CÉLULAS DE MAMÍFEROS	PROF. DR. DIMAS TADEU COVAS	20/12/2004 a 20/05/2008	01.04.0962.00	809.000,00
FINEP	INFRA ESTRUTURA BÁSICA PARA PESQUISA NO BIOCENTRO EM TERAPIA CELULAR E MOLECULAR	PROF. DR. DIMAS TADEU COVAS	14/04/2003 a 03/04/2007	01.03.0029.00	300.000,00
FINEP	PRODUÇÃO DE FATOR VIII DA COAGULAÇÃO POR TECNOLOGIA DE DNA RECOMBINANTE	PROF. DR. DIMAS TADEU COVAS	11/01/2001 a 30/12/2004	64.00.0487.00	1.040.000,00
FINEP	CLONAGEM E EXPRESSÃO DOS FATORES VIII E IX DE COAGULAÇÃO SANGÜÍNEA HUMANA EM CÉLULAS DE MAMÍFEROS	PROF. DR. DIMAS TADEU COVAS	21/11/2005 a 21/11/2008	01.05.0691.00	370.000,00
FINEP	CONSOLIDAÇÃO DO LABORATÓRIO DE CULTURA CELULAR PARA ESTUDOS MOLECULARES E PROTEÔMICOS DE CÉLULAS DENDRÍTICAS E CÉLULAS TRONCO	PROF. DR. DIMAS TADEU COVAS	26/08/2005 a 26/08/2008	01.05.0466.00	522.082,00
FINEP	ESCALONAMENTO DA PRODUÇÃO DOS FATORES VII E IX RECOMBINANTES EM BIORREACTORES E ENSAIOS PRÉ-CLÍNICOS EM CAMUNDONGOS HEMOFÍLICOS	PROF. DR. DIMAS TADEU COVAS	19/12/2007 A 19/12/2009	01.07.0652.00	2.384.565,82
FINEP	ANÁLISE BIOLÓGICA E MOLECULAR DE CÉLULAS TRONCO SOMÁTICAS PLURIPOTENCIAIS NO REPARO TECIDUAL	PROF. DR. DIMAS TADEU COVAS	04/12/2007 A 04/12/2009	01.07.0581.00	434.050,00
FINEP	INFRA-ESTRUTURA PARA GERAÇÃO E MANIPULAÇÃO DE MODELOS ANIMAIS GENETICAMENTE MODIFICADOS NO ESTUDO DE DOENÇAS HUMANAS	PROF. DR. MARCO ANTONIO ZAGO	25/09/2006 A 25/09/2008	01.06.0598.00	742.800,00
FINEP	PROTEÔMICA ESTRUTURAL E FUNCIONAL APLICADA À ÁREA BIOMÉDICA	PROF. DR. LEWIS JOEL GREENE	14/12/2007 A 14/12/2009	01.07.0595.00	720.000,00
FINEP	TRANSPLANTE DE CÉLULAS TRONCO HEMATOPOÉTICAS EM DOENÇAS AUTO-IMUNES	PROF. DR. JULIO CESAR VOLTARELLI	10/03/2004 a 04/03/2008	01.04.0042.00	424.484,88
CNPQ	BANCO DE CÉLULAS DE CORDÃO UMBILICAL: CARACTERÍSTICAS DAS CÉLULAS HEMATOPOÉTICAS PROGENITORAS DO CORDÃO UMBILICAL	PROF. DR. MARCO ANTONIO ZAGO	16/05/2001 a 30/06/2005	62.0019/99-9	202.000,00



CNPQ	ISOLAMENTO, CARACTERIZAÇÃO, CULTURA, EXPANSÃO E AVALIAÇÃO DO POTENCIAL VASCULOGÊNICO "IN VIVO" E "IN VITRO" DE CÉLULAS TRONCO PLURIPOTENCIAIS DO ADULTO COM CAPACIDADE DE DIFERENCIAÇÃO ENDOTELIAL	PROF. DR. MARCO ANTONIO ZAGO	11.011/2006 a 11/01/2008	552186/2005-8	399.527,78
CNPQ	TRANSPLANTE DE CÉLULAS TRONCO HEMATOPOÉTICAS PARA DIABETE MELITO TIPO I E DOENÇAS NEURO-DEGENERATIVAS	PROF. DR. JULIO CÉSAR VOLTARELLI	01/10/2005 a 01/10/2008	552266/2005-1	375.000,00
CNPQ	ISOLAMENTO, CARACTERIZAÇÃO, CULTURA, EXPANSÃO E AVALIAÇÃO DO POTENCIAL VASCULOGÊNICO "IN VIVO" E "IN VITRO" DE CÉLULAS TRONCO PLURIPOTENCIAIS DO ADULTO COM CAPACIDADE DE DIFERENCIAÇÃO ENDOTELIAL	PROF. DR. DIMAS TADEU COVAS	FEV/2008 A FEV/2010	558137/2008-3	54.158,80
CNPQ	ISOLAMENTO E CARACTERIZAÇÃO DOS FATORES DE CRESCIMENTO PARA DIFERENCIAÇÃO DE CÉLULAS TRONCO	PROF. DR. DIMAS TADEU COVAS	11/07/2005 a 11/07/2007	477650/2004-0	25.000,00
CNPQ	AVALIAÇÃO DA EXPRESSÃO GÊNICA DE CÉLULAS PROGENITORAS ENDOTELIAIS ISOLADAS A PARTIR DE SANGUE DE CORDÃO UMBILICAL	PROF. DR. DIMAS TADEU COVAS	26/10/2007 A 25/12/2009	480770/2007-7	56.000,00
CNPQ	PERFL QUANTITATIVO DA EXPRESSÃO DE MICRORNAS EM POPULAÇÕES DE LINFÓCITOS T DE PACIENTES INFECTADOS PELO HTLV-1	PROFA. DRA. SIMONE KASHIMA HADDAD	NOV/2006 A NOV/2008	475091/2006-0	38.600,00
CNPQ	APLICAÇÃO DA QUÍMICA DE PROTEÍNAS A PROBLEMAS BIOLÓGICOS E CLÍNICOS	PROF. DR. LEWIS JOEL GREENE	04/10/2000 a 30/10/2006	661132/1998-6	746.500,00
FAPESP	INICIAÇÃO CIENTÍFICA JÚNIOR	PROF. DR. DIMAS TADEU COVAS	01/04/2004 a 30/08/2005	03/11.814-7	4.480,00
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VITAE	CONSOLIDAÇÃO DO MuLEC – Museu e Laboratório de Ensino de Ciências	PROF. DR. DIMAS TADEU COVAS	De maio a novembro/2005	B-13694/1	243.241,00

## 8. Webpage - Regional Blood Center



**HEMOCENTRO DE RIBEIRÃO PRETO**  
Centro Regional de Hemoterapia do HCFMRP-USP  
*Tecnologia e Pesquisa a serviço da vida*

Apresentação
Assistência
Canal do Doador
Ensino
Fundação de Apoio
Links
Legislação
Pesquisas Científicas

Segunda-feira, 28 de Abril de 2008

Início
 S.A.C. / Ouvidoria
 Webmail
 Intranet
 Mapa

Dê vida de presente!  
**Doe sangue! ✓**

**- Editais e Licitações**

**- Processos Seletivos**

**- Biblioteca**

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**MULTIMÍDIA** Ver Todos



**Não deixe para amanhã...**

◆ Terapia Celular no HCRP.  
◆ Ministério Público afirma que "sistemas administrativos, financeiro e contábil do Hemocentro são excelentes".

**NOTÍCIAS** Ver Todas

- ▶ 24/04/2008 Xuxa doa sangue para ajudar vítimas da dengue no Rio
- ▶ 15/04/2008 Campanha Não deixe para amanhã o que se pode fazer hoje: Salve uma Vida, Doe Sangue
- ▶ 18/04/2008 Programa Biblioteca da FUNDHERP

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- ◆ Curso de Hemoterapia Aplicada
- ◆ Setembro - Tutorial Linfóides



Pesquisadores da USP de Ribeirão Preto localizam células-tronco adultas na parede dos vasos sanguíneos. **Leia mais...**

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- ◆ Colégio Brasileiro de Hematologia

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- ◆ Guia do doador
- ◆ Agende sua doação de sangue
- ◆ Datas e locais das próximas coletas externas
- ◆ Perguntas frequentes

**PESQUISA**

- ◆ Introdução
- ◆ Artigos
- ◆ Produção
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## ENSINO



O Centro Regional de Hemoterapia desenvolve extenso e permanente programa educacional que inclui o treinamento e desenvolvimento de profissionais médicos, enfermeiros, biólogos, fisioterapeutas e técnicos de laboratório. Estas atividades são estendidas a especialistas e pesquisadores da área, dentre eles alunos de pós-graduação, residentes em hematologia e hemoterapia, alunos de graduação.

Destaca-se também os cursos e treinamentos e desenvolvimento oferecidos aos profissionais de ciência do ensino médio e fundamental da região de Ribeirão Preto.

Oferece de forma sistemática e permanente cursos para a formação de especialistas em biologia molecular, em nível de mestrado e doutorado.

Apoia a Escola Brasileira de Hematologia desde 1992, com o objetivo de promover a educação continuada de médicos nas áreas de hematologia e hemoterapia, trazendo benefícios não só na experiência científica, mas especialmente na prática clínica.



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**O CTC abre inscrições para Pós-doutorado no Hemocentro.**


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**Células Tronco e Diabetes**  
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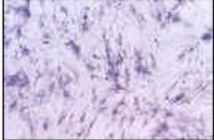



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