CTC

Centro de Terapia Celular

Center for Cell-Based Therapy

RELATÓRIO CIENTÍFICO
PROCESSO Nº 98/14247-6

Universidade de São Paulo

Hemocentro de Ribeirão Preto

2002

Junior Investigators

Name	Institution	Subproject
Maria Cristina Ramos Costa	FUNDHERP – FAPESP	▪ Identification of single base polymorphisms (SNPs) in the coding regions of genes expressed in tumor cells using the HCGP-ORESTES data bank. ▪ Identification of genes differentially expressed in dendritic and precursor CD34+ cells of human umbilical cord blood.
Rita de Cássia Viu Carrara	FUNDHERP - FAPESP	▪ Identification of genes differentially expressed in CD34+ BCR-ABL+ cells of patients with chronic myeloid anemia.
José César Rosa	FMRP/USP	▪ 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.
Clarice Izumi	FMRP/USP	▪ 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	▪ Analysis of differential gene expression in cells treated *in vitro* with antitumoral drugs.
José Augusto Baranauskas	FMRP/USP	▪ Genome data mining.

PhD Students

Name	Institution	Adviser
Adriano J Holanda	FUNDHERP – CAPES	Wilson Araújo da Silva Júnior
Ana Silvia Gouveia	FMRP/USP	Eduardo Magalhães Rego
Andrea Aparecida Garcia	FMRP/USP – CNPq	Rendrik França Franco
Carolina Boschi Cabral	FMRP/USP – FAPESP	Lewis Joel Greene
Eduardo Ramaciotti	FUNDHERP	Rendrik França Franco
Gustavo Antonio de Souza	FMRP/USP – FAPESP	Lewis Joel Greene
José Sebastião Ismael	HCRP/USP	Roberto Passeto Falção
Kiyoko Abe Sandes	FMRP/USP – CAPES	Marco Antonio Zago
Lyris Martins Franco de Godoy	FMRP/USP – FAPESP	Lewis Joel Greene
Maria Giziani Fagundes	FAPESP	Marco Antonio Zago
Sandra Rodrigues Pereira	FMRP/USP - FAPESP	Lewis Joel Greene
Simone Kashima Haddad	FUNDHERP	Dimas Tadeu Covas
Vitor Marcel Faça	FMRP/USP – FAPESP	Lewis Joel Greene
Greice A Molfetta	FMRP/USP – FAPESP	Marco Antonio Zago
Kelson Roberto Kodama	FMRP/USP - CAPES	Wilson Araújo da Silva Júnior
Rodrigo Alexandre Panepucci	FMRP/USP – FAPESP	Marco Antonio Zago
Rodrigo Tocatins Calado S Rodrigues	FMRP/USP-FAPESP	Roberto Passetto Falcão
Barbara Amélia Santana	FMRP/USP-FAPESP	Eduardo Magalhães Rego
Gil de Santis	FUNDHERP	Eduardo Magalhães Rego

FUNDHERP	Fundação Hemocentro de Ribeirão Preto
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
HCRP/USP	Hospital das Clínicas de Ribeirão Preto / Universidade de São Paulo
FMRP/USP	Faculdade de Medicina de Ribeirão Preto / Universidade de São Paulo
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.
CEPID	Centro de Pesquisa Inovação e Difusão.

CNPq Conselho Nacional de Desenvolvimento Cientifico e Tecnológico.

Principal Investigators

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

Aparecida Maria Fontes	**FUNDHERP**	**Subproject Coordinator:** ▪ Cancer Vaccine for Chronic Myeloid Leukemia. ▪ Cloning and Expression of HTLV-1 structural genes in mammalian cells ▪ Recombinant antigen p24 of HIV-1 expressed in mammalian cells ▪ Cloning and Expression of recombinant human coagulation factor IX in mammalian cells
Dimas Tadeu Covas	**FUNDHERP**	**Subproject Coordinator:** ▪ Drug therapy approach to purging Ph^- progenitor cells from chronic myelogenous leukemia patients. ▪ Profiling changes in TPO expression level and CD34 cell numbers in normal donors after plateletapheresis. ▪ Sequence characterization of an HTLV type I isolate from Ribeirão Preto. ▪ Molecular epidemiology of TT virus (TTV) and characterization of the TTV genotypes in Ribeirão Preto. ▪ Cloning and expression of recombinant human coagulation factor VIII in mammalian cells using retrovirus as a vector. ▪ Brazil Cord Blood Bank. ▪ Microchimerism in multitransfused patients. ▪ Phenotype-genotype relationship of the Duffy blood group in Caucasian, African-America Black and Asian populations.

Name	Institution	Position/Responsibility
Eduardo Magalhães Rego	FMRP/USPP	**Subproject Coordinator:** ▪ Study of oncogenesis induced by the NPM-RARα fusion protein. ▪ Analysis of differentiating and pro-apoptotic effects of Vitamin E isomers ▪ Analysis of the effects of Histone Deacetylases inhibitors on cell differentiation ▪ Analysis of cell cycle and apoptosis in myeloid progenitors of PML-RARα transgenic mouse model ▪ Study of the effect of histone deacetylase on gene transcription in acute promyelocytic leukemia cells. ▪ Analysis of cyclin A1 expression in acute promyelocytic leukemia: role of histones deacetylases. ▪ Use of transgenic PML-RARα animals in models of activation of coagulation and inflammation.
Júlio César Voltarelli	FMRP/USP	**Subproject Coordinator:** ▪ Immunological mechanisms involved in the therapy of chronic myelogenous leukemia with alpha-interferon. ▪ Monitoring of intracellular cytokines in lymphocytes and monocytes post-bone marrow transplantation. ▪ Comparison of quantitative methods for detection and monitoring of cytomegalovirus infection in immunocompromised patients. ▪ Hematopoietic stem cell transplantation for autoimmune diseases.
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..
Marisa Ramos Barbieri	FUNDHERP	**Subproject Coordinator:** ▪ The cells, the genome and you.

Name	Institution	Position/Responsibility
Marco Antonio Zago	FUNDHERP	**Subproject Coordinator:** ▪ Identification of Single Nucleotide Polymorphism in the coding region of genes expressed in cellular tumors utilizing Data Banks. ▪ Expression of tumor-specific antigens in lymphoid neoplasias. ▪ Gene expression in hematopoietic lineages and neoplasias. ▪ The relationship of single nucleotide polymorphisms with susceptiblity, clinical evolution and treatment response of hematological neoplasias.
Rendrik França Franco	FUNDHERP	**Subproject Coordinator:** ▪ Clinical and laboratory characterization of Von Willebrand's disease: molecular basis of Von Willebrand's disease. ▪ Genetic factors and thrombotic risk in patients with neoplastic diseases.
Roberto Passetto Falcão	FMRP/USP	**Subproject Coordinator:** ▪ Evaluation of glycoprotein P activity in precursor hematopoietic cells in acquired aplastic anemia and in myelodysplastic syndrome ▪ Comparison of the hypergranular forms of acute promyelocytic anemia.
Wilson Araújo da Silva Júnior	FUNDHERP/UFPA	**Subproject Coordinator:** ▪ Initiative to validate of the human transciptoma. ▪ Analysis of the sequences generated by the Human Genome of Cancer project. ▪ Characterization of leukemia transcripts in the 5q31 region. ▪ Clinical Genomics Project – Bioinformatics Laboratory. ▪ Genome Data Mining. ▪ Laboratório Associado de Bioinformática no Programa Humano do Câncer - CompBioNet

Senior Investigators

Name	Institution	Subproject
Evamberto Garcia de Góes	FUNDHERP - FAPESP	▪ Use of Telecobalt therapy for the prevention of graft versus host disease asociated with transfusion: dosimetry and quality control of irradiated blood ▪ Effects of diagnostic X-ray dose on peripheral blood mononuclear cells
Luciano Fontoura	UFSCAR	▪ Analysis and statistics applied to micro-arrays.
Míriam Lane de Oliveira Rodrigues Castilho	FMRP/USP	▪ Immunotherapy of melanoma.
Roger Chammas	FMRP/USP	▪ Immunotherapy of melanoma ▪ Expression of De-N-acetyl-GD3 Ganglioside in normal and neoplastic lymphoid cells.
Vanderson Rocha	EUROCORD	▪ 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).

B. RESULTS OBTAINED IN BASIC RESEARC

B.1 - Publications

B.1.1 Morelli VM, Lourenço DM, D'Almeida V, Franco RF, Miranda F Zago MA, Noguti MA, Cruz E, Kerbauay J. Hyperhomocysteinemia increases the risk of venous thrombosis independent of the C677T mutation of the methylenetetrahydrofolate reductase gene in selected Brazilian patients. Blood Coagul Fibrinolysis. 2002:271-275. *Enclosure*

B.1.2 Franco RF, Simões BP, Tone LG Gabellini SM, Zago MA, Falcão RP.The methylenetetrahydrofolate reductase C677T gene polymorphism decreases the risk of childhood acute lymphocytic leukaemia. Br J Haematol. 2001.115:616-8. *Enclosure*

B.1.3 Shindo N, Alcantara LC, Van Dooren S, Salemi M, Costa MC, Kashima S, Covas DT, Teva A, Pellegrini M, Brito I, Vandamme AM, Galvao-Castro B. Human retroviruses (HIV and HTLV) in Brazilian Indians: seroepidemiological study and molecular epidemiology of HTLV type 2 isolates. AIDS Res Hum Retroviruses. 2002. 1;18:71-7. *Enclosure*

B.1.4 Basso LR, Vasconcelos C, Fontes AM, Hartfelder K, Silva JA, Coelho PS, Monesi N, Paco-Larson ML. The induction of DNA puff BhC4-1 gene is a late response to the increase in 20-hydroxyecdysone titers in last instar dipteran larvae. Mech Dev. 2002; 110:15-26. *Enclosure*

B.1.5 Camargo AA, Samaia HP, Dias-Neto E, Simao DF, Migotto IA, Briones MR, Costa FF, Nagai MA, Verjovski-Almeida S, Zago MA, Andrade LE, Carrer H, El-Dorry HF, Espreafico EM, Habr-Gama A, Giannella-Neto D, Silva WA Jr, et al The contribution of 700,000 ORF sequence tags to the definition of the human transcriptome.Proc Natl Acad Sci U S A. 2001 9;98:12103-8. *Enclosure*

B.1.6 Scrideli CA, Kashima S, Cipolloti R, Defavery R, Bernardes JE, Tone LG. Minimal residual disease in Brazilian children with acute lymphoid leukemia: comparison of three detection methods by PCR. Leuk Res. 2002 ;26:431-8. *Enclosure*

B.1.7 Batista WC, Kashima S, Marques AC, Figueiredo LT. Phylogenetic analysis of Brazilian Flavivirus using nucleotide sequences of parts of NS5 gene and 3' non-coding regions. Virus Res. 2001, 75:35-42. *Enclosure*

B.1.8 Calado RT, Falcão RP, Garcia AB, Zago MA, Franco R. Influence of functional MDR1 gene polymorphisms on P-glycoprotein activity in CD34+ hematopoietic stem cells. Haematologica. 2002; v87:564-8. *Enclosure*

B.1.9 Calado RT, Franco R, Zago MA, Falcão RP. MDR1 gene C3435T polymorphism and the risk of acquired aplastic anaemia. British Journal of Haemathology. 2002; v117:769. *Enclosure*

B.1.10 Silva-JR WA, Bonatto SL, Ribeiro-dos-Santos AKC, Rodriguez-Delfin L, Paco-Larson ML, Petzl-Erler ML, Santos SEB, Zago MA. Mitochondrial genome diversity of native americans supports a single early entry of founder populations into America. American Journal of Human Genetics. 2002; v71:187-92. *Enclosure*

B.1.11 Gjetting T, Romstad A, Haavik J, Acosta AX, Silva-Jr WA, Zago MA, Guldberg P, Guttler F. A phenylalanine hydroxylase amino acid polymorphism with implications for melecular diagnostics. Molecular Genetics and Metabolism. 2001; v73: 280-4. *Enclosure*

B.1.12 Falcão RP, Rego EM. Leucemia linfoide aguda em adultos e crianças. Características morfologicas e imunofenotipicas. Serie de Monografias da Escola Brasileira de Hematologia. Brasil. 2002, v9:25-35. *Enclosure*

B.1.13 Rego EM, Falcão RP. Leucemia mieloide aguda - diagnostico, morfologia, imunofenotipo e citogenetica. Serie de Monografias da Escola Brasileira de Hematologia. Brasil. 2002; v.9:54-65. *Enclosure*

B.1.14 Rego EM. Mecanismos moleculares da leucemia promielocitica aguda. Serie de Monografias da Escola Brasileira de Hematologia. Brasil. 2002; v9:p15-24. *Enclosure*

B.1.15 Rego EM, Bernardi R, Grisendi S, Downing J, Shannon K, Kogan S, Pandolfi PP. Mouse models of human cancer: hematopoietic malignancies organ site. Mouse Models of Human Cancer Website, 2002. *Enclosure*

B.1.16 Rego EM, Pandolfi PP. Reciprocal products of chromosomal translocations in human cancer pathogenesis: key players or innocent bystanders?. Trends in Molecular Medicine. 2002; v8:396-405. *Enclosure*

B.1.17 Rego EM, Garcia AB, Falcão RP, Carneiro JJ. Immunophenotype of normal and leukemic bone marrow B-precursors in a Brazilian population. A comparative analysis by quantitative fluorescence cytometry. Brazilian Journal of Medical and Biological Research. Ribeirão Preto. 2001; v34:183-94. *Enclosure*

B.1.18 Januario AH, Filho ER, Pietro RC, Kashima S, Sato DN, Franca SC. Antimycobacterial physalins from Physalis angulata L. (Solanaceae). Phytother Res. 2002 Aug;16(5):445-448. *Enclosure*

B.1.19 Scrideli CA, Kashima S, Cipolloti R, Defavery R, Tone LG. Clonal evolution as the limiting factor in the detection of minimal residual disease by polymerase chain reaction in children in Brazil with acute lymphoid leukemia. J Pediatr Hematol Oncol. 2002 Jun-Jul;24(5):364-7. *Enclosure*

B.1.20 Ward RJ, Oliveira AHC, Bortoleto RK, Rosa JC, Faça VM, Greene LJ. Refolding and Purification of Bothropstoxin-I, a Lys49-Phospholipase A_2, Homologue, Expressed as Inclusion Bodies in *Escherichia coli.* Protein Expression and Purification. 2001; 21: 134-140. *Enclosure*

B.1.21 Lourenço EV, Pereira SR, Faça VM, Coelho-Castelo AAM, Mineo JR, Roque-Barreira MC, Greene LJ and Panunto-Castelo A. Toxoplasma gondii micronemal protein MICI is a lactose-binding lectin. Glycobiology. 2001;11(7):541-547. *Enclosure*

B.1.22 De Souza DA, Manço ARX, Marchesan WG and Greene LJ. Epidemiological data of patients hospitalized with burns and other traumas in some cities in the southeast of Brazil for 1991 to 1997. Burns 2002;28:107-114. *Enclosure*

B.1.23 Cabral CB, Imasato H, Rosa JC, Laure HJ, Paula da Silva, CHT, Tabak M, Garratt RC and Greene LJ. Fluorescence properties of tryptophan residues in the monomeric d-chain of Glossoscolex paulistus haemoglobin: an interpretation based on a comparative molecular model. Biophysical Chemistry. 2002; 97: 139-157. *Enclosure*

B.1.24 Andrião-Escarso SH, Soares AM, Fontes MRM, Fuly AL, Corrêa FMA, Rosa JC, Greene LJ and Giglio JR. Structural and functional characterization of an acidic platelet aggregation inhibitor and hypotensive phospholipase A2 from Bothrops jararacussu smnake venom. Biochemical Pharmacology. 2002;64: 723-732. *Enclosure*

B.1.25 Peitl P, SS, M., ML, C., GA, P., MP, H., RS, C., ES, S. Chromosomal rearrangements involving telomeric DNA sequences in Balb/3T3 cells transfected with the Há-ras oncogene. Mutagenesis. Oxford. 2002; v17. N1:67-72. *Enclosure*

B.1.26 RS, C., S, T., Peitl, P., T. G., ET, S. Evaluation of cromosomal aberrations, micronuclei, and sister chromatid exchanges in hospital workers chronically exposed to ionizing radiation. Teratogenesis Carcinogenesis and Mutagenesis. 2001;v21.n6:431-9 *Enclosure*

B.1.27 Zamaro PJA, Carvalho LV, Melo SMA, Silva Jr WA; Bonini-Domingos CRB. Hemoglobina fetal aumentada: um estudo familiar. Newslab (Brasil). 2002;52:90-96. *Enclosure*

B.1.28 Rizzatti EG, Garcia AB, Portieres FL, Silva DE, Martins SLR, Falcão RP. Expression of CD117 and CD11b in bone marrow can differentiate acute promyelocytic leukemia from recovering benign myeloid proliferation. 2002;118:31-37. *Enclosure*

B.1.29 Falcão RP, Garcia AB, Marques MG, Simões BP, Fonseca BA, Rodrigues ML, Foss NT. Blastic CD4 NK cell leukemia/lymphoma: a distinct clinical entity. Leukemia Research. 2002.26:803-807. *Enclosure*

B.1.30 Calado RT, Garcia AB, Gallo DAP, Falcão RP. Reduced function of the multidrug resistance P-glycoprotein in CD34+ cells of patients with aplastic anaemia. British Journal of Haematology. 2002; 118:320-6. *Enclosure*

B.1.31 *Voltarelli JC.* Transplante de células tronco hematopoéticas para doenças auto-imunes no Brasil. Revista Brasileira de Hematologia e Hemoterapia. 2002; 24(1):9-13. *Enclosure*

B.1.32 *Bizario JCS, Castro FA, Sousa JF, Fernandes RN, Damião AD, Oliveira MK, Palma PVB, Larson RE, Voltarelli JC, Espreafico EM*. Myosin-V colocalizes with MHC class II in blood mononuclear cells and is up-regulated by T-lymphocyte activation.. Journal of Leukocyte Biology. 2002; v71:195-204. *Enclosure*

B.1.33 *Souza SS, Ferriani RA, Santos CMP, Voltarelli JC*. Immunological evaluation of patients with recurrent abortion. Journal of Reproductive Immunology. 2002;56:111-121. *Enclosure.*

B.1.34 *Azevedo AM, Nucci M, Maiolino A, Vigorito AC, Simões BP, Aranha FJP, Tabak DG, Voltarelli JC, Souza CA.* Haemopoietic growth factors. A randomized, multicenter study of G-CSF starting on day +1 *vs* day +5 after autologous peripheral blood progenitor cell transplantation. Bone Marrow Transplantation. 2002; 29:745-751. *Enclosure*

B.1.35 *Ferraz AS, Saber LS, Voltarelli JC, Mytilineos J, Opelz G, Donadi EA.* Comparative study of HLA-DR typing by serology and sequence-specific primer analysis in a genetically highly diverse population of kidney transplant recipients. Transplatation Proceedings. 2002; 34:463-465. *Enclosure*

B.1.36 *Rizzatti EG, Zago MA.* Aplicação da Biologia Molecular às leucemias agudas. Série Monografias da Escola Brasileira de Hematologia. 2002; v9:1-14. *Enclosure*

B.2 - Books

B.2.1 Barbieri MR, Sicca NAL, Carvalho CP. A construção do conhecimento do professor – uma experiência de parceria entre professores do ensino fundamental e médio da Rede Pública e a universidade, editora Holos, Ribeirão Preto, 2001. *Enclosure*

B.2.2 Barbieri MR. Laboratório de Ensino de Ciências – 20 anos de história, editora Holos, Ribeirão Preto, 2002. *Enclosure*

B.3 – Papers in press

B.3.1 Franco RF. (2001) Reitsma, P. Review Article: Genetic Factors of Venous Thrombosis.. Human Genetics, *in press.*

B.3.2 Kashima S, Soares AM, Roberto PG, Pereira JO, Astolfi-Filho S, Contra AO, Fontes MRM, Giglio JR, França SC. cDNA sequence and molecular modeling of anerve growth factor from Bothrops jararacussu venom gland. Biochimie, 84 (2002), *in press*

B.3.3 Orellana MD, Fontes AM, Palma PVB, Morais FR, Gomes GG and Covas DT (2002). High yield of dendritic cells from healthy donors in short term culture. *in press*

B.3.4 Molfetta GA, Pina-Neto JM, Silva Jr WA. Clinical, cytogenetical and molecular analysis of Angelman syndrome. Genetic Counseling (*in press*) 2002.

B.3.5 Watanabe MAE, Milanezi CM, Silva Jr WA, Angulo IL, De Santis GC, Kashima S, da Costa JAC, Neto MM; Covas DT. Molecular investigation of GB virus C RNA in hemodialysis and thalassemics patient from Brazil. Renal Failure (*in press*), 2002.

B.3.6 Calado RT, Pintão MC, Silva-Jr WA, Falcão RP, Zago MA – Lack of association of acquired aplastic anaemia and mutations of the hTR telomerase gene. Lancet (letter), *in press.*

B.3.7 Molfetta GA, Félix TM, Riegel M, Ferraz VEF, Pina Neto JM. A Further case of a prader-willi syndrome phenotype in a patient with angelman syndrome molecular defect. Arq Neuropsiquiatr. 2002, *in press*.

B.3.8 Molfetta GA, SilvaJR WA, Pina Neto JM. Clinical cytogenetical and molecular analyses of angelman syndrome. Genet Counselling. 2002, *in press*.

B.3.9 Molfetta GA, Hojas MVM, Silva JR WA, Pina-Neto JM. Characterization of a novel mutation causing angelman syndrome: a familial dupGAGG. Am J Med Genet. 2002, *in press*

B.3.10 Rego EM. Molecular Basis of Acute Myelogenous Leukemia, *in press*

B.3.11 Rocha V, Franco RF, Porcher R, Bittencourt H, Silva Jr WA, Dvergie A, et al. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplant. Blood 2002. First Edition Paper, prepublished online July 25, 2002; DOI 10.1182/blood, *in press*.

TECHNOLOGICAL ACHIEVEMENTS

- Quality Control program ISO 9002 Certified GMP Self diagnosis software developed.
- Viral proteins cloned and expressed in mammalian cells.
- Quality Control program AABB will be implemented very soon.
- At the end of October/2002 will be set up one editorial for the installation of business incubator together with the Ribeirão Preto City House.
- On July 18-19/02 was performed the first National meeting of Brazilian Blood Center.
- Development of a susceptometric system to measure iron deposits in human tissues (in collaboration with A.Carneiro and O. Baffa)

1- Education Activites

Course "The Cell, the Genome and You, the Teacher":

Summer Course	Voyage to the Genome
Specialized Course	Molecular Biology
Register of CTC Researchers Lectures Through VHS Recording	Specialized support to high school teachers of science
Workshop – Results of Projects developed by Students and Teachers of the Educational Program	Oral and Poster presentation, Experience interchange
Science House – special event for the diffusion of science organized in São Paulo by FAPESP for elementary and high school students.	Activities for elementary school students. Presentation of two plays: The Clone e the Miracle of Life
V Meeting of Educational Programs on Science (Brazilian Center West Region)	Oral Presentation
48 National Meeting of Genetics	Teachers of Educational Program participated of the session for the general public Genetics on the Square
Meeting of CEPIDS representatives	**Experience Interchange**
Course for High School Teachers: (`The Cells, The Genome and You - Teacher')	Participation of faculties from USP Campus of Ribeirão Preto, Araraquara, São José do Rio Preto and Fiocruz
VII Show of Educational Material for Teaching Science in High Schools / Science House	Presentation of 38 posters describing the activities developed by high school teachers of The CTC educational Program
Special Activities for Elementary school students	Molecular Biology Classes
High School Students	Talent Hunt Project
Diffusion of Projects developed by High School Teachers and	Educational Program Link in the site http://ctc.fmrp.usp.br

Other Educational Activities

In addition to the educational activities related to primary and secondary Public Schools, activities directed at undergraduate and postgraduate students were carried out. The **Summer Course** this year entitled “Genome, Proteome and Cellular Universe” was taught for the third consecutive year to undergraduate students. The course was taught in January 2001 and counted with the participation of 24 undergraduate students from the most diverse areas and geographic regions (see enclosure). The course “Principle of Gene Therapy” was taught during August of 2002 to undergraduate students and higher level technicians. A total of 12 students participated in this course.

2. RESULTS OF BASIC RESEARCH

In 2 years of existence, the Center for Cell Based Therapy (CTC) succeeded in aggregating 8 basic research laboratories and one educational program from the University of São Paulo on a common objective: the study of therapeutic strategies based on the use of *ex-vivo* manipulated cells. The activities of the educational program will be described elsewhere. The on going basic research projects study several aspects involved with this approach such as:

A. Techniques for harvesting and expanding ex–vivo human progenitor cells;
B. Analysis *in vivo* and *ex-vivo* induction of cell differentiation and purging of leukemic cells;
C. Immunological approaches to treatment of cancer and autoimmune diseases;
D. Identification of clinical and laboratorial features with diagnostic and prognostic relevance for the treatment of hematological malignancies;
E. Development of mouse models of human cancer;
F. Analysis of the genome and proteome of normal and cancer cell, and cells that are used in cell therapy.

As result of the data (partial or definitive) obtained by CTC projects, other scientists from different laboratories and institutions are currently collaborating with our endeavor. Consequently, the number of projects increased from 65 to 104, broadening the reach of the original proposal. Moreover, ten projects were concluded, yelling 5 Ph.D. and 2 Ms thesis and several publications (enclosure).

The progress of related projects will be described bellow:

A. Techniques for harvesting and expanding ex–vivo human progenitor cells

Development of short term culture of human dendritic cells from healthy donors. This study was conducted to get high quality and sufficient numbers of mature dendritic cells from healthy donor peripheral blood. A monocyte population with 75-85% of purity was obtained from 23 healthy donors after a Percoll density gradient centrifugation. The resulting monocyte population were cultured in the presence of GM-CSF and IL-4 and after 5 days different maturation conditions were performed and analyzed for the morphology and the expression of a mature DC phenotype. To date, only in the cultures with TNF-α plus prostaglandin E2 or lipopolysaccharide an increased expression (80-99%) of CD1a, CD80, CD86 and HLA-DR was obtained.

Isolation and characterization of mesenchymal stem cells from human bone marrow and cord blood. The objective of this study is to characterize mesenchymal stem cells (MSC) from bone marrow and cord blood for use in research on the application of stem cells. The analysis of four mesenchymal culture from bone marrow showed an expression of 80-95% of CD13, CD29 and CD90. At this moment, these MSCs are being cultivated and differentiated into adipocytes and osteocytes. Related to cord blood source, the morphology analysis showed the typical characteristic, and the flow cytometry assay are underway.

Expansion of cord blood mononuclear cells in coculture with autologous human umbilical vein endothelial cells (HUVEC). Ex vivo expansion of human umbilical cord blood cells (HUCBC) is explored by several investigators to enhance the repopulating potential of HUCBC. We performed the expansion of HUVECs. Analysis from four experiments showed that the cells demonstrate the classical endothelial cell phenotype, including the expression of cadherin-5 (CD144), intercellular cell adhesion molecule-1 (ICAM-1 or CD54) and CD31 (PECAM-1) and the typical cobblestone monolayer

appearance. Our next steps include to perform experiments using either mononuclear cells or CD34+-selected HUCBC from the same donation in the presence of irradiated human umbilical vein endothelial cells (HUVEC) in order to optimize conditions for *ex vivo* expansion of those cells.

Profiling changes in TPO expression level, platelet recovery and hematopoetic progenitor cell numbers in normal donors after platelet apheresis. We evaluated the plateletapheresis products of 25 donors after 2 h, 1 day, and 2, 3, 4, 5, 7 and 9 days. The platelet count showed that the number of platelets was significantly lower after apheresis and become similar to pre-apheresis levels on day 7. The number of reticulated and activated platelet and the level of TPO showed no significant difference after apheresis when compared with pre-apheresis. However, the number of CFU-GM, BFU-E and CFU-MK colonies was significantly higher after different times of apheresis. The results obtained were published as a Thesis and are currently being prepared for submission.

B. Analysis *in vivo* and *ex-vivo* induction of cell differentiation and purging of leukemic cells

Analysis of differentiating and pro apoptotic effects of Vitamin E isomers. We are analyzing the effects of the treatment with alpha,

gamma and delta tocopherols and tocotrienols on granulocytic differentiation and induction of apoptosis of the normal and leukemic cells. We have detected that alpha tocopherol exerts a moderate differentiating effect, that is enhanced by the all-trans-retinoic acid (ATRA) cotreatment. We will expand this analysis to other vitamin E isomers.

Analysis of the effects of Histone Deactylase Inhibitors (HDACis) on cell differentiation. We analyzed the expression of adhesion molecules by hematopoetic cells treated with Trichostation A or Phenylbutirate. The analysis of cell markers showed that HDACis have a small activity as differentiating agents *per se*, but may have a synergistic action to classical differentiating agents such as ATRA and Vitamin D3. Functional assays were performed on endothelial cell monolayers, which indicated that HDACis induce cell adhesion. We are expanding these experiments and will analyze the gene expression profile of treated compared to untreated cells.

Evaluation of gene expression during differentiation and maturation of cord blood CD34-derived dendritic cells using proteomic analysis. *The goal of this work was to optimize dendritic cell (DC) preparations obtained from patients suffering from chronic myeloid leukemia (CML) and compare with patients under interferon, hydroxiurea and STI treatments and newly diagnosed CML patients. The analysis of 12 patients (3 from each category) showed that the percentage of mature CML-DC varied among patients between 9% and 83% with the higher percentage for newly diagnosed and patients under interferon and STI treatments. Also, we determine the optimal culture conditions. The yields of mature CML-DC were higher in the following cytokines requirements: five days in the presence of GM-CSF, SCF and FLt-3 following by two days of maturation with GM-CSF, IL-4, TNF-alpha and PGE. During this year we set up some of the basic methods required for the proteomic approach using the following systems: a) proteomic analysis of gene expression during differentiation and maturation of cord blood CD34-derived dendritic cells; b) proteome modification during the early stages of melanoma malignant transformation; and c) proteomic analysis of human metastatic cells treated with antitumoral drugs. We studied methods of cell and total protein extraction. The 2D-electrophoresis system has been set up and we demonstrated its reproducibility. The quantitation by imaging is being developed. At same time, methods for enzymatic "in situ" digestion and identification of some proteins by ESI/MS/MS were set up. Although it is not the ideal equipment, we already have been identified some proteins extracted from gel spots. Besides that, if necessary, we shall take samples to São Paulo to run on a MALDI-TOF equipment.*

Ex vivo drug therapy to purge Ph- progenitor cells from chronic myelogenous leukemia patients. *We are evaluating the effect of cytarabine (50 ng/ml), STI571 (1.0 uM) and staurosporine (5 mg/ml) after 48 of drug exposure in peripheral blood and bone marrow mononuclear cells from chronic myelogenous leukemia patients using two different approaches: a) clonogeneic cell assay and b) proliferation assay. The most effective treatment was obtained with cytarabine (50 ng/ml) which suppressed 77.4% of CFU-GM colony formation and 40% of in vitro proliferation when compared with untreated CML mononuclear cells. After STI571 the inhibition of CFU-GM colony formation and in vitro proliferation was 20 and 22%, respectively. Analysis of individual CML colonies for the presence of the hybrid BCR/ABL DNA insert by polymerase chain reaction (RT-PCR) is currently underway to demonstrate whether or not the selection of Ph$^-$ PB-MN cells was obtained after these in vitro drug treatments.*

C. Immunological approaches to cancer treatment

Immunological changes induced by IFNalpha treatment in chronic myelogeneous leukemia (CML) patients. *We analyzed IL-2, IL-4 and IFN gamma production, T and NK activation markers, NK cells activity, apoptosis induction and lymphocyte subsets distribution in 26 CML patients treated with IFN alpha. Several measures were done during a 9 months follow up period. The immunologic data were correlated with the hematological and cytogenetic responses. We detected a positive and direct correlation between immunological activation and disease remission. IFN alpha effect was detectable from the third month of the beginning of the treatment. These results were published as a Ph.D. thesis and are currently being prepared for submission.*

Treatment of auto-immune disorders with stem cell transplantation (SCT). The Brazilian cooperative trial of autologous hematopoietic stem cell transplantation for autoimmune diseases was successfully launched, nine patients (4 lupus, 3 multiple sclerosis, 2 systemic sclerosis) were transplanted, 5 in our institution, six patients are alive and improving very much in relation to the pretransplant status. Laboratory studies to understand immunological mechanisms involved in

the therapeutic effect of stem cell transplantation in autoimmune diseases are underway. We are launching a new protocol of hematopoietic stem cell transplantation for hemoglobin disorders (thalassemia and sickle cell anemia) and also starting animal models to examine engraftment of hematopoietic and mesenchimal stem cells in NOD/SCID mice.

In addition, we are analyzing the intracellular production of inflammatory cytokines (IL-1 and TNF-alpha) in patients submitted SCT for hematologic malignancies, and correlating this production with occurrence of acute graft-versus-host disease. In parallel, we are monitoring the cytomegalovirus infection in these patients by three methods, immunocytochemistry, flow cytometry and PCR, aiming to establish the prevalence of this infection in our transplant population and choose a method for routine detection of CMV in immunocompromised patients.

Post-transfusional microchimerism in multitransfused patients. Previous studies demonstrated that significant immunodepression may occur after cell infusion. To investigate the possible mechanisms involved, we opted to analyze the post-transfusional microchemerism in multitransfused patients. The sequence of chromosome Y SRI was investigated by a highly sensitive nested PCR in 12 female patients with hereditary hemoglobinopathies (β-thalassemia, S-β thalassemia or sickle cell anemia) transfused with male packed red cells. Sequential samples were analyzed from each patient. Microchimerism was detected longer (20-60 days) than previously reported in the literature (5-7 days), but not one year after transfusion. The use of filtered blood did not prevented the development of microchimerism. Therefore, the kinetics of microchimerism in multitransfused thalassemia major patients resembles that observed in immunosuppressed patients.

Inducing immunologic response against BCR-ABL fusion gene products. CML is a paradigm of anti-tumoral response. We aim to generate *ex-vivo* T cytotoxic cells specific to BCR-ABL$^+$ leukemic cells. We cloned BCR-ABL cDNAs encoding for different domains of the fusion protein. After sequencing, these sequences will be essential for the development of retrovirus vectors, capable of transfecting dendritic cells. These specific antigen presenting cells (APCs) could be used to activate and expand cytotoxic T-cells.

D. Identification of clinical and laboratorial features with diagnostic and prognostic relevance for the treatment of hematological malignancies.

In order to identify patients, who would benefit from more aggressive therapies, such as SCT, as well as to categorize them according to their risk of disease relapse and/or treatment morbidity, we analyzed several gene polymorphisms, immunophenotypic features of leukemic cells, and methods of disease monitoring.

We demonstrated that gene polymorphisms of cytokines [(TNF-α and β), IL-1 receptor antagonist (IL-1Ra), IL-6 and IL-10)], adhesion molecules (CD31 and CD54), Fcγreceptors (FcγRIIa, IIIa, IIIb), mannose-binding lectin (MBL) and myeloperoxidase (MPO) are informative genetic risk factors for infections, acute and chronic graft versus host disease and, early mortality in HLA-identical bone marrow transplantation.

Similarly, we demonstrated that *MDR-1* and methylenetetrahydrofolate reductase gene polymorphisms were risk factors for the development of acquired aplastic anemia and childhood acute lymphoblastic leukemia, respectively.

Monitoring of leukemia treatment can be performed by several methods and has an important prognostic impact. We developed a quantitative flow cytometric method, analyzing 5 different markers, for the detection minimal residual disease in acute lymphoblastic leukemia (ALL). We succeeded in distinguishing normal and leukemic B-precursors from the bone marrow 39/40 cases of ALL analyzed. In another project, 3 different methods based on the PCR technique: (1) using consensus primers for the detection of a clonal population for TCRgamma; (2) clone-specific primers for the junctional region of TCRgamma; and (3) a semi-nested reaction with an initial cycle with consensus primers followed by a second cycle with clone-specific primers. MRD presence was associated with a shorter event-free survival and was the major independent prognostic factor in most of the phases analyzed. The use of consensus primers for the detection of TCRgamma clonality, although less sensitive, proved to be a simpler, faster and less costly method whose positivity was associated with more than 90% relapse rates during all phases analyzed.

We are analyzing *FLT-3* and *c/EBPalpha* mutations in patients with acute myelogenous leukemia, *ABCB1* and *hTR* gene (which encodes for the MDR-1 protein and for RNA moiety of telomerase, respectively) in patients with aplastic anemia and myelodysplastic syndromes.

In addition, we have compared the expression of several myeloid markers in acute promyelocytic leukemia cells and in normal promyelocytes from recovering benign myeloid proliferation. Our results demonstrated that the analysis of CD11b and CD117 expression distinguishes between these 2 conditions.

E. Development of mouse models of human cancer.

Analysis of cell cycle and apoptosis in myeloid progenitors of PML-RAR transgenic mice during the pre leukemic phase. Acute Promyelocytic Leukemia (APL) is characterized by the expansion of malignant myeloid cells that morphologically resemble promyelocytes**.** In the vast majority of APL cases, the t(15;17) is present and causes the fusion between retinoic receptor a (RAR) gene locus located on chromosome 17 with the promyelocytic leukemia (PML) gene locus located on chromosome 15, yielding to a fusion gene translated in an aberrant PML-RAR fusion protein, which is thought to act as biologically distinct RAR mutant leading to active transcriptional repression. We study a transgenic mouse model of APL generated by Prof. Pier Paolo Pandolfi from the Memorial Sloan Kettering Cancer Center, in which the fusion gene PML-RARalpha is expressed under the control of a cathepsin G promoter. Ten percent of these transgenic mice (TM) develop a form of acute promyelocytic leukemia that closely resemble human APL. The long pre-leukemic phase and low incidence of leukemia, suggest that other mutagenic events must occur before the development of overt leukemia. We are analyzing the apoptosis, cell cycle and the expression of cell cycle regulators, such as p16 and p15, in myeloid progenitors from the bone marrow of TM at different time points (at birth, 6, 9, 12 months and after the development of leukemia). In addition, we are performing comparative genomic hybridization (CGH) of TM leukemic cells, in order to identify genetic abnormalities that may represent additional mutagenic events. We have detected aneuploidy in half of the leukemic TM tested so far, with non significant increase of the percentage of cells in S phase. The precise chromosomal aberrations are being characterized.

Analysis of the coagulation cascate in PML-RARα Transgenic Mice. An important feature of APL is the high frequency of disseminated intravascular coagulation (DIC) at presentation. The best clinical management of this situation is controversial and randomized clinical trials are hampered by ethical considerations, therefore the development of an animal model of DIC in APL is important for the development of new therapeutic strategies. We are currently analysing coagulation factors and pro-inflammatory cytokines in PML-RARα Transgenic Mice treated with LPS.

Analysis of the NPM-RAR oncogenic activity on a transgenic mouse model. In the first phase of the project, we have determined that transgenic mice harboring the NPM-RARα fusion gene expressed under the control of the Cathepsin G promoter develop a myelomonocytic leukemia, characterized by the infiltration of blood, bone marrow and lymph nodes by blasts CD68+, MPO+, lysozyme+. Recently, it has been demonstrated by Pellici´s group that the wild type NPM protein interacts physically with the p53 protein. We are currently trying to determine whether the expression of the fusion protein NPM-RARα results in functional inactivation of p53**.**

F. Analysis of the genome and proteome of normal and cancer cells

Analysis of gene expression using a ORESTES based strategy. Open reading frame expressed sequences tags (ORESTES) differ from conventional ESTs by providing sequence data from the central protein coding portion of transcripts. We generated a total of 696,745 ORESTES sequences from 24 human tissues and used a subset of the data that correspond to a set of 15,095 full-length mRNAs as a means of assessing the efficiency of the strategy and its potential contribution to the definition of the human transcriptome. We estimate that ORESTES sampled over 80% of all highly and moderately expressed, and between 40% and 50% of rarely expressed, human genes. In our most thoroughly sequenced tissue, the breast, the 130,000 ORESTES generated are derived from transcripts from an estimated 70% of all genes expressed in that tissue, with an equally efficient representation of both highly and poorly expressed genes. In this respect, we find that the capacity of the ORESTES strategy both for

gene discovery and shotgun transcript sequence generation significantly exceeds that of conventional ESTs.

Identification and confirmation of new SNPs (Single Nuclotide Polymorphisms) in human ESTs obtained from normal and neoplastic tissues. Based on the data generated by the project Genome of Human Cancer (Projeto Genoma Humano do Câncer), 538 candidate SNPs were identified, sixteen of which resulted in modification of the aminoacid sequence, confirmed experimentally. Among others, we detected and confirmed the substitution of a leucine by a isoleucine in the codon 307 of the *Tax1BP1* (Human T-cell leukemia virus type 1-binding protein) gene.
Leukemia Gene Index (LGI). In this project we aim: a) to characterize the transcriptome from leukemic cells, b) identify and characterize new SNPs, c) Map ESTs detected in genomic regions harboring genes that, when mutated, may be associated with the devlopment of leukemia, d) analysis of gene expression "in silico". e) analysis of gene expression by Microarray and SAGE (Serial Analysis of Gene Expression). Until now (about a year) we have generated 17.000 ESTs from bone marrow cells of healthy subjects and patients with Chronic Myelogenous Leukemia.

Research projects not directly related to cell therapy are being developed in our center. Most of them result from the active collaboration with other investigators, who by their turn are involved in CTC projects. We consider this interchange of technical information an important contribution to the Brazilian Academy Community. These projects are:

a) Based on the sequencing of 8.8 Kb mithochondrial DNA of 40 individuals in a collaborative project coordinated by our Center, we obtained results that demonstrate that peopling of Americas was probably done by a single wave of Asian migrants who arrived around 20 thousand years ago, much earlier than generally accepted. This work was the focus of considerable attention by the lay press (Follha de São Paulo, O Estado de São Paulo, both important daily newspapers) and was the object of a cover article by the monthly science review edited by FAPESP (Pesqisa FAPESP).
b) Sequencing the *Drosophila melanogater* and *Apis mellífera* genome. We have until now generated 10.000 ESTs of *Drosophila.*
c) Analysis of risk of venous thrombosis associated with C677T mutation of the methylnetetrahydrofolate reductase gene. We demonstrated that fasting hyperhomocysteinemia is a risk factor for venous thrombosis in patients without known acquired thrombophilia and other genetic risk factors for venous thrombosis. – Concluded, article enclosed.
d) Analysis of HTLVI/II, HIVI/II, and TTV Retroviruses Genome. Some of most common viral infections are caused by retroviruses such as HTLVI/II, HIVI/II, and the hepatropic virus TTV. This project aims: 1) to characterize the genotype of a human immunodeficiency virus type 1 (HIV-1) isolated from Ribeirão Preto; 2) to sequence the HTLV type I isolate from Ribeirão Preto and, 3) to determine the molecular epidemiology of the TT virus (TTV) in Ribeirão Preto. We have until now studied 122 HTLV and 52 TTV patients. Proviral DNAs of three genomic regions (the long terminal repeat, pol and tax regions) were amplified and sequenced. The TTV amplified PCR products were subjected to sequence analysis: 75% of them were classified into genotype 1 and 25% into genotype 2.
e) Structural and functional characterization of an acidic platelet aggregation inhibitor and hypotensive phospholipase A(2) from Bothrops jararacussu snake venom. – Concluded, article enclosed.
f) Fluorescence properties of tryptophan residues in the monomeric d- of Glossoscolex paulistus hemoglobin: an interpretation based on a comparative molecular model. – Concluded, article enclosed.

Technology Transfer Project

Ongoing Programs

Quality Control Programs

The Regional Blood Center developed and installed a quality control program (ISO 9002) certified in 10/29/1999. The resulting experience is available to all public blood centers of the State of São Paulo through the ***Blood Coordination of São Paulo State Health Secretary***. The Certificate itself is presented (enclosure)

Development of a Good Manufacturing Practices (GMP) Self-Diagnosis Software

The Regional Blood Center developed in partnership with GMP, a private company, a software intended to perform the auto-diagnosis of the Good Manufacturing Practices. The software was installed in 72 units of the Blood Center network and two GMP evaluations have been performed thus far in each unit. The preliminary results were presented at a Seminar conducted by FAPESP August 2001. This program was supported by a Public Policy grant from FAPESP. A version of the software is included.

Development of a Software for Financial Management of Research Projects (Via Expressa / Expressway)

Based on UP (Unified Process) concept, UML- Unified Modeling Language and CASE Rational Rose and RequisitePro Tools, we developed a Software for Financial Management of Research Projects (Via Expressa / Expressway). This software is being tested in CTC for material requisition and purchase, research progress updates and statistics.

Umbilical Cord Blood Bank (UCBB) and Brazil-Cord Program

The Center set up a UCBB that is now operating in a pilot phase in an adapted laboratory situated in the fractionation sector of the Blood Center . By the end of the year the UCBB will move to a specific new facility located in the Blood Center. The Center UCBB is part of the Brazilian network of UCBBs called BRAZIL-CORD which is now being organized.

Biotechnology Projects

A. Cloning and expression of proteins of biotechnological interest

1. ***Production of HIV-1 recombinant proteins***
   The objective of this project is to obtain proteins that might be used in diagnostic tests (ELISA and Western Blot) of HIV-1 infection. The HIV-1 p24 capsid protein is an important early marker of HIV infection. We recently obtained the isolation of a 666-bp fragment corresponding to the p24 HIV. This DNA product was isolated by nested PCR from DNA isolated from peripheral blood mononuclear lymphocytes of an asymptomatic HIV-1 seropositive human subject from University Hospital of Ribeirão Preto. This 666-bp fragment was further sub-cloned in the expression vector and transfected into HEK293 cell line. By RT-PCR we have detected the expression of mRNA p24 in the cell population after selection of geneticin. At this moment, western blot assay is being conducted to evaluate the level of expression of the P24 protein.

2. **Production of HTLV-1 recombinant proteins**
   As we reported before an expression vector with the region coding for gp21 of HTLV-1 was obtained. After, this plasmid vector was transfected to the HEK293 cell line by in vitro electroporation. mRNA p24 expression was detected in the cell population after selection with geneticin. Work is currently underway to obtain the cell clone with highest expression level. Also, we have cloned the 635-bp fragment which codifies p24 capsid protein of HTLV-1 in mammalian expression vector. This DNA product was isolated by nested PCR from DNA isolated fromHTLV-1 infected MOLT-4 cell line. Further, this fragment was transfected by eletroporation into HEK293 and at this moment geneticin selection is being performed.

3. Production of human clotting factors VIII and IX by gene recombination.
   The objective of this project is to clone and express human clotting factors VIII and IX. These factors are absent or altered in patients with hemophilia A and B, respectively, who depend on infusion of exogenous factors for the normalization of the coagulation process. The greatest concern in hemophilia treatment has been viral safety. Related to FVIII, our propose consists of generating a recombinant FVIII with B- domain deleted. To make that we isolated N- and C- terminal domains of FVIII and at this moment we are performing the fusion of these parts at the site of amino acids Ser 743 and Ser 1637. Our next step includes to introduce this cDNA into appropriate expression vector. Related o FVIX, a human liver cDNA library of 1 x 10(6) clones was screened with an insert of 1.4 kb, representing the whole FIX cDNA. Four individual hybridizing cDNA clones were maintained through fourty screening. All of them contains the DNA insert of 1.4 kb as analysed by PCR. One clone, C4, was selected for further study, and at this moment, the DNA sequencing is being performed. This project also counts with financial support from FINEP.

B.Development of a non invasive method of measurement of iron in human liver.

Iron overload is a common problem in patients with chronic anemia who are submitted to regular blood transfusion and in patients with hereditary hemochromatosis. Chelation therapy has shown to be quite effective in eliminating the effects of iron toxicity, thus increasing life expectancy. Regular and accurate determination of iron overload is the basis of medical treatment of these patients. Due to the large amounts of iron usually stored in liver, this organ is often subjected to the quantitative analysis of iron

overload. The current technique for direct measurement of hepatic iron is liver biopsy, which causes significant discomfort and risk to the patient to be routinely used, therefore we aim to develop a non invasive method of measurement of iron in human liver. We evaluated 4 configurations of susceptometer: homogenous and non homegenous magnetizing field coupled with an axial second-order and planar first-order gradiometric magnetic detector. The results obtained until now suggest that all configurations have potential to evaluated the level of hepatic iron, with some technical limitation. Although further studies are necessary, we believe that the methods analyzed may lead to development of devices that can accurately determine liver iron load. The article describing the results obtained so far is enclosed.

Other Activities in the Transfer Area

During this period, the Transfer Coordinator promoted meetings with the company *Indústria Farmacêutica JP* (JP Pharmaceutical Industry) which is a partner in the development of plastic products for cell collection and culture. Some projects have been outlined and efforts are currently underway to make this cooperation operational.
Contacts were also made with the Ribeirão Preto City Hall, which officially invited the Center to participate in an “Incubator of Small Businesses” project focusing on the biotechnology sector. A Seminar about the Economic Development of the Ribeirão Preto Region was held in June 2001, where the Transfer Coordinator gave a lecture about the Center and the possibility of partnership with private enterprises.

Dissemination Activities (Press and Television)

Information for the general public is an important item in the CTC program. In numeros occasions the CTC has been the focus or the source of news and information in the press and television.

CTC Educational Project

History

The educational project "The Cells, the Genome and You" developed at the Blood Center Foundation of Ribeirão Preto, in the CTC, consists since April 2001 of four fundamental programs: the course "The Cell, the Genome and You, the Teacher" with 7 research groups, the Science Teaching Site, the Science Journal, and the Talent Scout program.

The course is taught on Saturdays morning in the amphitheater of the Blood Center. In 2001 the course was weekly and was attended by 27 teachers from state and city public schools (21) of the Regional Teaching Districts of Ribeirão Preto and Sertãozinho. In 2002, there are a total of 94 basic education teachers (from 63 schools), as well as post-graduate students. These are teachers of the macro-region of Ribeirão Preto belonging to the public and private networks.

In 2002, as also done in 2001, the 7 research groups are oriented by CTC investigators. The idea is that, in addition to receiving updating information about specific contents centered around molecular biology/genome, the teachers will be helped to "transform" these contents through a pedagogic approach, so that they may be worked upon in the classroom.

In 2001, the teachers brought their students who sat in on the classes in a highly participative manner. The growing number of students and the major proposal of the Talent Scout "forced" the team of the Science House to open a special space only for these students. Thus, in 2002, the students participated in a class program separate from that of their teachers. On this basis, we included the contents directly related to the project but expanded in order to permit the students to elaborate activities, with questions leading to scientific exercise, to be answered within 3 months under the orientation of the investigators, a fact that triggered the beginning of the Talent Scout program. The teachers expect support from their students to perform the proposed activities, which are discussed and even elaborated by research groups. This creates a "productive dialogue" between teachers and students (not always their own) in the extra-class spaces. The documentation and presentation of the process will create the so-called inter-school networks *(Enclosure 1).*

Another important element is the Science Journal which has acquired its own characterization with its own editorial line elaborated during this period. The journal always "portrays" the Saturday classes, the participation of the students, and the work of the teachers in the school starting from the research groups. The Journal contains an interview with some investigators about a relevant topic.

In the Science Teaching Site of the CTC (with 3665 accesses) there is a discussion forum – BioForum CTC – that permits interaction between teachers and investigators. The forum approaches the question of science, technology and society, and intends to make available in full the work of the teachers, with a more dynamic and up to date access.

The students are already being oriented by the teachers and investigators, as done for the research groups. Thus, the Talent Scout program has already been articulated and the inter-school network is beginning to be formed, permitting a longer-reaching type of learning within a shorter interval of time.

The research groups have developed activities in school, especially in the extra-class space, so far as a group. To this end, the teachers, individually or in groups, give classes outside normal hours about what is being treated in the groups *(Enclosure 2).* Play activities were elaborated in 2001, such as games, scale models, schemes, kits and even theater plays about the most diverse topics considered. Contents have been planned in a more detailed manner for each research group and instruction materials have been elaborated according to appropriate methods and techniques such as seminars, practical activities in the CTC laboratories, and presentation in data shows. Thus, it is a task for the teachers to adapt the process to a format that can be implemented in basic schools. On this basis, many teachers already trained in the pedagogic method during their work in 2001, have developed activities in the schools and have invested in "contamination" work among the students triggered by the Talent Scout program. It is the responsibility of the Science House and of the group coordinators to provide the conditions that will permit the resulting data to return to the project. To create this "fractal" theme we count with the expansion of the student team. In addition, new theater plays have already been elaborated.

It is interesting to comment that in one of these plays the teacher set a date for interested persons to come for preselection. To her surprise, more than 90 students appeared and only 22 were selected. The classes for the students of Sertãozinho schools continue to be held during extra hours.

According to the teacher, the engagement and dedication of an ex-pupil (a 2001 pupil) was essential because he was the one who mobilized the entire team. Despite all this dedication, the pupil has a vocation for the theater and not necessarily for science. And this is the purpose of the Talent Scout program, since talents already exist and what is necessary is to discover them in the schools; obviously we focus on those who have a vocation for science, but the most diverse vocations exist.

Since this is a specialization course, it is based on certain requirements such as evaluations with a minimum note of 7.0 and 85% attendance. In general, specialization courses require the elaboration of a monograph or term paper at the end of the course. However, in the course "The Cells, the Genome, and You, the Teacher", "the final evaluation is based on the different activities carried out during the process. Although each research group requires an activity, the importance of dissemination must be clear, i.e., the socialization of our learning by means of explanatory support texts. We consider for evaluation the records of the activities, the involvement of teachers and students in the Talent Scout program, the participation in shows, exhibits and workshops, the execution of tests and methodologic exercises, and the commitment to, and participation in, the Saturday classes and in the research groups, as well as documentation, data analysis and presentation of the results.

In 2001, “standard classes”/”pathway classes” were elaborated by the research groups and a workshop was held.

In March of this year we presented the results of 2001 at the Science Station in São Paulo, in September we participated in the 7th Show of Material for Science Dissemination and Teaching, and we are invited to a special presentation in November. We also intend to organize an exhibit of all work projects at the end of this semester in Ribeirão Preto. This exhibit will consist not only of the teachers’ work in the schools, but also of the work carried out by the students stimulated by the Talent Scout program, i.e., an investigative question on which they worked over a period of 3 months.

Development

Taking as reference the first group that took the specialization course “The Cells, the Genome and You, the Teacher”, changes were made in the structure of the Saturday classes in order to expand the knowledge of the teachers in topics of Cell and Molecular Biology based on the research groups which meet weekly *(Enclosure 3)*

The classes occupied a new space, with a specific program of biotechnology topics articulated with classical and consecrated biology themes, and found support for the associations between the themes treated by the research groups, which detail and consolidate the new concepts learned. Interaction between these two “biologies” occurs in the research group, thus providing the teachers with the necessary articulation to complement the classes of the course and with self-confidence in the presence of their students in the classroom. *(Enclosure 4).*

This year, the interaction between research groups, advisers and teachers is more defined in view of the interaction expected among the members of each

group and between their group and the others. The action of the teachers involved in the course is involving the school, the activities start to be disseminated, to be reported to the advisers of the research group and to the team of the Science House, and therefore to be evaluated.

In contrast to 2001, the students have gained their own space, with dynamics similar to that of the course for the teachers, although with an emphasis on experimental work. Investigators, teachers, and graduate and postgraduate students who teach the classes for teachers and students are the potential advisers who direct the questions of the students, thus promoting proximity of basic and higher education, and denying the myth of the distant role of science and scientists.

With this new space, the students start on their path following the steps of the scientific method, from the question raised to the written report, including a review of the bibliography, with a period of 3 months until the presentation of their results.

This type of work is oriented by a specialist in the area and/or by the teacher himself, who frequents the research groups and works with his students on the knowledge acquired. In view of the difficulties in performing cell and molecular biology experiments with elementary school children, many of the questions are answered during interviews with the researchers on the occasion of visits to the working environment of the Blood Center, where the students have the opportunity to familiarize themselves with this state-of-the-art technology.

Evidence

In 2002, the specialization course "The Cells, the Genome and You, the Teacher" was recognized through the Department of Internal Medicine (resolution CoCEx 3878/91) in March 2002.

The advances exceeded the initial project in terms of number of teachers enrolled, students, schools and towns, and consequently in terms of meetings and visits to the Blood Center and the Science House, and meetings in the Schools *(Enclosure 4).*

It is a project that has created an atypical reality since, in addition to classes, it includes research groups, documentation of the activities and dissemination of the results.

The project is characterized by a sort of pioneering spirit in developing work of dissemination – a partnership between investigators, teachers and students whose results are insistently "tapped".

There seems to be articulation between three scientific spaces developed at the same time: specific content, pedagogic work and dissemination in various formats. All three must be programmed for publication. This process is repeated in the classes, in the research groups, in the schools, and in the Science House as if it were a "fractal". The data are being collected for dissemination; instruction materials such as models, games and play acting must be accompanied by written texts.

The proposed method, according to which the teachers investigate their own work, has permitted them to perceive the complexity of the task involved in planning, writing objectives and raising hypotheses. The advancement of this project is the exercise of documenting the process, in addition to analyzing data and writing a paper.

In contrast to training courses for teachers, which usually neglect the questions related to evaluation, the proposal is to permit the teachers to investigate their work and to learn to disseminate it. The project has led to changes in the schools involved.

Through this project, under the guidance of the investigators, the teachers have created conditions favorable to the growth of learning, participation and interest in the schools.

During this period there were changes in the schools brought about by the activities performed by the teachers and students involved in the project. The teacher does not perceive these changes if he is not evaluated. During their interaction with the investigators and with the team of the Science House the teachers and the students learn to perceive the results obtained.

For us it is not enough to see about 200 teachers and elementary school children frequenting the premises of the Blood Center during the week and on Saturdays. We want to disseminate what they do and how they are able to achieve results that alter the routine of the schools and awake the interest of many, even the parents of the pupils.

Activities

- March 4, 2002 – Interview with the journalist Rose – Press Office of USP/RP. Reported in the USP Ribeirão Journal, nº 740, March 1, 2002, p.8-9.

- March 5, 2002 – TV Rede Família (Family Network TV) – Interview of Prof. Marisa Ramos Barbieri about the profile of 2002 freshmen of the University of São Paulo.

- March 14, 2002 – S.B.T. – Interview of Prof. Marisa Ramos Barbieri in the program "Noticidade"

- July 3, 2002 – Local EPTV Station – Participation of Prof. Marisa Ramos Barbieri in the "Tchamders" program – New technology means in education.

- O desafio de compartilhar (Thwe challenge of sharing). Revista Pesquisa/Fapesp, julho de 2002, p.20-5.

- December 12, 2001 – Workshop in the red amphitheater of the Blood Center Foundation – presentation of work by teachers and students involved in the project, sponsored by the Education Director's Office, with the presence of 84 persons among teachers, students, postgraduate students, and teaching Supervisor.

- March 22, 2002 – Visit to the Science Station: 2 buses with teachers and their students went to the Station to present 10 interactive activities to approximately 400 students of basic schools in the city of São Paulo. The two theater plays presented, "The clone" and "The miracle of life", were applauded by full houses.

- V Meeting on Research in Education of the Center-West, June 11-14, 2002, Uberlândia, MG. – Presentation of a paper.

- 48th National Congress of Genetics in Águas de Lindóia. – September 16 to 20 – participation of elementary school teachers linked to the projects in the activity "Genetics in the Square", with registration waived.

- October 5, 2002 – Meeting with representatives of 3 CEPIDs (Ribeirão Preto, São Paulo and São Carlos) in São Carlos

- 3 Coseas/USP "Work-Fellowship" recipients – Participation in support activities for the schools.

- 3 Biology students of FFCLRP – participation in activities of project evaluation.

- 7th Show of Science dissemination and teaching material – Estação Ciência/São Paulo, September 26 to 29, 2002. Thirty-eight panels about the activities carried out in the schools by the teachers under the orientation of the research groups were brought to the Show.

Works

Extracurricular groups:

Books –

Barbieri, M. R. Laboratório de Ensino de Ciências – 20 anos de história (Laboratory of Science Teaching – 20 years of history), Ed. Holos, 2002, 64p.

Barbieri, M. R. A construção do conhecimento do professor (The construction of teacher knowledge) Ed. Holos, 2002, 100p.

Jornal das Ciências – numbers 2 to 7 (in press).

Scheduled publications:

Saturday classes – a book with articles by the speakers.

Antenna – programs, evaluations, and methods of the project.

Science Web – work by the research groups and in the schools.

Science Trail – students' work

Writing workshop – support by journalists to the Saturday classes, to the Talent Scout program and to the Research Groups, and to the activities scheduled by the Science House

Science House

As we reported before the incorporation of the Science House had not been programmed by Cepid. At this moment the tean is temporarily installed in the Science Workshop/MuLEC, located at Rua Carlos de Campos, 1474. But, brieffly the definitive installation will be in the Bosque Municipal Fábio Barreto.

CEPID PROJECT – PROCESS 98/14247-6

TECHNICAL RESERVE (Complementary Budget)

Amount granted for two years**:** R$ 257.038.54

Amount spent in the second year R$ 140.786,50

PERMANENT MATERIAL ADQUIRED IN THE NATIONAL MARKET

QUANT	DESCRIPTION OF THE MATERIAL PURCHASED	VALUE R$
01	**Notebook "Toshiba Tecra 8200/750"**	7.500,00
02	**Timer triple DSPLY**	100,00
07	**No break 600VA**	2.030,00
01	No break model SU-100 with software	1.890,00
01	Printer model Deskjet 840C	369,00
01	**Pipetman P2, volume 0,1 a 2 uL**	1.055,00
01	Pipetman P10, volume de 0,5 a 10 uL	1.055,00
02	**Kit Pipetman Start Kit with 02 pipets P20, 02 P200, 02 P1000 e acessórios**	5.180,00
01	Refrigerator R250 – 243 litros, 100 volts – Eletrolux	430,00
01	VCR camera model NVRZ315BR - Panasonic	950,00

01	TV 29 inches, model TC29A9/A10/A11STMV/VCR – Panasonic	850,00
01	Vídeo Cassete model NVFJ615BRSTHFCR	389,00
01	Voltage stabilizer for microcomputador 1 KVA	50,00
01	Laser Printer model 1200	1.700,00
01	Pentium III 900 MHz Microcomputers	2.299,00
01	Water bath with termometer, model 100-220 volts.	462,00
01	Water bath with capacity for 120 tubes, model 102/6 – 220 volts	649,00
01	OPT DVD Rom, 10X, model 1000 – cód. X6168A	1.560,45
01	Voltage stabilizer 2 KVA	64,00
01	Precision Scale with precision of 0,01g e maximal weight of 2 kg, model AS2000C	1.478,40
01	Photographic camera, model Prima Zoom 60 kit	335,00
01	No break senoidal on line, isolado de 5000 KVA	7.195,00
01	No break 1.4 KVA, 120V, model SU-1400 NET	2.170,00
01	No break, 2.2 KVA, 120V, model SU-2200 NET	3.100,00
01	No break, 1 KVA	380,00
01	Cordeless phone 900 MHz, model KX-TC 1461LAB	178,00
01	Zip drive external 100 Mb USB	305,00
01	Oven, model LUNA 8446	175,00
01	Water filter	148,00
06	Pentium III 1.1 GHz Microcomputers for the educational project	14.100,00
	Total:	**58.146,85**

CONSUMMABLES ACQUIRED IN THE NACIONAL MARKET

Mainly reagents and other consummables for lab research	53.231,35

THIRD PARTY SERVICES

Description	Value R$
Repair VDS Videodocumentador for the educational program	4.068,45
Repair of VDS Videodocumentador, with replacement of lamps	1.299,40
Sorologic Screening for Transgenic Mice at the Charles River Institute , USA	2.099,52
Repair of Central air conditioning system of the animal facility	797,67
Repair of Central air conditioning system of the animal facility	572,48
Repair of Central air conditioning system of the animal facility	201,00
Production of 500 issues of the “Science Newsletter” for the educational program	495,00
Research assistants	4.613,83
Total:	**14.147,35**

HOTEL EXPENSES

Description	Value R$
Hotel expenses of Dr. Richard Burt, on October 11 2001, Hotel Shelton Inn, Seminar	50,00
Hotel expenses of Dr. Sandro Bonatto, November 11 to 14 2001, Hotel Shelton Inn, Seminar	227,80
Hotel expenses of Dr. Pandolfi Pier, May 29 to 31, 2002, Hotel Plaza Inn, Seminar	233,18

Total:	**510,98**

AIRPLANE TICKETS

Tickets for visiting researchers either to carry on research projects (Roger Chammas, Vanderson Rocha, Paulo Pandolfi, S Bonatto) and/or to participate in the annual seminar program (list of seminars enclosed). No ticket was used for travelling by any of the researchers or students of the center.

Total: R$ 14.749,97

OTHER RESOURCES

1. RIBEIRÃO PRETO BLOOD CENTER FOUNDATION	**R$**
Payment of labor fees and meal tickets for two hired Biologists and an Animal House Assistant	7.200,00
Rent of the building where the “Science House” is installed	6.760,70
Insurance in the “Science House”	274,95
Administrative staff and clerical and technical support paid by the Regional Blood Center (Fundação Hemocentro)	240.503,76
Total:	**254.739,41**

2. FINEP	Process nº 64.00.0487.00	1,040.330,00
3. PADCT	Process nº 62.0019/99-9	**202.000,00**
4. FINEP	Process nº 0659/02	**300.000,00**
5. PRONEX – CNPq	Process nº 66.1132/1998-6	746.500,00
	Total:	**1.988.830,00**