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

2001

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 .
Clarice Izumi	FMRP/USP	▪ Proteome modification during the differentiation of dendritic cells from CD34+ cells of human umbilical cord blood.
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
Fabíola Attié de Castro	FUNDHERP	Júlio César Voltarelli
Helen Julie Laure	FMRP/USP - FAPESP	Lewis Joel Greene
José Sebastião Ismael	HCRP/USP	Roberto Passeto Falção
Kiyoko Abe Sandes	FMRP/USP – CAPES	Marco Antonio Zago
Marcelo Oliveira de Morais	FAPESP – CEPID	Wilson Araújo da Silva Júnior
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

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.

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.

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.
Eduardo Magalhães Rego	FUNDHERP	**Subproject Coordinator:** ▪ Study of oncogenesis induced by the NPM-RARα fusion protein. ▪ Study of the effect of histone deaceylase on gene transcription in acute promyelocytic leukemia cells. ▪ Analysis of cyclin A1 expression in acute promyelocytic leukemia: role of histones deacetylases. ▪ Study of the expression of the cyclins and protein p16 and p15 genes in myeloid precursors of transgenic PML-RARα mice. ▪ Use of transgenic PML-RARα animals in models of activation of coagulation and inflammation.
Name	**Institution**	**Position/Responsibility**
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.
Marisa Ramos Barbieri	FUNDHERP	**Subproject Coordinator:** ▪ The cells, the genome and you.
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	FUNDHERP/UFPA	**Subproject Coordinator:**

Júnior	▪ 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.

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 Padovani JC, Pazin-Filho A, Simões MV, Marin-Neto JA, Zago MA, Franco R. Gene polymorphisms in the TNF locus and the risk of myocardial infarction. Thrombosis Res. 2001; 100:263-9 - *Enclosure*

B.1.2 Martinelli AL, Zago MA, Roselino AM, Villanova MG, Secaf M, Tavela MH, Zucoloto S, et al. Porphyria cutanea tarda in Brazilian patients: association with hemochromatosis C282Y mutation and hepatitis C virus infection. Am. J. of Gastroenterology 2001; 95:3516-21 - *Enclosure*

B.1.3 Zago MA, Silva-Jr WA, Gualandro S, Araujo AG, Tavela MH, Gerard N, Krishnamoorthy R., Elion J. Rearrangements of the beta-globin gene cluster in apparently typical beta-S haplotypes. Haematol. 2001; 86:142-5 – *Enclosure*

B.1.4 Martinelli AL, Franco R, Villanova MG, Figueiredo JFC, Secaf M, Tavela MH, Zucoloto S, Zago MA. Are haemochromatosis mutations related to the severity of liver disease in hepatits C virus infection? Acta Haematol. 2000; 102:152-6 - *Enclosure*

B.1.5 Rodriguez-Delfin L, Rubin CV, Zago MA. Genetic diversity in an Andean population from Peru and regional migration patterns of Amerindians in South America: data from Y chromosome and mitochondrial DNA. Human Heredity 2000; 51:97-106 – *Enclosure*

B.1.6 Souza SJ, Camargo AA, Briones M, Costa FF, Nagai MA, Verjovski-Almeida S, Zago MA, Dias-Neto E, Simpson AJ. Identification of human chromosome 22 transcribed sequences with ORF expressed sequence tags. Proc. Nat. Acad. Sci. of the USA 2000; 97:12690-3 - *Enclosure*

B.1.7 Dias-Neto E, Garcia-Correa R, Verjovski-Almeida S, Briones M, Nagai MA, Silva-Jr WA, Zago MA, et al. Shotgun sequencing of the human transcriptome with ORF expressed sequence tags. Proc. Nat. Acad. Sci. of the USA 2000; 97:3491-6 – *Enclosure*

B.1.8 Simpson AJ, Reinach FC, Arruda P, Briones M, Camargo AA, Costa FF, Dias-Neto E, Nagai MA, Zago MA. Setubal Jc The genome sequence of the plant pathogen Xylella fastidiosa. Nature 2000; 406:151-9 - *Enclosure*

B.1.9 Ribeiro-Dos-Santos AKC, Guerreiro JF, Santos SEB, Zago MA. The split of the Arara population: comparison of genetic drift and founder effect. Human Heredity 2000; 51:79–84 – *Enclosure*

B.1.10 Calado R, Franco RF, Pazin Filho A, Simões M, Marin Neto J, Zago MA. HFE Gene Mutations In Coronary Atherothrombotic Disease. Braz. J. Med. Biol. Res 2000; 33:301–6 – *Enclosure*

B.1.11 Chen F, Covas DT, Baffa O. Dosimetry of blood irradiation using an alanine/ESR dosimeter. Appl Radiat Isot 2001; 55:13-6 – *Enclosure*

B.1.12 Covas DT, Biscaro TA, Nasciutti DC, Guerreiro JF, Santos SE, Zago MA. Gene frequencies of the HPA-3 and HPA-5 platelet antigen alleles among the Amerindians. Eur J Haematol 2000; 65:128-31 – *Enclosure*

B.1.13 Defovery R, Lemos JÁ, Kashima S, Bernardes JE, Scridelli CA, Covas DT, Tone LG. Analysis of the p53 gene by PCR-SSCP in ten cases of Wilms' tumor. São Paulo Med J. 2000; 118:49-52 - *Enclosure*

B.1.14 Fontes AM, Riedl A, Jacobs-Lorena M. Devolopmental regulation of na instability element from the Drosophila fushi tarazu mRNA. Genesis 2001; 30:59-64 - *Enclosure*

B.1.15 Rego EM, Pandolfi PP. Analysis of the Molecular Genetics of Acute Promyelocytic Leukemia in mouse models. Sem. In Hematology.2001; 38:154 -70 – *Enclosure*

B.1.16 Rego EM, Garcia AB, Falcao R.P, Carneiro JJ. Immunophenotype of normal and Leukemic Bone Marrow B-Precursors in a brazilian population. a comparative analysis by quantitative fluorescence cytometry. brazilian J. Med. Biol. Res. 2001; 34:183-94 – *Enclosure*

B.1.17 Rego EM, Wang Z, Peruzzi D, HE L, Cordon-Cardo C, Pandolfi PP. Role of Promyelocytic Leukemia (PML) Protein in Tumor Suppression. Journal of Experimental Medicine.2001; 193:521-30 – *Enclosure*

B.1.18 Rego EM, HE L, RP Warrell, Wang Z, Pandolfi PP. Retinoic Acid (RA) and As2O3 treatment in transgenic mouse models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RARα and PLZF-RARα oncoproteins. Proc. Nat. Acad. Sci. of the USA 2000; 97-18:10173-8 - *Enclosure*

B.1.19 HE L, Bhaumik M, Tribioli C, Zelent A, Rego EM, Pandolfi PP, Ivins S. Two critical hits for promyelocytic leukemia. Mol. Cell. 2000; 6-5:1131–41 – *Enclosure*

B.1.20 Rego EM., Garcia AB, Carneiro JJ, Falcao RP. Immunophenotype of normal and leukemic bone marrow B-precursors in a Brazilian population. A comparative analysis by quantitatuve fluorescence cytometry. Braz. J. Med. Biol. Res 2001; 34:183-94 – *Enclosure*

B.1.21 Voltarelli JC, Ahmed H, Paton E, Stracieri AB, Holman P, Bashey A, et al. Beneficial effect of intravenous lidocaine in cutaneous chronic graft-versus disease secondary to donor lymphocyte infusion. Bone Marrow Transplant 2001; 28:97-9 - *Enclosure*

B.1.22 Souza SS, Castro FA, Mendonca HC, Palma PV, Morais FR, Ferriani RA, Voltarelli JC. Influence of menstrual cycle on NK activity. J Reprod Immunol 2001; 50:151-9 - *Enclosure*

B.1.23 Voltarelli JC – Applications of flow cytometry to hematopoietic stem cell transplantation. Mem Inst Oswaldo Cruz 2000; 95:403-14 - *Enclosure*

B.1.24 Troncon EA, Dantas RO, Figueiredo JFC, Ferrioli E, Moriguti JC, Martinelli ALC, Voltarelli JC. A standardized, structured long-case examination of clinical Competence of senior medical students. Medical Teacher 2000; 22:380-5 - *Enclosure*

B.1.25 Voltarelli JC, Stracieri ABPL. Aspectos imunológicos dos transplantes de células hematopoéticas. Medicina Ribeirão Preto 2000; 33:443-62 - *Enclosure*

B.1.26 Donadi EA, Smith AG, Louzada-Junior P, Voltarelli JC, Nepom GT. HLA class I and class II profiles of patients presenting with Sydenham's cho. J Neurol 2000; 247:122-8 - *Enclosure*

B.1.27 Cereia M, Terenzi HF, Jorge JA, Greene LJ, Rosa JC, Polizeli MLTM. Glucoamylase activity from the thermophilic fungus *Scytalidium thermophilum.* Biochemical and regulatory properties. J. Basic Microbiol 2000; 40:83-92 - *Enclosure*

B.1.28 Amorim CRN, Matsuura MSA, Rosa JC, Greene LJ, Leite DS, Yano T. Purification and characterization of the fimbria F18ac (2134P) isolated from Escherichia coli enterotoxigenic (ETEC). Veterinary Microbiology 2000; 76:41-9 - *Enclosure*

B.1.29 Panunto-Castelo A, Almeida IC, Rosa JC, Greene LJ, Roque-Barreira MC. The Rubino test for leprosy is a ß2-glycoprotein 1-dependent antiphospholipid reaction. Immunology 2000; 101:147-53 – *Enclosure*

B.1.30 Ward RJ, Oliveira AHC, Bortoleto RK, Rosa JC, Faça V, Greene LJ. Expression and the purification of a disulphide rich protein in a hydrophobic resin environment, bothropstoxin-I a Lys49-phospholipase A2 homologue. Protein Expression and Purification 2001; 21:134-40 – Enclosure

B.1.31 Lourenço EV, Pereira SR, Faça VM, Coelho-Castelo AAM, Mineo JR, Roque-Barreira MC, Greene LJ, Panunto-Castelo A. Toxoplasma gondii micronemal protein MICI is a lactose-binding lectin. Glycobiology 2001; 11:541-7 - *Enclosure*

B.1.32 Franco RF, Fagundes MG, Reitsma PH, Lourenco D, Morelli V, Maffei FH, Piccinato CE, Silva-Jr WA, Zago MA. Identification of polymorphisms in the 5'-untranslated region of the TAFI gene: relationship with the plasma TAFI levels and risk of venous thrombosis. Haematologica 2001; 86:510-7 - *Enclosure*

B.1.33 Attie-Castro FA, Zago MA, Lavinha JE, J Rodriguez-Delfin L, Guerreiro JF, Franco RF. Ethnic heterogeneity of the factor XIII Val 34 Leu polymorphism. Thrombosis and Haemostasis 2000; 84:601-3 – *Enclosure*

B.1.34 Franco RF, Pazin-Filho A, Tavela MH, Simoes MV, Marin-Neto JA, Zago MA. Factor XIII Val 34 Leu and the risk of myocardial infarction. Haematologica 2000; 85:67-71 – *Enclosure*

B.1.35 Franco RF, Lourenço D, Morelli V, Maffei F, Piccinato C, Zago MA, Fagundes MG, Reitsma P, Meijers JCM. Identification of Polymorphisms In The 5'-Untranslated Region of The Tafi Gene Relationship With Plasma Tafi Levels and Risk of Venous Thrombosis Haematologica 2001; 86:510-7 - *Enclosure*

B.1.36 Franco RF, Middeldorp SPHM. JVPER. Factor XIII Val34leu and the risk of venous thromboembolism in factor V leiden carriers. Brit. J. Haematol 2000; 111:118-121 – *Enclosure*

B.1.37 Martins SL, Falcao RP. A importância da imunofenotipagem na leucemia mielóide aguda. Revista da Associação Médica Brasileira 2000; 46:57–62 - *Enclosure*

B.1.38 Falcao RP, Simões BP, Garcia AB, Terrafilho J. Aggressive variant of morphologically typical T-large granular lymphocyte leukemia lacking NK cell markers. Acta Haematologica 2000; 104:115-8 – *Enclosure*

B.1.39 Falcao RP, Calado RT. Anemia aplástica grave diagnóstico e fisiopatologia. Séries de Monografias da Escola Brasileira de Hematologia. 2000; 7:1-18 - *Enclosure*

B.1.40 Murta EFC, Andrade JM, Falcao RP, Bighetti S. Lymphocyte subpopulations in patients with advanced breast cancer submitted to neoadjuvant chemotherapy. Tumori 2000*;* 86:403-7 – *Enclosure*

B.1.41 Rego EM, Garcia AB, Falcão RP. Determination of the CD10, CD19,TdT, CD34 and CD79a densities by quantitative cytometry in B- precursors in the bone . Brazilian Journal of Medical and Biological Research. 2001; 34: 183-94 - *Enclosure*

B.1.42 Silva-Jr WA, Costa MC, Valente V, Paco-Larson ML, Espreafico EM, Zago MA, Simpson AJG, Dias-Neto E. PCR template preparation for capillary DNA Sequencing. *Biotech.*. 2001;30:540-2 - *Enclosure*

B.1.43 Zago MA, Silva-Jr WA, Gualandro S, Hutz MH, Tavela MH, Araujo AG, et al. Krishnamoorthy R. Atypical beta^{S} haplotypes are generated by diverse genetic mechanisms. Am. J. Hematol 2000; 63:79-84 - *Enclosure*

B.1.44 Acosta AX, Silva-Jr WA, Carvalho TM, Zago MA. Tem novel mutations in the phenylalanine hidroxylase gene (PAH) observed in Brazilian patients with phenylketonuria. Human Mutation 2001; 17:77-8 – Enclosure

B.2 - Books and Chapters

B.2.1 Zago MA - Eritropoese e Eritropoetina. Produção e Destruição de Hemácias. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 23 – 31.

B.2.2 Zago MA - Granulócitos: Produção, Dinâmica e Função. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 33 – 43.

B.2.3 Zago MA - Monócitos e Macrófagos. Sistema de fagócitos mononucleares. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 45 – 49.

B.2.4 Zago MA - O Paciente com Anemia. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 103 – 113.

B.2.5 Zago MA - O Paciente com Esplenomegalia. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 115 – 123.

B.2.6 Zago MA - Deficiência de glicose-6-fosfato desidrogenase. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp.265 - 268.

B.2.7 Zago MA - Estrutura, Síntese e Genética das Hemoglobinas. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 269 - 277.

B.2.8 Zago MA - Defeitos Hereditários das Hemoglobinas. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 279 - 287.

B.2.9 Zago MA – Talassemias. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 309 – 328.

B.2.10 Covas DT - Suporte Transfusional de Pacientes com Neoplasias Hematolopoéticas. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp.413 - 417.

B.2.11 Covas DT – Retrovírus. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 691 – 704.

B.2.12 Covas DT, Zago MA - Antígenos Eritrocitários, Leucocitários e Plaquetários. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 951 – 975.

B.2.13 Covas DT - Doenças Infecciosas Transmissíveis por Transfusões Sangüíneas. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 977 - 1043.

B.2.14 Rego EM, Falcao RP. (2001) Classificação Das Neoplasias Hematológicas. Marcadores. Imunofenotipagem. **In:** Hematologia. Fundamentos E Prática..1ª Ed.São Paulo : Ateneu

B.2.15 HE L, Rego EM**,** Pandolfi PP. Acute Promyelocytic Leukemia **In:** Encyclopedic Reference of Cancer. 1º ed. Heidelberg Springer-Verlag ,2001.

B.2.16 Rego EM**,** Falcão RP. Classificação das Neoplasias Hematológicas marcadores Imunofenotipagem **In:** Hematologia, Fundamentos e Prática 1º ed. São Paulo: Ateneu, 2001.

B.2.17 Rego EM. Hematopoese Regulação e Microambiente. em Hematologia. **In:** Hematologia. Fundamentos e Prática.1ª ed.São Paulo : Ateneu, 2001

B.2.18 Martins S L, Rego EM, Falcão RP. Leucemias agudas classificação: citologia, citoquímica e imunofenotipagem **In:** Hematologia. Fundamentos e Prática.1ª ed.São Paulo Ateneu, 2001

B.2.19 Rego EM - Hematopoese. Regulação e Microambiente. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 15 – 22.

B.2.20 Franco RF, B Garicochea Choque: Distúrbios da Coagulação **In:** Choque. Ed.Porto Alegre : Edipuc-Rs, 2001

B.2.21 Franco RF. Defeitos Moleculares Nas Hemofilias A E B. **In:** Hematologia: Fundamentos e Pratica Ed.Sao Paulo : Atheneu, 2001

B.2.22 Franco RF. Diagnóstico De Doenças Hereditárias Multigênicas. Trombofilias **In**: Clínica Médica. Medicina Celular e Molecular Ed.São Paulo (In Press), 2001

B.2.23 Franco RF. Fisiologia da Coagulação E Fibrin'olise. **In:** Hematologia Fundamentos e Prática.1º Ed.São Paulo Atheneu.

B.2.24 Franco RF. EG. Rizzatti. O Paciente com Manifestações Hemorrágicas **In:** Hematologia: Fundamentos e Prática.1 Ed.São Paulo Atheneu, 2001.

B.2.25 Franco RF, AA Garcia. Trombofilias Adquiridas **In:** Doenças Vasculares Periféricas (Terceira Edição).3º Ed.São Paulo Medsi, 2001.

B.2.26 Franco RF, AA Garcia. Trombofilias Adquiridas. **In:** Hematologia: Fundamentos e Prática Ed.Sao Paulo Atheneu, 2001.

B.2.27 Franco RF. Trombofilias Hereditárias **In:** Doenças Vasculares Periféricas (Terceira Edição).3 Ed.São Paulo : Medsi, 2001.

B.2.28 Franco RF. Trombofilias Bases Moleculares **In:** Hematologia: Fundamentos e Prática.1 Ed.São Paulo : Atheneu, 2001.

B.2.29 Franco RF – Bases Moleculares das Hemofilias A e B. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 797 - 802.

B.2.30 Franco RF – Trombofilias. Bases Moleculares. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 843 - 854.

B.2.31 Falcão RP, Calado RT - Heterogeneidade das Células do Sangue. Órgãos hematopoéticos e linfopoéticos. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 3 – 13.

B.2.32 Falcão RP, Voltarelli JC - Ontogênese e Diferenciação do Tecido Linfóide. Dinâmica dos Linfócitos. Imunidade Celular e Humoral. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 51 – 67.

B.2.33 Falcão RP - O Paciente com Linfonodomegalia. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 125 – 131.

B.2.34 Falcão RP, Calado RT - Anemia Ferropriva. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp.223 - 229.

B.2.35 Falcão RP, Rego EM - Classificação das Neoplasias Hematológicas. Marcadores. Imunofenotipagem. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 383 - 400.

B.2.36 Falcão RP - Leucemia Linfocítica Crônica. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 569 - 575.

B.2.37 Rizzatti EG, Franco RF - O Paciente com Manifestações Hemorrágicas. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 133 – 142.

B.2.38 Malvezzi M, Zago MA – Deificências de Vitamina B_{12} e de Folatos: Anemias Megaloblásticas. In Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 195 – 210.

B.2.39 Calado RT, Alberto FL, Falcão RP - Metabolismo do Ferro. In Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp. 213 - 222.

B.2.40 Garicochea B, Zago MA - Bases Moleculares e Citogenéticas. Oncogenes e Antioncogenes. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp.367 - 381.

B.2.41 Martins SLR, Rego EM, Falcão RP - Classificação das Leucemias Agudas: Citologia, Citoquímica e Imunofenotipagem. **In** Zago MA, Falcão RP, Pasquini R (eds) Hematologia – Fundamentos e Prática, Editora Ateneu, Rio de Janeiro, 2001, pp.433 - 445.

B.3 – Papers in press

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TECHNOLOGICAL ACHIEVEMENTS

- Quality Control program ISO 9002 Certified GMP Self diagnosis software developed.
- Umbilical Cord Bank in now operating.
- Viral proteins cloned and expressed in mammalian cells.

1- Education Activites

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

Video of presentation of the course	Visit of the Pro-Dean of USP, Hernan Chaimovich
CD on the research lines of CTC	Presentation of the CTC researchers
CD with lectures by the researchers of USP/Blood Center/CTC	Opening of the course
CD of the presentation of the topics selected by the groups	Formation of teacher groups advised by researchers
Class for teachers about "Life Rhythms - Chronobiology"	Exhibition of "Life Rhythms - Chronobiology"
Class for students that acted as monitors at the exhibition of Chronobiology	Exhibition of "Life Rhythms - Chronobiology"
Biosafety class for students	Talent Hunt Project
Class given by Prof. Ana Rosa Orellana Soprani (São Paulo)	**Diversified activities in the classroom**
Class given by Prof. Dr. Dalton de Souza Amorim –FFCLRP/USP	Partnerships with the other faculties of the Campus
Class given by the CTC researchers in schools	Activities of the research groups
Exhibit of part of the LEC patrimony	Visit of the Pro-Dean of Culture and Extension of USP, Prof. Adilson Avansi de Abreu, and of Prof. José Carlos Teixeira de Barros Moraes
Class given by Dr. Marina Coutinho on bone marrow transplantation	Activity of the group that studies the topic "Umbilical Cord Cells"
Video about the participating schools (material for the EPTV TV Journal, Rede Globo Network)	Dissemination of the CTC Educational Project
Work by teachers and students	Science Teaching Site http://ctc.fmrp.usp.br, then click on Talent Hurt and Science Journal
"I Meeting on HIV and Society – Education and Prevention"	Activities of the group that studies the topic "HIV and Society"

Science Workshop - MuLEC

Participation in congresses: “47th National Congress of Genetics” and “Meeting on Research in Education, Communication and Scientific Dissemination in Museums”.	Dissemination of scientific papers
Summer Course	Trip to the Genome
Specialization Course	Molecular Biology

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** entitled “Voyage to the Genome” was taught for the second consecutive year to undergraduate students. The course was taught in January 2001 and counted with the participation of 23 undergraduate students from the most diverse areas and geographic regions (see enclosure). As part of the activities, the students compiled a newsletter called “Genoma News” which was widely distributed and which is available on the home page of the Center. The course “Techniques of Molecular Biology” was taught during the first semester of 2001 to postgraduate students and higher level technicians. A total of 35 students participated in this course, with 40 hours of activity.

2. RESULTS OF BASIC RESEARCH

The Center for Cell Based Therapy did not exist as a unit before being funded by FAPESP. It was formed by the association of four previously existing research groups that collaborated and had interests in broadly overlapping areas (hematology, blood transfusion, cancer, genome, protein chemistry), but the researchers had not worked together with a common objective. Since the setting up of the center by FAPESP the researchers started to work on their specific projects with the common target of cell therapy. In nine months since the beginning of the activities various projects covering the research objectives of the center proposal are underway, and some solid results have already been obtained, although the publications list does not reflect this progress yet, because most of the published articles of the researchers are the consequence of previous research activities that are being completed or going on.

The research program includes 5 cell biology projects directly related to cell-terapy: 1) Cancer vaccine for chronic myeloid leukemia; 2) Drug therapy approach to purging Ph^- progenitor cells from chronic myelogenous leukemia patients; 3) Evaluation of gene expression during differentiation and maturation of cord blood CD34-derived dendritic cells using proteomic analysis, 4) Profiling changes in TPO expression level and CD34 cell numbers in normal donors after plateletapheresis, and 5) Use of umbilical cord cells for transplantation.

The principal results obtained are: 1) we evaluated the ability to differentiate CB-CD34+ progenitor cells and PB-monocyte cells into dendritic cells (DC) using the appropriate cytokine requirements from normal blood and CML patients. Human mature DC with typical morphology and surface antigen phenotype ($CD1a^+$, $CD83^+$ and $CD86^+$) were obtained from PB-monocyte cells after 7-11 days of culture in a yield of 52-86%, and from CB-CD34+ progenitor cells in a yield of 21-32% from normal donors. However, from leukemia patients the yield of DC differentiated from PB-monocyte cells was 6-21% and from CB-CD34+ was 5-14% and the typical morphology was less pronounced; 2) we are evaluating the effect of cytarabine (5 ng/ml, 50 ng/ml e 250 ng/ml), STI571 (0.1, 1.0 and 10.0 mM) and staurosporine (5 mg/ml) after 24, 48 and 72 h of drug exposure in peripheral blood mononuclear cells from chronic myelogenous leukemia patients using three different approaches: a) clonogeneic cell assay; b) flow cytometric analysis to detect induction of apoptosis, and c) FISH. The most effective treatment was obtained with cytarabine (5 ng/ml) after 48-72 h of drug exposure which suppressed 52 and 89% of CFU-GM and BFU-E colony formation, respectively, and increased the level of apoptosis to 16% when compared with untreated CML PB mononuclear cells. Analysis of individual CML colonies for the presence of the hybrid BCR/ABL mRNA by reverse transcription-polymerase chain reaction (RT-PCR) and FISH is currently underway to demonstrate whether or not the selection of Ph^- PB-MN cells was obtained after these in vitro drug treatments; 3) we evaluated different methods for isolation, selection and purification of $CD34^+$ and monocyte cells from PB donors. A 1% recovery of CD34+ cells from the total nucleated cells collected was obtained with a purity of 99%. Also, we compared two different methods to purify monocytes by density centrifugation: a) Ficoll-Hypaque or b) Percoll. Using flow cytometry, we determined that the percentage of CD14-positive cells was significantly greater after Percoll centrifugation (60-76%) when compared with Ficoll centrifugation (6-13%). These results suggest that the Percoll protocol should be performed on in vitro DC differentiation in order to generate in vitro culture of human DC for proteomic analysis; 4) we evaluated the plateletapheresis products of 17 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 the third day. The number of reticulated platelet and CD 34+ cells showed no significant difference after apheresis when compared with pre-apheresis. At the moment we are evaluating the number of megakaryocyte colonies and the level of TPO to calculate the correlation with the above results.

In another study, we showed that the therapeutic effect of alpha-interferon on the chronic phase of chronic myelogenous leukemia is associated with activation of several components of the immune system (NK killing, intracellular cytokines and expression of DR and Fas molecules in immunocompetent cells).

Cord Blood (CB) cells have been used as a source of hematopoietic stem cells for transplantation. Delay of engraftment and probability of graft failure after CB transplants have been associated with the number of nucleated and CD34+ cells infused. Bone marrow (BM) T CD8+, CD8+TCRαβ , and CD8 non T-cells TCRαβ – facilitate engraftment of progenitors BM cells. The capacity of homing of stem cells has been attributed to the presence of CXCR4 in CD34+ and in CD3+ cells. In order to evaluate CB subpopulations that have the capacity of homing and facilitation of CB engraftment, we analyzed 22 cord blood units. A panel of antibodies for the above cell subset was used in flow cytometry. Results were compared with peripheral blood (PB) from mothers using the Kruskal-Wallis test. Results: The mean number of white blood cells and lymphocytes was 10.2x109/L (3.0-19.0) and 4.4x109/L (1.8-9.8), respectively, in CB and 14.0x109/L (6.0-24.0) and 2.3x109/L (0.7-7.5), respectively, in mothers. The mean percentage of CD34+CXCR4+ and CD3+CXCR4+ cell numbers was 1.3% and 7.5%, respectively, in CB and 1.1% (p=0.76) and 27.0% (p=0.003) in mothers. The mean percentage of CD3+Cd8+TCRαβ cells was 16.5% in CB and 28.2% in mothers (p>0.001) and the mean percentage of CD3-CD8+TCRαβ - cells was 9.5% and 5.4%, respectively (p=0.002). In conclusion, these results demonstrate the presence of subpopulations of facilitating cells for engraftment and homing in cord blood units. Percentages of CD3+CXCR4+ and CD3+Cd8+TCRαβ + cells are lower and percentages of CD3-Cd8+TCRαβ - cells are higher in CB compared to mother PB. Since the number of cells present in the CB graft is 10 times less than in the BM graft and approximately 100 times less then in the PB graft, quantitative and functional assays must be performed on animals to demonstrate that these cell subsets can facilitate and improve engraftment of a CB graft.

The search for immunological approaches to treat neoplasias includes the evaluation of antigens expressed by normal and abnormal cells. We are examining the expression of tumor antigens in chronic lymphoid neoplasias. Thus far we observed the modest expression of some genes of the MAGE series, and a more important expression of genes of the PRAME antigens. Also, the analysis of expression of GD3 ganglioside and its derivative de-N-acetyl-GD3 (d-NAc-GD3) in normal and abnormal lymphoid cells revealed unexpected results. GD3 displays similar distribution between normal and leukemic cells while de-NAc-GD3 accumulates in normal T CD3+ and CD 38+ cells but not in B CD19+ cells. Lymphocytes from leukemia patients display an inversion in this pattern, with CD19+ cells accumulating more d-NAc-GD3 than CD3+ and Cd38+ cells.

Acute promyelocytic leukemia (APL) is a useful model for the study of leukemogenesis. Currently, 5 subprojects are underway on this subject: a) Analysis of the effect of PML inactivation of APL leukemogenesis; b) Analysis of the NPM-RARα oncogenic activity on a transgenic mouse model; c) Analysis of the effect of histone deacetylases inhibitors on gene expression of APL cells; d) Analysis of hemostasis and pro-inflammatory factors in transgenic mouse models of leukemia; e) Study of cyclin A1 expression in APL cells.; f) Analysis of cell cycle and apoptosis in myeloid progenitors of PML-RARα transgenic mice during the pre leukemic phase. The main results were: 1) By crossing PML-RARα TM with PML knock-out mice, we demonstrated that PML inactivation leads to an increase of

the leukemogenic activity of the PML-RARα fusion protein. 2) Leukemic cells harboring PML haploinsufficiency or PML complete inactivation are resistant to the pro-apoptotic effect of Fas and to the pro differentiating effect of retinoic acid and vitamin D_3. 3) For subproject (b), we observed that NPM-RARα TM develop a form of disease characterized by adenopathy, hepato-splenomegaly, anemia and thrombocytopenia. Some of these mice also present leukocytosis with circulating tumor cells; 4) The morphologic, cytochemical and immunophenotypic characterization of tumor cells in lymph nodes, spleen, bone marrow and liver demonstrated monocytic involvement.

The MDR-1 gene is the most important responsible for drug resistance in chenotherapy. We are particularly interested in studying the pattern and regulation of expression of this gene. We evaluated the activity of p-gp on CD34+ bone marrow cells from aplastic anemia patients. The activity of P-gp was decreased in CD34+ progenitor cells from patients with aplastic anemia at diagnosis and in remission after immunosupressive therapy; and was restored after bone marrow transplantation. These findings suggest that the decreased activity of P-gp cells may contribute to increasing the susceptibility of CD34+ stem cells to drugs and other toxic substances.

Another research line concerns the characterization of the transcriptome of normal and cancerigenous cells. These studies were started in the Human Cancer Genome Project (Projeto Genoma Humano do Câncer - PGHC: http://www.ludwig.org.br/ORESTES), in which we actively participated by sequencing a total of 267 thousand expressed sequence tags (ESTs) from different cancers. As part of a larger project that produced over 1.2 million ESTs from different cancer tissues, we constructed a set of 51,102 ESTs obtained from bone marrow cells of patients with CML and CLL, which represent partial expressed gene sequences that are biased toward the central coding regions of the resulting transcripts. The ESTs were classified on the basis of the annotation of the matched sequences into 8 functional categories (cell cycle 5.0%, cell motility and structure 9.3%, signaling and communication 31.0%, DNA metabolism 3.8%, RNA metabolism 10.3%, defense and homeostasis 7.9%, metabolism 24.7%, protein metabolism 7.9%).

Several projects are being developed using this data base. An example is the project aiming at the identification and validation of single nucleotide polymorphisms (SNPs), which has already identified 278 SNP candidates distributed among 163 genes, 176 of which are mutations of the non-synonymous type. Using the same approach as that of PGHC, we have started a project for the characterization of the transcriptome of hematopoietic neoplasias. A total of 7,836 ESTs were generated and annotated as known genes (6,503), paralogues (11), orthologues (10), and unknown genes (1,312 no-matches). Some analyses will be carried out to characterize these transcripts, such as the functional classification of the transcripts, identification of alternative splicing, paralogues, a search for functional domains in unknown sequences, genomic mapping etc. The results of these analyses will be of fundamental importance for the udnerstanding of the genetic model of hematopoietic neoplasias. As a continuation of this project, we ar now carring out sequencing EST obtained from a larger spectrum of hematopoietic neoplasias.

Research on the biological value of polymorphisms of selected genes has produced two results that are relevant to cell therapy and neoplastic diseases. The myeloperoxidase gene is highly expressed in granulocytes, where it has an important bactericidal role. A polymorphism in the promoter region of the gene (A/G at – 463) influences the level of expression. In patients submitted to bone marrow transplantation for leukemia, we demonstrated that the allele with lower enzyme expression in the donor cells is associated

with a significantly higher incidence of bacterial infection immediately post-transplantation. We also demonstrated that the common thermolabile variant of methylenetetrahydrofolate reductase (C677T) is associated with a lower risk for the development of acute lymphoblastic leukemia in childhood, possibly because the lower enzyme level reduces uracyl misincorporation into DNA (owing to the reduced pool of methyl-tetrahydrofolate).

Some of most common viral infections are caused by retroviruses such as HTLVI/II, HIVI/II, and the hepatropic virus TTV has been the subject of study at this cell therapy center in the following projects: 1) Molecular characterization of a human immunodeficiency virus type 1 (HIV-1) isolated from Ribeirão Preto; 2) Sequence characterization of an HTLV type I isolate from Ribeirão Preto; 3) Cloning and expression of HTLV-1 structural genes in mammalian cells and 4) Molecular epidemiology of TT virus (TTV) and characterization of the TTV genotypes in Ribeirão Preto. The principal results obtained were: 1) and 2) Primary cultures of peripheral blood mononuclear cells (PBMC) from HIV+ and HTLV-1^{+} patients were set up with two immortalized primary human lymphocytes, Molt-4 and Jurkat cells. The presence of human retrovirus HIV-1 and human T-lymphotropic retrovirus I was demonstrated: a) by detection of cytopathic effects by light microscopy; b) by indirect immunofluorescence assays and c) by determination of the V3 region of gp120 of HIV and of the long terminal repeat (LTR) of HTLV by the polymerase chain reaction after infection. The optimal time for HIV-1 and HTLV-1 infection was obtained between 13 and 30 days. In addition, we determined the DNA sequence of 37 and 93% of the HIV and HTLV genome, respectively. At this time, molecular epidemiology and phylogenetic studies have been conducted on 50 HTLV patients by amplification and sequencing of proviral DNAs of three genomic regions: the long terminal repeat, pol and tax region; 3) we have cloned two structural genes from HTLV-1, env and gag gene into a DNA plasmid vector. At this moment we are analyzing the DNA sequence of these clones, and 4) we determined the prevalence of TT virus (TTV) DNA among 270 blood donors, 43 transfused recipients and 41 hemophilic patients which was 11, 33 and 44%, respectively, by the polymerase chain reaction (PCR) with primers derived from a conserved region. Also, the amplified PCR products of 11 blood donors were subjected to sequence analysis. Nine of them were classified into genotype 1 and 2 into genotype 2. At this moment, we are conducting the genotyping of TTV isolates both from transfused recipient and hemophilic patient populations.

Two other studies are currently in the phase of standardization of laboratory methodology. We are measuring intracellular cytokine levels (pro- and anti-inflammatory) in populations of blood monocytes and lymphocytes after allogeneic bone marrow transplantation. The sequential results will be correlated with clinical events experienced by the patients. We are comparing three methods of detection of cytomegalovirus infection (antigenemia, PCR and flow cytometry) in immunocompromised patients. The results will show the regional epidemiology of CMV infection in these patient populations and also provide means of early therapeutic intervention.

Finally, the program of hematopoietic stem cell transplantation (HSCT) for autoimmune diseases (AIDs) started with a refractory nephrotic syndrome lupus patient receiving an autologous transplantation after conditioning with cyclophosphamide and antithymocyte globulin. Clinical and immunologic recovery is being monitored and

plasma/cell samples are being stored for future studies of the immunopathogenesis of AIDs and the effects of intensive immunosuppression.

What about deleterious effects of cell therapy? In the last year there has been considerable controversy as to the occurence of immunodepression after the simplest form of cell therapy: red cell transfusion. To investigate the possible mechanisms involved, 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. Two samples were analyzed from each patient, one early after transfusion and the other after one year. Microchimerism was detected longer (20-60 days) than previously reported in the literature (5-7 days), but not one year after transfusion. These results suggest a role for microchimerism in prolonged transfusion-related immunomodulation, but not in the induction of autoimmunity, as recently shown for systemic sclerosis (fetal/maternal chimerism).

In addition to research directed at cell therapy, a significant activity of research on cell and molecular biology is currently being carried out at the center, focused on: a) human population and medical genetics, especially the genetic diversity of Brazilian Amerindian and African-derived populations, b) thrombophilia, c) polymorphism of blood group antigenes For instance, 10 new mutations in the gene of phenylalanine hydroxylase have been reported by us, observed in families with phenylketonuria, and the role of single nucleotide polymorphisms of various genes has been explored in thrombophilia and in porphyria. We also described novel polymorphisms in the promoter region of the TAFI gene that are associated with the level of the TAFI protein in plasma and possibly with the risk of thrombosis. Two projects are focused on population screening in order to determine variation, genetic distance, and gene flow, parameters useful for the genetic characterization of Brazilian populations: 1) Differential contribution of Indigenous, Caucasian and African populations to the formation of an urban population in the Bahia and Ribeirão Preto as revealed by mtDNA and Y-DNA, and 2) Phenotype-genotype relationship of the Duffy blood group in Caucasian, African-American black and Asian populations. The principal results obtained were: 1) The analyses of 7 markers of mitochondrial DNA and 8 markers of Y-DNA from 509 individuals demonstrated that the contribution of the African population to the formation of the Bahia group was 78%, which supports historical data; 2) The PCR and DNA sequence of the FY region from 35 Caucasians, 48 African-American blacks and 33 Asians have been determined. At the moment, we are studying additional samples for further phylogenetic analysis of Duffy blood group in these three populations.

Taken together, these results show that most of the methodologies required to develop research projects related to cell therapy were successfully set up. In addition to results obtained from continuing previous research activities, novel lines specifically aimed at cell therapy are producing their first results.

Technology Transfer Project

Specific Projects already developed

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 from the ***Blood Coordination of São Paulo State Health Secretary***. The Certificate itself is presented (enclosure)

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 last August. This program was supported by a Public Policy grant from FAPESP. A version of the software is included.

Umbilical Cord Blood Bank (UCBB)

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.

New Projects

Cloning and expression of proteins of biotechnological interest

After the Center started its activities, special emphasis was placed on the development of research with immediate biotechnological applications. Several projects were started with the main objective of expressing *in vitro* several proteins that might be used for therapeutic or diagnostic purposes. Among the projects currently underway are the following:

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. We recently obtained the expression of glycoprotein 160 (gp160) of the viral envelope of HIV in mammalian cells (a summary of the study is enclosed). Other HIV regions are being cloned. The final objective is to obtain a set of recombinant antigens that will be used for the development of chemoluminescent diagnostic tests, as previously done for the diagnostic test of Chagas' diseases developed and patented by this Center.

2. **Production of HTLV-1 recombinant proteins**

Similar to the previous one, an expression vector with the region coding for gp21 of HTLV-1 was recently obtained. Work is currently underway to transfect mammalian cells with this vector to test its expression efficiency.

3. **Development of diagnostic tests for HIV-1 and HTLV-1 infection**

Another project complementing the previous ones is the development of chemoluminescent diagnostic tests (ELISA and Western Blot) using antigens derived from viral lysis. Viral cultures for HIV-1 and HTLV-1 have been established for this purpose (summary enclosed).

4. **Production of human clotting factor VIII by gene recombination.**

The objective of this project is to clone and express human clotting factor VIII. This factor is absent or altered in patients with hemophilia A, who depend on infusion of exogenous factors for the normalization of the coagulation process. These exogenous factors are obtained by processing human plasma or, more recently, by recombinant techniques. In the current phase of the project the cDNA of factor VIII was isolated from a human liver library and work is underway to introduce this cDNA into appropriate expression vectors. This project also counts with financial support from FINEP.

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 na important item in the CTC program. In numerows occasions the CTC has been the focus or the source of news and information in the press and television.

CTC Educational Project

History

Since the beginning, the Cell Therapy Center (CTC) Educational Project has counted with the participation of teachers and representatives of the Education Director Offices of the Ribeirão Preto region in order to define the basis for the elaboration of the Project, as required by Cepid. **(Enclosure 1)**

The teachers promptly adhered to the project since they realized that its justification was based on previous evidence, in particular that contained in continued training programs developed in the Laboratory of Science Education (LSE), Faculty of Philosophy, Sciences and Letters/USP). Another relevant factor was the lecture given by the journalist Mônica Teixeira. When explaining her engagement in the study of a scientific matter, she convinced the teachers that, even if they are not researchers they are in a position to carry out scientific educational work. In her speech, the journalist proved that she has become a specialist in the Genome subject by visiting the sites involved and by documenting the stages of scientific research **(Enclosure 2)**.

Development

The Educational Project is being structured in such a way as to test the hypothesis that a teacher does research on education and produces knowledge. This agrees with the proposal contained in the Cepid project which has incorporated the results obtained by the LSE by investigating the content about the Genome and developing appropriate methods for the teachers to organize their classes and monitor student learning.

By learning the topic under study, which is part of the Science and Biology disciplines, the teachers have the opportunity of acquiring professional training in the scientific area, thus creating the conditions necessary to deal with and study other topics.

The differential factor is represented by the possibility of teacher to do research. This possibility is rare in primary and secondary schools and even in the university, where research in specific areas is highly valued. Neither the routine of the school nor the licensing courses offer the teachers or the students conditions at the level of their potential for the preparation of classes that will improve the educational process. What is observed is that these potentials, even with courses and training workshops, would require permanent

guidance in order to be actually developed. In the daily routine of the school, textbooks continue to be consulted and challenging questions continue to wait for an answer. In this process, textbooks cannot be fully excluded at this time since there are no resources for this. However, they may simply be a resource and not the major objective of many classes, as observed thus far.

Evidence

At the CTC, the location of the Educational Project, teachers learn about scientific work, experience the routine of laboratories, carry out their own research, and extend it to the classroom to stimulate the students for scientific initiation **(Enclosure 3)**

The basis of the Project is the course called "The Cells, the Genome, and You, the Teacher", given on Saturdays at the Blood Center. The major topic approached in this course is based on two questions answered by the advisers, who are Blood Center researchers. The questions are: *"in your experience as a researcher what do you consider to be most significant for presentation to the teachers?"* and *"what topics and methodologies would you suggest to the teachers you will eventually advise?"* "This strategy has permitted a general and anticipated perception of the work of the research groups of the Blood Center and has also been used as a criterion for the teachers to choose topics and advisers **(Enclosure 4)**.

As expected, there was interest in all topics (some still waiting for teachers). The groups were defined according to time availability, since the study meetings occur during the week **(Enclosure 5)**. The teachers were divided into six groups studying, under the guidance of the researchers, transgenic products, ethnic groups, umbilical cord cells, genetic diversity, HIV, Genome, cancer, and hereditary diseases. The activities of the course are : updating of concepts, lectures given by the researchers, and presentation of work carried out by the schools and by the research groups. The "educational clippings" consist of the transposition of raw material (specific content) developed by the research groups to "appropriate formats" for the work of the teachers in the schools. Furthermore, in each situation in which the "formatted material" is applied, the teachers are encouraged to organize and evaluate such material

according to the investigative proposal of the project. The documentation of oral and written manifestations (notebooks), experiments, and the presented elements can be analyzed and made available on the occasion of the Saturday class itself during the course. The material used for the class is rapidly made available by advanced technology for discussion in the presence of specialists and educators.

Some strategies were used during the course, among them newspaper and magazine readings of reports such as those on cloning in the magazines Veja, Isto É and the newspaper Folha de S.Paulo **(Enclosure 6)**, also made available on line during the class. Time for questions is allowed, and the responses are referred to Prof. Dimas, coordinator of the Project. The responses also required concepts of evolution that extrapolated the topic itself, confirming the need for the presence of professionals from the most diverse areas of knowledge in the area of biology in the class.

One of the assumptions is that, from the diversity of professionals present with "multivariate visions" new knowledge is constructed, which progressively organizes the relationship between the student's – or the teacher's – notebook and the paper of a researcher, corresponding to a space of continued training.

The teachers started to bring students to the course since the schools themselves and their directors were involved. Approximately 20 teachers currently take the course, a number that increases each Saturday, with the class becoming a space for all: students from the 6th grade to the last year of high school, for graduating students, teachers, and researchers. It is assumed that each one selects what to learn according to his/her ability to understand. By clarifying the doubts of the students, the teachers organize their own knowledge while still in the class **(Enclosure 7)**.

This confirms the hypothesis that teachers feel they are part of a group whose major interest is to develop programs and to evaluate the participants, confirming their learning. The creation of resources and their availability are thus one of the objectives. The evaluation of teachers' and students' work and even of the researchers results in the elaboration of Newsletters, Support Texts and material for the Science Journal **(Enclosure 8)**. This documentation permits an evaluation of the project and dissemination of the results so that teachers not involved may also acquire the knowledge produced.

Results

Within four months, teachers and students assimilated the development of research as a way (method) of improving teaching in the classroom. They attend presentations by the researchers and learn about their research lines and suggestions, They schedule their own research and study groups for other periods of time in addition to Saturdays and accept the challenge offered by the researchers to present their texts and to use notes for the acquisition of new knowledge. The teachers then return to their schools and demand materials to be made available in Cds, they access the Science Site (www.pegasus.fmrp.usp.br) and intend to have their research published in the Science Journal. All of this leads to the development of skills previously non-existing in the schools.

Today, about fifteen public schools of the Ribeirão Preto and Sertãozinho districts participate in the project, corresponding to more than three thousand primary and secondary school students.

The schools where the teachers work became involved in the Project and are currently organizing to reactivate their laboratories and to use the Internet. After six weeks of participation in the Project, students from the Prof. Eugênia Vilhena de Moraes State School of Ribeirão Preto asked for the opportunity to speak at the inauguration of the Science House **(Enclosure 9)**, addressing scientists and authorities. A statement repeated several times was: "By participating in the Projects with my teachers I was able to understand what occurs to us. But I started late, because I am already in the third year of middle school." Similarly, the laboratory activities of the Prof. Nestor Gomes de Araújo State School of Dumont were documented by the students while supporting their teachers in laboratory activities, the result being material for the local TV news program of the EPTV station, which is part of the Rede Globo network.

On the occasion of the inauguration of the Science House in August, the Pro-Dean's office of the Committee of University Culture and Extension of USP, São Paulo, contacted the House for the implementation of the Science Museum of USP in order to consolidate the concept of a museum of a virtual nature consisting of the networking of the patrimony of USP, in this case the material of the LSE **(Enclosure 10)**.

The Blood Center, with its frontier research in a high technology environment, did not inhibit, but rather challenged the teachers, who soon familiarized themselves with the resources available such as multimedia methods (Internet, data show, projections). Support by the Blood

Center/CTC guarantees the possibility of doing science to the teachers, as well as giving classes and guiding the students in scientific initiation skills concerning the topic. The teachers consult and borrow books from the USP library during the reduced time available to them, and write papers for congresses **(Enclosure 11)** in order to disseminate their findings.

Thus, exceeding expectations, the students started to accompany their teachers to Saturday classes, a fact that led to the assignment of a special weekly period for them involving programmed activities at the Blood Center. In view of student demands, the installation of a house for the activities was hastened, resulting in the Science Workshop, which shelters part of the LSE patrimony and permits the execution of Science activities such as those concerning the project. The Workshop was later called Museum and Laboratory of Science Education (MuLSE) **(Enclosure 12)**.

Enclosure 1

Photographs of the meeting with teachers and professional educators.

- **Enclosure 2**

The lecture of the journalist Mônica Teixeira, of the Cultura TV Network of São Paulo, was motivated by the "Project of cooperation for the transfer of knowledge to Cepid" and was given on April 19, 2000 at the Ribeirão Preto Blood Center. The journalist produced the documentary "Genome: a search for the dreams of science", aired on the national educational TV network from August 16 to 20, 1999.

- **Enclosure 3**

Photographs of the students attending a class in the laboratory with researchers.

- **Enclosure 4**

Barbieri, M.B et a.i. "Construção do Conhecimento" (Building up Knowledge). Ed. Holos. Ribeirão Preto, 2001, in press.

- **Enclosure 5**

A total of 109 teachers from 55 public schools of Ribeirão Preto and surrounding region enrolled in the course, in which 22 Blood Center researchers acted as advisers. Twenty teachers from 15 schools are currently taking the course under the guidance of six researchers.

- **Enclosure 6**

Reports from the magazines Veja and Isto É and the newspaper Folha de S. Paulo (Folhinha) about cloning. Downloaded from the Internet and from the printed edition on August 15, 2001.

- **Enclosure 7**

Class on transgenic products given by Prof. Dr. Aparecida Fontes. During the presentation the students asked heterogeneous, and even confused, questions about the topic, mixing the concepts of transgenic products and cloning. Some of the questions asked were: "which animals contain growth hormone?" , "what is the longevity of cloned sheep?".

- **Enclosure 8**

The Jornal das Ciências (Science Journal) is a bimonthly publication with a circulation of 3000, distributed free of charge to public schools for use in the classroom. The section "The Teacher Speaks" is devoted to the activities of teachers in the classroom, with text and a newsletter. The "Students' Space" is directed at the work carried out by students in school.

- **Enclosure 9**

Inauguration of the Science House, located in the Bosque Municipal Fábio Barreto, with the Itinerant Exhibit "Chronobiology – Life Rhythms", of the Science Station (SP), from July 3 to August 28. This was a partnership between the CTC/Blood Center of Ribeirão Preto and LSE-FFCLRP and the City Hall of Ribeirão Preto. Students from the project participated in a course and acted as monitors during the Chronobiology exhibit. The House will be open until the end of the year after some adaptations.

- **Enclosure 10**

During the visit by the Pro-Dean of Culture and Extension, Prof. Adilson Avansi de Abreu, and by Prof. José Carlos Teixeira de Barros Moraes, an exhibit was shown using the historical patrimony of the LSE since 1978, with reports and records, together with the documentation about the Educational Project.

- **Enclosure 11**

As a consequence of the activities carried out by the researchers, teachers and students, papers with partial results were sent to the "47th National Congress of Genetics" to be held in Águas de Lindóia in October 2001, and to the "Meeting about Research in Education, Communication and Scientific Dissemination in Museums" to be held in Rio de Janeiro, September 26-28, 2001.

- **Enclosure 12**

Setting up of the Science Workshop, Museum and LSE in September, located at Carlos de Campos street, 1474, telephone: (16) 633-8183 – close to USP. With part of the patrimony of the LSE, it will be a space for exhibits, with a teaching laboratory, informatics room and Writing room, where the students will participate in the elaboration and confection of the Science Journal.

Science House

The Blood Center subsidized the installation of the Science House (patrimony, capital, costs and consumable material) as well as its maintenance. The House was set up using the patrimony incorporated into the LEC, which includes research material and the results of projects. The incorporation of this patrimony had not been programmed by Cepid. The definitive installation will be in the Bosque Municipal Fábio Barreto, after some reforms, according to a contract in partnership with the Ribeirão Preto City Hall. The team is temporarily installed in the Science Workshop/ /MuLEC, located at Rua Carlos de Campos, 1474.

CEPID PROJECT – PROCESS 98/14247-6

TECHNICAL RESERVE

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

Amount spent in the first year	R$ 73,056.62
National Market	R$ 38,017.99
Importation	R$ 35,433.43

NATIONAL MARKET PERMANENT MATERIAL

QUANT	DESCRIPTION OF THE MATERIAL PURCHASED	VALUE R$
01	*Duplex refrigerator, 350 liters, frost-free – Eletrolux*	1,300.00
01	*Duplex refrigerator, 350 liters –Eletrolux*	930.00
01	*Pentium III 750 Mhz microcomputer*	2,845.00
01	Air conditioner, 7500 BTUs –Eletrolux – for the animal house.	429.00
01	Deskjet 670 printer, HP	318.11
02	*Bench microcentrifuge for Eppendorf tubes - Bioagency*	1,600.00
01	Pentium 866 Mhz Microcomputer	2,367.00

01	Nobreak, 600VA –MCM	228.00
11	Various books to be used in the Science House – Transfer of Knowledge –	209.23
01	Printer, model 252 - Lexmark	572.00
01	Pentium III 800 Mhz Microcomputer	2,360.00
05	Pentium III 750 Mhz Microcomputer	8,240.00
01	Bench digital pHmeter - Mettler	1,155.00
02	Automatic micropipette – Labysystem	833.20
01	Pentium III 933 Mhz Microcomputer	3,140.00
01	Pipetman Kit containing 3 automatic micropipettes with accessories - Gilson.	2,290.00
	Total:	**28,816.54**

NATIONAL MARKET MATERIAL

02	Anatomical forceps	12.80

THIRD PARTY SERVICES

Description	Value R$
Corrective services in the air conditioning system of the Animal Facilities of the Faculty of Pharmacy.	5,500.00
Glass openings in 12 doors.	192.00
Payment to Solange A. Bispo dos Santos for bibliography services and organization of teaching and paradidactic material for the Itinerant Exhibit “Life Rhythms”	852.00
Total:	**6,544.00**

HOTEL EXPENSES

Description	Value R$
Hotel expenses for Dr Silvana Chiavegatro, USP Campus, and Prof. José Alexandre M. Barbuto, ICB-USP, for their participation in the regular program of seminars at the Center.	107.30

AIRPLANE TICKETS	**Total:**	*R$ 2,142.55*

IMPORTATION

PRODUCT	R$	US$
Homogenizer equipped with a generator - Brinkman	7,332.75	3,259.00
Spectrophotometer for nucleic acids – Gene Suant	7,459.86	3,815.00
Laminar flow hood with accessories – Tabeonco	7,051.18	3,606.00
Electrophoresis cuvettes, two dry baths with 4 solid blocks, one power supply and one micromax centrifuge – Fisher	13,589.64	6,949.80
Total:	**35,433.43**	**17,629.80**

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 – July and August	2,959.37
Fellowships	90.252.50
Rent of the building where the “Science House” is installed – July and August	1,200.00
Insurance and alarm against robbery in the “Science House”	1,021.00
Traveling and hotel expenses for lecturers in the course “Transfer of Knowledge”	3,301.80
Payment of two fell – timeresearchs (including fringe benefits)	217,800.12
General furniture for the “Science House”	1,720.20

	Total:	**318,254.99**
2. FINEP	Process nº 64.00.0487.00	1,040.330.00
3. PADCT	Process nº 62.0019/99-9	***202,000.00***
4. PRONEX – CNPq	Process nº 66.1132/1998-6	***746,500.00***
	Total:	***1,988.830.00***