CTC
Centro de Terapia Celular

HEMOCENTRO DE RIBEIRÃO PRETO

RELATÓRIO CIENTÍFICO

PROCESSO Nº 98/14247-6

USP

FAPESP

2003

Principal Investigators

Name	Institution	Position/Responsibility
Marco Antonio Zago	FMRP/USP	Coordinator Center of Cell-Based Therapy.
Dimas Tadeu Covas	FMRP/USP	Coordinator of Technology Transfer.
Marisa Ramos Barbieri	FUNDHERP	Coordinator of Education and Dissemination.
Marco Antonio Zago	FMRP/USP	**Subproject Coordinator:** ▪ Functional genomics of B-cell malignancies: the gene expression profiles of chronic lymphocytic leukemias and mantle cell lymphomas ▪ The impact of gene polymorphisms on the response to treatment with cell therapy. ▪ The impact of gene polymorphisms on the susceptibility to hematological diseases ▪ The functional genomics of cells used for cell therapy: the gene expression profiles of human mesenchymal stem cells obtained from different sites ▪ The functional genomics of cells used for cell therapy: the comparison of the gene expression profiles of human CD34+ cells obtained from bone marrow, umbilical cord and peripheral blood ▪ The early gene expression changes in the hematopoiesis: the erythroid and granulocytic-monocytic pathways
Dimas Tadeu Covas	***FMRP/USP***	**Subproject Coordinator:** ▪ Generation, characterization and in vitro manipulations of mesenchymal stem cells aiming at their use for cell therapy ▪ The impact of gene polymorphisms on the response to HIV and HTLV infection. ▪ The functional genomics of cells used for cell therapy: the gene expression profiles of human mesenchymal stem cells obtained from different sites ▪ The functional genomics of cells used for cell therapy: the comparison of the gene expression profiles of human CD34+ cells obtained from bone marrow, the umbilical cord and peripheral blood ▪ Cloning and expression of recombinant human coagulation factor VIII in mammalian cells using retrovirus as a vector. ▪ Brazil Cord Blood Bank. ▪ Development of an animal model for the study of mesenchymal stem cell differentiation in vivo. ▪ Assessment and treatment of iron overload in β thalassemia homozygous patients.

Name	Institution	Position/Responsibility
Eduardo Magalhães Rego	FMRP/USP	**Subproject Coordinator:** ▪ Animal model of dyskeratosis congenita ▪ Analysis of the molecular basis of leukemogenesis in the transgenic model of acute promyelocytic leukemia ▪ Analysis of leukemic cells adhesion and tethering upon Histone Deacetylases inhibitors and G-CSF treatment in acute promyelocytic leukemia ▪ Analysis of FLT-3 mutations in acute myelogenous leukemia by single strand polymorphism ▪ Analysis of the effect of vitamin E isomers in acute promyelocytic leukemia ▪ Development of an animal models for testing cell therapy for lung disorders. ▪ Study of the pathogenesis of disseminated intravascular coagulation in the transgenic model of acute promyelocytic leukemia.
Roberto Passetto Falcão	FMRP/USP	**Subproject Coordinator:** ▪ The expression of adhesion molecules in the leukemic phase of non-Hodgkin's lymphomas ▪ Analysis of blasts adhesion and tethering upon histone decaetylase inhibitors and G-CSF treatment in acute promyelocytic leukemia
Júlio César Voltarelli	FMRP/USP	**Subproject Coordinator:** ▪ Treatment of immunological diseases by high dose chemotherapy and autologous bone marrow transplantation. ▪ Treatment of late onset type II diabetes mellitus by bone marrow transplantation ▪ Development of animal models for testing cell therapy for lung disorders.
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
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
Wilson Araújo da Silva Júnior	FMRP/USP	**Subproject Coordinator:** ▪ Initiative to validate of the human transciptome ▪ The comparison of gene expression in human acute leukemias, with especial emphasis in interluekines, adhesion molecules and angiogenesis. ▪ 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. ▪ Bioinformatics - CompBioNet

Senior Investigators

Name	Institution	Subproject
Roger Chammas	FM/USP	▪ The use of pulsed autologus dendritic cells for the treatment of melanoma ▪ Gene expression profile during melanoma progression ▪ Analysis of cell membrane molecules changes during melanoma progression ▪ Gangliosides in hematological malignancies and normal lymphoid cells
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). ▪ The impact of gene polymorphisms on the response to treatment with cell therapy. ▪ The impact of gene polymorphisms on the susceptibility to hematological diseases
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

Junior Investigators

Name	Institution	Subproject
Greice A Molfetta	FMRP/USP - FAPESP	▪ Changes of gene expression in the early differentiation of CD34+ along the erythroid and the granulocytic-monocytic pathways
Rita de Cássia Viu Carrara	FUNDHERP - FAPESP	▪ Identification of genes differentially expressed in CD34+ Bcr/Abl+ cells of patients with chronic myeloid leukemia
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	▪ Changes of gene expression in human cells treated *in vitro* with antitumoral drugs
José Augusto Baranauskas	FMRP/USP	▪ Genome data mining.

Post Doctoral Fellows

Name	Institution	Adviser
Greice A Molfetta	FMRP/USP-FAPESP	Marco Antonio Zago
Paulo Peitl Jr	FMRP/USP-FAPESP	Marco Antonio Zago
Paulo H Godoi	FMRP/USP-FAPESP	B.1.1 Marco Antonio Zago
Rita de Cássia Viu	FMRP/USP-FAPESP	Dimas Tadeu Covas
Kiyoko Abe Sandes	UFSBA	Marco Antonio Zago

Ph D Students

Name	Institution	Adviser
Adriano J Holanda	FUNDHERP – CAPES	Wilson Araújo da Silva Júnior
Andrea Aparecida Garcia	FMRP/USP – CNPq	Rendrik França Franco
Carolina Boschi Cabral	FMRP/USP – FAPESP	B.1.2 Lewis Joel Greene
Gustavo Antonio de Souza	FMRP/USP – FAPESP	Lewis Joel Greene
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
Kelson Roberto Kodama	FMRP/USP - CAPES	Wilson Araújo da Silva Júnior
Rodrigo Alexandre Panepucci	FMRP/USP – FAPESP	Marco Antonio Zago
Barbara Amélia Santana	FMRP/USP-FAPESP	Eduardo Magalhães Rego
Rodrigo Abreu e Lima	FMRP/USP-FAPESP	Eduardo Magalhães Rego
Lorena Lobo e Figueiredo	FMRP/USP-FAPESP	Eduardo Magalhães Rego
Gil de Santis	FUNDHERP	Eduardo Magalhães Rego
Rodrigo Proto Siqueira	FMRP/USP-FAPESP	Marco Antônio Zago
Daniel Mazza	FMRP/USP-FAPESP	Roberto Passetto Falcão
Edgar G Rizzatti	FMRP/USP	Roberto Passetto Falcão
Virginia Proença Picanço	FUNDHERP	Dimas Tadeu Covas

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.

B. RESULTS OBTAINED IN BASIC RESEARC

B.1 - Publications

B.1.a.1 Covas DT, Siufi JLC, Silva ARL, Orellana MD. Isolation and Culture of Umbilical Vein Mesenchymal Stem Cells. ***Brazilian Journal of Medical and Biological Research,*** 36(9):1179-1183, 2003.
Enclosure

B.1.a.2 Rocha V, Franco RF, Porcher R, Bittencourt H, Silva Jr WA, Devergie A. at all. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow tansplant. ***Blood,*** 100(12):3908-3818, 2002.
Enclosure

B.1.a.3 Voltarelli JC, Ouyang J. Hematopoietic stem cell transplantation for autoimmune diseases in developing countries: Current status and future prospectives. ***Bone Marrow Transplantation,*** Supl 1:S69-71, 2003.
Enclosure

B.1.a.4 Ruggero D, Grisendi S, Piazza F, Rego EM, Mari F, Rao PH, Cordon Cardo C, Pandolfi PP. Dyskeratosis Congenita and Cancer in Mice Deficient in Ribosomal RNA Modification. ***Science,*** 299:259-262, 2003.
Enclosure

B.1.a.5 Calado RT, Machado CG, Carneiro JJ, Garcia AB, Falcão RP. Age-related changes of P-glycoprotein-mediated rhodamine 123 efflux in normal human bone marrow hematopoietic stem cells. ***Leukemia,*** 17(4):816-18, 2003.
Enclosure

B.1.a.6 Azevedo A, Nucci M, Maiolino A, Vigorito A, Simoes BP, Aranha F, Tabak D, Voltarelli JC, Souza C. A randomized multicenter study of G-CSF starting on day +1 vs. day +5 after after autologous peripheral blood progenitor cell transplantation. ***Bone Marrow Transplantation,*** 29:745-751, 2002.
Enclosure

B.1.a.7 Castro FA, Moraes F, Palma PV, Voltarelli JC. Immunological effects of interferon-alpha in chronic myelogenous leukemia. ***Leukemia and Lymphoma,*** 2003.
Enclosure

B.1.a.8 Proto-Siqueira R, Falcão RP, Souza CA, Ismael SJ, Zago MA. The expression of PRAME in chronic lymphoproliferative disorders. ***Leukemia Research,*** 27(5):393-6, 2003.
Enclosure

B.1.a.9 Verde DMSV, Silvamonteiro E, Jasiulionis M, Oliveira DAF, Brentani RR, Savino W, Chammas R. Galectin-3 modulates carbohydrate-dependent thymocyte interaction with the thymic microenvironment. ***European Journal of Immunology,*** 32(5):1434- 44, 2002.
Enclosure

B.1.a.10 Calado RT, Pintão MC, Silva Jr WA, Falcão RP, Zago MA. Aplastic anaemia and telomerase RNA mutations. ***Lancet,*** 360(9345):1608, 2002.
Enclosure

B.1.a.11 Cordeiro AT, Godoi PHC, Silva CHTP, Garratt RC, Oliva G, Thiemann. Crystal structure of human phosphoglucose isomerase and analysis of the initial catalytic steps. ***Biochimica et Biophysica Acta, 1645***:117-122, 2003.
Enclosure

B.1.a.12 Nagai MA, Barbosa HS, Zago MA, Silva Jr WA, Nishimoto IN, Salaorni S, Costa LNFG, Araújo MS, Oliveira AGC, Mourão Neto M, Brentani MM. TP53 mutations in primary breast carcinomas from white and African-Brazilian patients. ***International Journal of Oncology,*** 23(1):189-96, 2003.
Enclosure

B.1.a.13 Nucci M, Anaissie EJ, Simoes BP, Oliveira JS, Voltarelli JC, Maiolino A, Pasquini R, Souza C. Outcome predictors of 84 patients with hematological malignancies and Fusarium infection. ***Cancer,*** 98(2):315-9, 2003.
Enclosure

B.1.a.14 Peitl P, Mello SS, Camparoto ML, Passos GAS, Hande MP, Cardoso RS, Sakamoto-Hojo ET. Chromosomal rearrangements involving telomeric DNA sequences in Balb/3T3 cells transfected with the H-ras oncogene. ***Mutagenesis,*** 17(1)1:67-72, 2002.
Enclosure

B.1.a.15 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 bening myeloid proliferation. ***American Journal of Clinical Pathology,*** 118:31-7, 2002.
Enclosure

B.1.a.16 Silva Jr WA, Bonatto SL, Holanda AJ, Ribeiro-dos-Santos AKR, Paixão B M, Goldman GH, Abe-Sandes KA, Rodriguez-Delfin LR, Barbosa M, Paço-Larson MLP, Petzl-Erler MLP, Valente V, Santos SEB, Zago MA. Correction Mitochondrial DNA Variation in Amerindians. ***American Journal of Human Genetics,*** 72:1346-1348, 2003.
Enclosure

B.1.a.17 Voltarelli JC, Stracieri ABP, Coutinho MA, Paton EJ, Almeida K, Vieira OM, Dantas M. Acute renal dysfunction associated with antithymocyte globulin in the conditioning for hematopoietic stem cell transplantation. ***Biology of Blood and Marrow Transplantation.*** Charlottesville, VA, USA, 8(2):110, 2002.
Enclosure

B.1.a.18 Watanabe MAE, Milanezi CM, Silva Jr WA, Angulo IL, Santis GC, Kashima S, Costa JAC, Moisés Neto M, Covas DT. Molecular Investigation of GB Virus C RNA in Hemodialysis and Thalassemics Patients from Brazil. ***Renal Failure,*** 25(1):67-75, 2003.
Enclosure

B.1.a.19 Fonseca LM, Brunetti IL, Campa A, Catalani LH, Calado RT, Falcão RP. Assessment of monocytic component in acute myelomomocytic and monocytic/monoblastic leukemias by a chemiluminescent assay. ***The Hematology Journal,*** 4(1):26-30, 2003.
Enclosure

B.1.a.20 Huang N, Marie SK, Livramento JA, Chammas R, Nitrini R. 14-3-3 protein in the CSF of patients with rapidly progressive dementia. ***Neurology,*** 61(3):354-357, 2003.
Enclosure

B.1.a.21 Farias KCRM, Saelens X, Pruijn GJM, Vandenabeele P, Venrooij WJ. Caspase-mediated cleavage of the U snRNP-associated Sm-F protein during apoptosis. ***Cell Death and Differentiation,*** 10:570-9, 2003.
Enclosure

B.1.a.22 Konozy EHE, Mulay R, Faça V, Ward RJ, Greene LJ, Roque-Barriera MC, Sabharwal S, Bhide SV. Purification, some properties of a D-galactose-binding leaf lectin from *Rythrina indica* and further characterization of seed lectin. ***Biochimie,*** 84(10):1035-43, 2003.
Enclosure

B.1.a.23 Novak EM, Metzger M, Chammas R, Costa M, Dantas K, Manabe C, Pires J, Oliveira AC, Bydlowski SP. Downregulation of TNF-a and VEGF expression by Sp1-decoy oligodeoxynucleotides in mouse melanoma tumor. ***Gene Therapy,*** v.10, 2003.
Enclosure

B.1.a.24 Covas DT, Kashima S. Complete Nucleotide Sequences of the Genomes of Two Brazilian Specimens of Human T Lymphotropic Virus Type 2 (HTLV 2). ***AIDS Research and Human Retroviruses,*** 19(8):689-697, 2003.
Enclosure

B.1.a.25 Konozy EHE, Bernardes ES, Rosa C, Faça V, Greene LJ, Ward . Isolation, purification, and physicochemical characterization of a D-galactose-binding lectin from seeds of *Erythrina speciosa.* ***Archives of Biochemistry and Biophysics,*** 410(2):222-9, 2003.
Enclosure

B.1.a.26 Boson WL, Romano-Silva MA, Correa H, Falcão RP, Teixeira-Vidigal PV, De Marco L. Thiopurine methyltransferase polymorphisms in a Brazilian population. ***The Pharmacogenomics Journal,*** 3(3):178-82, 2003.
Enclosure

B.1.a.27 Alcantara JR LC, Dooren SV, Gonçalves MS, Kashima S, Costa MC, Santos FL, Bittencourt AL, Dourado I, A Filho A, Covas DT, Vandamme A. M, Castro B G. Globin Haplotypes of Human T Cell Lymphotropic Virus Type I Infected Individuals in Salvador, Bahia, Brazil, Suggest a Post Columbian African Origin of This Virus. ***Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology,*** 33(4):536-542, 2003.
Enclosure

B.1.a.28 Boturão-Neto E, Marcopito LF, Zago MA. Urinary iron excretion induced by intravenous infusion of deferoxamine in B-thalassemia homozygous patients. ***Brazilian Journal of Medical and Biological Research,*** 35(11):1319-28, 2002.
Enclosure

B.1.a.29 Carneiro AAO, Baffa O, Fernandes JP, Zago MA. Theoretical evaluation of the susceptometric measurement of iron in human liver by four different susceptometers. ***Physiological Measurement,*** 23(4):683-93, 2002
Enclosure

B.1.a.30 Carneiro AAO, Fernandes JP, Zago MA, Covas DT, Angulo IL, Baffa O. An alternating current superconductor susceptometric system to evaluate liver iron overload. ***Review of Scientific Instruments,*** 74(6):3098-3103, 2003.
Enclosure

B.1.a.31 Machado LBP, Silva Jr WA, Manfrin MH, Sene FM. Microsatellite loci in the cactophilic species Drosophila antonietae (Diptera; Drosophilidae). ***Molecular Ecology Notes***, 3(1):159-161, 2003.
Enclosure

B.1.a.32 Malmegrim KCR, Pruijn GJM, Venrooij WJ. The fate of the U1 snRNP autoantigen during apoptosis: implications for systemic autoimmunity. ***Israeli Medical Association Reviews,*** 4:706-712, 2002.
Enclosure

B.1.a.33 Palatnik M, Silva Jr WA, Estalote AC, Oliveira JE, Milech A, Zago MA. Ethnicity and type 2 diabetes in Rio de Janeiro, Brazil, with a review of the prevalence of the disease in amerindians. ***Human Biology,*** 74(4):533-544, 2002.
Enclosure

B.1.a.34 Watanabe MA, Cavassin GG, Orellana MD, Milanezi CM, Voltarelli JC, Kashima S, Covas DT. SDF 1 gene polymorphisms and syncytia induction in Brazilian HIV 1 infected individuals. ***Microbial Pathogenesis,*** 35(1):31-4, 2003.
Enclosure

B.1.a.35 Zampieri S, Mahler M, Blüthner M, Qiu Z, Malmegrim K, Ghirardello A, Doria A, Venrooij WJ, Raats JMH. Recombinant anti-P protein autoantibodies isolated from a human autoimmune library: reactivity, specificity and epitope recognition. ***Cellular and Molecular Life Sciences,*** 60:588-98, 2003.
Enclosure

B.1.a.36 Molfetta GA, Silva-Jr WA, Pina-Neto JM. Clinical, cytogenetical and molecular analyses of Angelman syndrome. ***Genetic Counseling,*** 14(1):45-56, 2003
Enclosure

B.1.a.37 Scartezini M, Zago MA, Chautard-Freire-Maia EA, Pazin-Filho A, Marin-Neto JA, Hotta JK, Nascimento AJ, Dos-Santos JE. The X-X-/E+E+ genotype of the XbaI/EcoRI polymorphisms of the apolipoprotein B gene as a marker of coronary artery disease in a Brazilian sample. ***Brazilian Journal of Medical and Biological Research,*** 36(3):369-75, 2003
Enclosure

B.1a – Articles Published in Brazilian Journals with Selective Editorial Politics

B.1.a.1 Lousada JRP, Magalhaes M, Takaki L, Silva LM, Voltarelli JC, Carvalho IF, Donadi E A. Pesquisa de anticorpos linfocitotóxicos em pacientes com lúpus neuropsiquiátrico e não-neuropsiquiátrico. ***Revista Brasileira de Reumatologia,*** 43:8-13, 2003.
Enclosure

B.1.a.2 Rego EM. Molecular Basis of Acute Myelogenous Leukemia. ***Revista Brasileira de Hematologia e Hemoterapia***, 24: 160-165, 2002.
Enclosure

B.1.a.3 Voltarelli JC, Stracieri ABP, Oliveira MCB, Paton EJ, Coutinho MA, Dantas M, Ribeiro A, Popovici M, Scheinberg M, Hamerschlak N. Transplante autólogo de células tronco hematopoéticas para nefrite lúpica: Resultados brasileiros iniciais. ***Jornal Brasileiro de Nefrologia***, 25:65-72, 2003.
Enclosure

B.2 - Books

B.2.1 Burt RK, Kenyon N, Voltarelli JC, Kaufman D. Hematopoietic stem cell transplantation as treatment for type I diabetes. Stem cell therapy for autoimmune diseases ed.Georgetown, TX : Landes Bioscience, 2003

B.2.2 Chammas R, Brentani RR. Cell-matrix interactions. Encyclopedia of Cancer.2 ed. : Elsevier Science (USA), 2002, v.1, p. 405-414.

B.2.3 Falcão RP, Rego EM, Ismael SJ. "Leucemia Linfóide Aguda do Adulto e Leucemias de Linhagem Ambígüa". Atualização Terapêutica.21 ed.São Paulo : Artes Médicas, 2003, p. 737.

B.2.4 Han SW, Moraes JZ, Silva CL, Chammas R, Rodrigues MM. DNA vaccines. Artificial DNA: Methods and Applications ed.Boca Raton : CRC Press, 2002, p. 329-361

B.2.5 Melo FHM, Junqueira MS, Chammas R. Mecanismos de Invasão e Metástases. Bases da Oncologia.2 ed.São Paulo : Editora Marina e Tedmedd Editora, 2003, p. 201-226.

B.2.6 Oliveira Filho RS, Festa Neto C, Paschoal FM, Tovo LFR, Ferreira LM, Enokihara MMSES, Tovo Filho R, Camponero R, Chammas R. Melanoma Cutâneo Localizado e Linfonodo Sentinela. São Paulo :LeMar, 2003, v.1. p.182.

B.2.7 Voltarelli JC, Donadi EA, Martinez JAB, Vianna EO, Sarti W. Bronchial asthma and idiopathic pulmonary fibrosis as potential targets for hematopoietic stem cell transplantation. Stem cell therapy for autoimmune disease.1 ed.Georgetown, TX : Landes Bioscience, 2003, p. 91-100.

B.3 – Articles in press

Silva Jr WA, Covas DT, Panepucci RA, Proto-Siqueira R, Siufi JLC, Zanette DL, Santos ARD, Zago MA. The profile of gene expression of human marrow mesenchymal stem cells. ***Stem Cells***, *in press*

Molfetta GA, Hojas MVM, Silva Jr WA, Wagstaff J, Pina-Neto JM. Discordante phenotypes in first cousins with UBE3A frameshift mutation. *in press*

Abe-Sandes K, Silva Jr WA, Zago MA. Heterogeneity of the Y chromosome in Afro-Brazilian populations. ***Human Biology***, *in press*

Souza GA, Oliveira PSL, Trapani S, Santos ACO, Rosa JC, Laure HJ, Faça VM, Correia MTS, Tavares GA, Oliva G, Coelho LCBB, Greene LJ. Amino acid sequence and tertiary structure of *Cratylia mollis* seed lectin. *in press*

Fontes AM, Orellana MD, Palma PVB, Covas DT. Maturation of dendritic cells following exposure to different maturational stimuli. *in press*

Rizzati EG, Portieres FL, Martins SLR, Rego EM, Zago MA, Falcão RP. The Microgranular and the t(11;17)/PLZF-RARα Variants of Acute Promyelocytic Leukemia also Present the Flow Cytometric Pattern of CD13, CD34 and CD15 Expression Characteristic of PML- RARα Gene Rearrangement. *in press*

Lieschke GJ, Pandolfi PP, Varma S, Rego EM, Winkler IG, Levesque JP, Came N. "Suppression of lethal myeloid leukemia development by granulocyte colony-stimulating factor deficiency" *in pres*

C TECHNOLOGICAL ACHIEVEMENTS

- A self-diagnosis software was developed for the Quality Program ISO 9002 certified GMP.
- Viral proteins cloned and expressed in mammalian cells (HIV and HTLV-1 recombinant proteins).
- In September/2003 the Regional Blood Center was evaluated and preliminarly certified. by the AABB (American Association of Blood Banks)
- Production of human cloting factors VIII na IX by gene recombination
- Development of a non invasive method of measurament of iron human liver.

1- EDUCATIONAL ACTIVITIES

Course: The Cells, the Genome and You, the Teacher.

Vacation Course	Genome, Proteome and the Cell Universe
Specialization Course	Molecular Biology
VHS Recording of the Classes and of the Writing Workshop	Specialized support for science teachers in written communication and the writing of papers
Results of the Projects Carried out by the Teachers of the Educational Program	Presentation of papers among the teachers
Educational Directorship of Sertãozinho – SP, Dissemination of Results and Teacher Qualification.	Technical Orientation with Kits for the Proceed Group
49th National Genetics Congress	Participation of Teachers of the Educational Project
Classes and technical orientations	Participation of speakers of the Ribeirão Preto USP Campus, of UNESP and of Fiocruz
Classes by the teachers and monitors of the Course The Cell, the Genome and You for students of the Talent Hunt Project	***Verification of learning for the groups of students who attend Saturday classes***
Visits to the schools of the teachers who are involved in the activities of the project, with different characteristics	Assessment of the results of the Course.
Orientation of papers, presentations, theater, lectures, exchange of didactic material	Exchange of teachers and students in the schools
A Pre-Congress of students and their teachers was held	Talent Hunt
Dissemination of the Projects developed by teachers and students of the Educational Project	Educational Program Link at the site http://ctc.fmrp.usp.br/education/

OTHER EDUCATIONAL ACTIVITIES

In addition to the educational activities carried out at the Cell Therapy Center, courses for undergraduate and graduate students have been included. The Vacation Course entitled Genome, Proteome and the Cell Universe was offered to university students for the 3rd consecutive year. The course, held in January 2003, counted with the participation of 00 university students in the areas of Biological Sciences.

During the month of May 2003, the course of Introduction to Computational Biology, with 130 participants, was given in order to present a general view of the applications of physics, mathematics and computation methods to the area of Health Sciences and Biotechnology.

Also, weekly Seminars of Molecular Biology are offered to graduate students, together with the Joint Seminar of CTC on Thursdays, at the Blood Center of Ribeirão Preto, with the participation of an average of 30 to 40 students per lecture.

The II Scientific Seminar of the Cell Therapy Center was also held, with 18 researchers presenting their work throughout the day in sessions of a maximum of 10 minutes. One-hundred abstracts were submitted, giving an idea about the activities of the Cell Therapy Center.

2. Results of Basic Research

This year was marked by two advances in the center: a)- definite progress in the basic research started in the preceding years, and b)- a significant change in the profile of the research, increasing the focus of the research team on the subject of the center, described in detail in chapter "**5. Changes in Plan"** of this report. Although the main purpose of the center, stated in the initial document, is to carry out research on basic cellular mechanisms (cell differentiation, cell recognition, cell-to-cell interaction, cell mediators, inflammation, and apoptosis) and processing (isolation, expansion, selection, purging) that are relevant for cell-based therapy and its relation with gene structure and protein expression, we are progressively increasing the clinical and applied component of the center research.

The main progress in the basic research of the center is summarized below.

Generation and characterization of stem cells.

Mesenchymal stem cells (MSC) have been obtained from different sources. Mesenchymal stem cells (MSC) are multipotent precursors present in adult bone marrow, that differentiate into osteoblasts, adipocytes and myoblasts, and play important roles in hematopoiesis. MSC obtained from human bone marrow have been characterized in relation to their gene expression profile, that has also been compared with the gene expression of CD34+ hematopoietic stem cells (Silva-Jr *et al*, 2003, Stem Cells, in press). We examined the gene expression of these cells by SAGE (serial analysis of gene expression), and found that collagen I, SPARC (osteonectin), transforming growth factor beta-induced, cofilin, galectin 1, laminin-receptor 1, cyclophilin A and matrix metalloproteinase 2 are among the most abundantly expressed genes. Comparison with a library of CD34+ cells revealed that MSC had a larger number of expressed genes in the categories of cell adhesion molecule, extracellular and development. The two types of cells share abundant transcripts of many genes. IL-11, IL-15, IL-27 and IL-10R, IL-13R and IL-17R were the most expressed genes among the cytokines and their receptors in MSC, and various interactions can be predicted with the CD34+ cells. This study identified the important contribution of extracellular protein products, adhesion molecules, cell motility, TGF-beta signaling, growth factor receptors, DNA repair, protein folding and ubiquination as part of the MSC transcriptome.

In addition to bone marrow, MSC can be obtained from other sites in the adult or the fetus. There is controversy if MSC can be obtained from the umbilical cord (UC). Instead of using cord blood, we (Covas *et al*, 2003) obtained MSC starting from cells detached from the UC vein, in a similar manner as for initiating HUVEC cultures. The cells had morphological features, immunophenotypic markers and differentiation ability similar to BM-MSC, and we used SAGE to compare the gene expression profile of BM-MSC and of the MSC derived from umbilical cord vein (UC-MSC). The two libraries share almost all of the first thousand most expressed transcripts, some of which were validated by RT-PCR, including the genes VIM, LGALS1, SPARC, COL1A1, COL1A2, TPT1, TAGLN, TAGLN2, ANXA2 and MMP2. Nevertheless, a set of genes related to anti-microbial activity, to osteoblast differentiation and adherence to the matrix, and to osteogenesis were expressed at higher levels in BM-MSC, whereas higher expression in UC-MSC was observed for genes that participate in pathways related to matrix remodeling via metalloproteinases and angiogenesis. Thus, MSC with similar

morphologic and immunophenotypic characteristics can be obtained from BM and from the UC vein.

The similarities observed between cultured MSC derived from the UC vein and from BM identifies UC-MSC as a new potential source of cells for use in cell-based therapies or tissue engineering and we are now starting to explore this. Umbilical cord is a more accessible source than bone marrow, and its availability tends to increase. In spite of their similarities, the genes differentially expressed between the two types of MSC may reflect functional differences related to their sites of origin: BM-MSC would be more committed to osteogenesis whereas UCV-MSC would be more committed to angiogenesis. If confirmed, this would imply that MSC from a specific source may be more efficient for a particular therapeutic target; for instance, UC MSC could be more appropriate for the treatments directed towards increasing revascularization.

We also concluded the study of the age-related changes of P-glycoprotein-mediated rhodamine 123 efflux in bone marrow stem cell from normal subjects. P-gp is expressed by stem cells and thought to represent a physiological mechanism of protection against toxic substances and metabolites. A positive correlation between P-gp activity and age was detected, with stem cells from older individuals expressing significantly higher Rh123 efflux (Calado *et al.*, 2003). These findings reinforce the idea of the existence of differences in the cellular biology of normal stem cells.

Cell markers of prognosis in cell therapy

Our findings reveal the clinical relevance of gene polymorphisms to the outcome of bone marrow transplantation (BMT) and suggest that therapeutic strategies should be individualized on the basis of genetic and clinical factors in order to decrease toxicities and graft-versus-host disease (GvHD).

The important contribution of polymorphisms of genes related to defense and the immunologic and inflammatory responses was demonstrated in 107 pairs of donors and patients with acute (n = 39) or chronic leukemia (n = 68). Genotyping was performed for gene polymorphisms of cytokines (tumor necrosis factor-α [TNF-α] and TNF-β, interleukin-1 receptor antagonist [IL-1Ra], IL-6, and IL-10), adhesion molecules (CD31 and CD54), Fcγ-receptors (FcγRIIa, IIIa, IIIb), mannose-binding lectin (MBL), and myeloperoxidase (MPO). In multivariate analysis, first overall infections were increased in patients with the FcγRIIa R-131 genotype, and severe bacterial infections were increased when the MPO donor genotype was AG or AA. Viral and invasive fungal infections were not influenced by any genetic factor studied. Interestingly, we also found that (1) time to neutrophil recovery was shorter when donors were FcγRIIIb HNA-1a/HNA-1b; (2) donor IL-1Ra (absence of IL-1RN*2) increased the risk for acute graft-versus-host disease (GVHD) (II-IV); and (3) recipient IL-10 (GG) and IL-1Ra genotypes increased the risk for chronic GVHD. Finally, 180-day transplantation-related mortality rates were increased when donors were FcγRIIIb HNA-1a/HNA-1a or HNA-1b/HNA-1b and donor MPO genotype was AA.

More recently, we demonstrated in the same 107 patients the role of polymorphisms of genes that may interfere with drug metabolism used in the preparative regimen and GvHD prevention of HLA-identical sibling BMT. The following candidate genes were investigated: P450 cytochrome family (CYP2B6*2, *3, *4, *5, *6), glutathione-S-transferases (GSTM1, GSTT1, GSTT1), thiopurine-S-methyl transferase

TPMT* (B and C), multiresistance drug (MRD1), methylenetetrahydrofolate reductase (MTHFR C677T) and vitamin-D receptor (VDR: ApaI, TaqI and BsmI). Univariate and multivariate analyses, using death as competing event, were performed and adjusted for potential clinical confounder factors. In multivariate analysis, increased incidence of hemorrhagic cystitis was associated with a gene involved in CY metabolism and in chronic leukemia. Venous occlusive disease was associated with donor to recipient CYP2B6*6 (GG). No clinical or genetic factors were associated with intersticial pneumonitis. We found increased GVHD associated with specific alleles of recipient MTHFR, VDR, IL10, donor GSTP1 and IL1-Ra, lower bone marrow cell dose, patient age and female donor. Thus we demonstrated that polymorphisms of genes that interfere with the toxicity and efficacy of drugs used in the preparative regimen and GvHD prophylaxis are important genetic factors for outcomes after HLA identical BMT.

In a different group of disorders, characterized by the failure of hematopoiesis, we also studied the relevance of genetic markers. We demonstrated that acquired aplastic anemia, an important target for hematopoietic transplantation, is not associated with mutations of the RNA telomerase gene (hTR), as had been suggested by others, neither is Fanconi anemia related to those mutations. We sequenced the relevant segment of the hTR gene in 42 unselected patients with aplastic anemia and none of the three mutations previously described were observed in this population. Although intriguing, our findings were later confirmed in a larger population. Finally, in 45 patients with Fanconi anemia we found only one example of the described mutation, that should more appropriately be interpreted as a clinically silent polymorphism.

Also related to the identification of cell markers with prognostic significance, we analyzed the immunophenotypical profile of 71 patients with acute promyelocytic leukemia. Our aim was to determine whether the intensity of expression of myeloid antigens could predict the occurrence of the retinoid syndrome, a life-threatening complication observed rarely in APL patients receiving all *trans* retinoic acid (ATRA) treatment. Neither the fluorescence intensity nor the coefficient of variation of CD33, CD13 and CD117 fluorescence in the blasts from APL patients were significantly correlated to a higher risk for the development of retinoid syndrome.

Cell markers in hematopoietic neoplasias.

The identification and characterization of tumor-associated antigens are important because they may elicit an immune response that may be the basis of a therapeutic approach. We studied PRAME (Preferentially Expressed Antigen in Melanoma) expression in chronic lymphoproliferative disorders. The PRAME gene encodes an antigen recognized by autologous T lymphocytes and is frequently expressed in human solid tumors and acute leukemias. We detected PRAME expression in 7 out of 16 cases of mantle cell lymphoma, 2 out of 4 cases of prolymphocytic leukemia and 6 out of 38 cases of chronic lymphocytic leukemia. Therefore, PRAME may be a target for immunological therapy of chronic lymphoproliferative disorders, and we are now starting studies with this purpose.

Regarding the diagnosis of hematopoietic malignancies, our group analyzed CD117 and CD11b expression profile for the distinction between acute promyelocytic leukemia (APL) and recovering benign myeloid proliferation. Whereas the CD117-negative and CD11b-positive phenotype was detected in the bone marrow cells from all 5

patients recovering from acute agranulocytosis, only one of 31 APL cases presented a similar profile (Rizzatti *et al*., 2003).

Finally, our group also developed a different approach for the diagnosis and classification of acute leukemias based on a chemiluminescent assay. The hydrolysis of 2-methyl-1-propenylbenzoate mediated by monocytic esterases generates acetone phosphorescence, which can be quantified with a luminometer, and applied to the assessment of monocytic lineage commitment. The results obtained with this method were the same as those obtained with α-naphthyl acetate esterase (ANAE) in 97% of 23 monocytic or myelomonocytic leukemias and the method was capable of distinguishing between acute myelogenous leukemias, with and without monocytic commitment from a pool of 66 AML cases.

Dendritic cell vaccines

As described previously, we established a method for the induction of dendritic differentiation from peripheral blood mononuclear cells based on *in vitro* culture in the presence of GM-CSF + IL-4 for 5 days, followed by TNF-α + PGE-1 or LPS treatment. In the last year, we compared the dendritic differentiation of mononuclear cells from patients with melanoma and normal subjects using the above method. The pattern of expression of CD1a, CD83 and CD86 upon stimulation was similar between the 2 groups, indicating that autologous cells from melanoma patients may be used as a source for *ex-vivo* dendritic differentiation.

Moreover, the proteomic analysis of differentiation and maturation of monocyte-derived dendritic cells was performed. For this purpose, after treatment with GM-CSF, IL-4 and LPS, 1 mg of total protein extract from each cell type [monocytes (Mo), immature and mature dendritic cells (DCs)] was applied to 2D-gels and run at pH 4-7. The reproducibility analysis of 2D electrophoresis was carried out using three gels and the coefficient of variation was 0.7% for pI, 2% for MW and 40% for colloidal Coomassie blue-staining intensity. Three-hundred-and-thirty-six spots were visualized in Monocyte gel, 385 spots in immature DC gel and 260 spots in mature DC gel. Eighty-four spots were common to all cell types, and 58 spots presented differential expression >100% for Mo x DCi comparison, 62 for DCi x DCm and 39 for Mo x DCm. For protein identification, some spots of interest were digested with trypsin and submitted to ESI/MS/MS and/or MALDI-TOF analysis. Six proteins were identified and each one proved to be characteristic of a different phase of maturation: actin and disulfide isomerase were overexpressed in Mo, vimentin was overexpressed in immature DCs, thioredoxin was overexpressed in mature DCs, and galectin-1 was overexpressed in both DCs. The protein tropomyosin was identified in 3 isoforms differentially regulated in each cell type.

Chapter 5 summarizes the projects for experimental and clinical uses of these cells.

Innovative use of hematopoietic stem cell transplantation

Few effective therapeutic alternatives are available for patients with autoimmune disorders refractory to conventional therapy. Therefore, the possibility of using Hematopoietic Stem Cell (HSC) transplantation for these diseases would be of great benefit. Moreover, due to the high prevalence of some autoimmune diseases such as rheumatic fever and some forms of pemphigus, the success of such approach would be

of great relevance. HSC transplantation was performed for 3 cases of refractory lupus erythematosus with impairment of renal function. The conditioning regimen consisted of cyclophosphamide plus antilymphocytic globulin (ALG) followed by the infusion of autologous HSC. Two of the 3 patients achieved clinical remission of the disease and do not require further immunosuppression at day + 360 and + 700 post transplant. The third patient died of respiratory insufficiency due to alveolar hemorrhage. In addition, 7 patients with refractory multiple sclerosis were transplanted. The conditioning regimen consisted of BEAM (BCNU, Etoposide, Aracytin and Melphalan) plus ALG. In five out of the seven patients the progression of neurological impairment was halted by the transplant and the patients became independent of the use of immunosuppressors. Two patients died of transplant-related disorders. Finally, the same approach was applied to one patient with Takayasu´s arteritis and one with pemphigus. However, the follow-up is still too short to be evaluated. In general, our results suggest that HSC transplantation is feasible and may be a therapeutic option for refractory severe autoimmune disorders. Partial results were reported by Voltarelli *et al*., in *Bone Marrow Transplation* and *Biology of Blood and Marrow Transplantation* (enclosed).

Regarding the pathogenesis of autoimmune disorders, our group was interested in the hypothesis that dying cells could act as a potential reservoir of modified autoantigens that initiate and drive systemic autoimmunity in susceptible hosts. The uridine-rich small nuclear ribonucleoprotein (U-snRNP) complex is a common target for autoantibodies in systemic lupus erythematosus. We studied the effects of caspase-mediated cleavage of spliceosomal SM proteins during apoptosis and demonstrated the generation of a 9 kDa fragment, which remained associated with the U-snRNP and which may be recognized by the sera of patients with autoimmune disorders. These results were reported by Malmegrim de Farias *et al* in *Cell Death and Differentiation* and a review about the subject was published by Malmegrim *et al.* in *IMAJ.*

Animal models of human cancers

Dyskeratosis Congenita is a rare disease characterized by premature aging and increased tumor susceptibility. The disease is treated by hematopoietic transplantation and represents an interesting model of failure of hematopoiesis and increased cancer susceptibility. It is an X-linked recessive disease caused by point mutations in the *DKC1* gene, which codes for dyskerin, a putative pseudouridine synthetase that mediates posttranscriptional modification of ribosomal RNA (rRNA) . In addition, dyskerin can bind to the RNA component of telomerase. In a collaborative effort between the Memorial Sloan-Kettering Cancer Center, the Baylor College of Medicine and the Center for Cell-Based Therapy, we generated hypomorphic *Dkc1* mutants. Cells from hemizygous male and heterozygous female mutants displayed a decreased level of Dkc1 expression. About 60% of the *Dkc1* mutants presented bone marrow failure, with reduced number of erythroid and B colony-forming units in BM clonogenic assays. Moreover, the mutants were highly prone to tumors and developed a variety of them, the most common being lung and mammary gland tumors. To determine the molecular basis for dyskeratosis congenita, we analyzed the telomere status/telomerase activity and the ribosomal RNA pseudouridylation. Whereas the impairment in ribosome biogenesis was detected from the first generations of mutant mice, reductions of telomere length became evident only in later generations. These results were reported by Ruggero *et al.* in the journal *Science* and represent the first demonstration that the deregulation of ribosomal function is

important for oncogenesis, being important in the initiation of dyskeratosis congenita, whereas telomere shortening may modify and/or exacerbate the disease,

We are also studying the role of granulocyte (G-) and granulocyte-macrophage (GM-) colony-stimulating factors (CSFs) in leukemogenesis. This may be important since the clinical application of cell therapy may require the use of these growth factors. With this aim, we generated G- and GM-CSF-deficient mice harboring the PLZF-RARα transgene that leads to the development of a form of acute myelogenous leukemia. CSF-replete and GM-CSF-deficient transgenic mice developed leukemia and died prematurely. In contrast, G-CSF deficiency suppressed leukemia development completely, indicating that endogenous G-CSF signals are essential for leukemogenesis. These results have been submitted for publication (Lieschke *et al.,* 2003).

3. 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)

In September/2003 the Regional Blood Center was evaluated and preliminarly certified by the AABB (American Association of Blood Banks).

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 and implemented in 121 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.

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 Requisite Pro 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. The presence at the protein was confirmed by immunological detection

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

   A vector system was constructed for the gp21 gene of HTLV-1 for expression in *E.coli* systems using Gateway™ Technology (Invitrogen Life Technologies) which is based on bacteriophage lambda site-specific recombination properties. First, the whole gp21 region isolated from MT-2 cell line was cloned in the pDONR™201 (Invitrogen) vector and then we used as destination vector pDEST 15™ (Invitrogen). The recombinant plasmid was transformed in Rosetta(DE3)pLYsS strain (Novagen). Cell cultures were grown and induced with 1,0 mM IPTG. After

induction, the cells were harvested and the soluble and insoluble fraction were analyzed by SDS-PAGE showing an intense band with an approximate molecular weight of 45,000 (expected size for this recombinant protein which was tagged with GST in the N-terminal portion). This recombinant fusion protein was identified by immunological detection using anti-gp21 monoclonal antibody. Further steps will be conducted as purification using GST columns and analysis of immunological properties against HTLV-1 serum samples.

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. We introduced this cDNA into appropriated expression vector and obtained two clones that are producing F VIII as determined by activity assays. Related o FIX, 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 forty screening. All of them contains the DNA insert of 1.4 kb as analyzed by PCR.We obtained a clone that is secreting active FIX. Next step includes optimization of the expression. 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 performed measurements in a group of 34 normal individual and 20 patients with iron overload showing the ability of the instrument to perform the measurements and to distinguish normal and pathological conditions. Now we are using the instrument routinely.

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.

Setup of a Small Business Incubator

In november/2002 the Small Business Incubator linked to the CEPID (INBIOS – Incubadora de Biotecnologia e Saúde) was officially launched. The Incubator is located in a space of 126 m^2 (figure) and should, initially, suport 5 small business initiatives.

Dissemination Activities (Press and Television)

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

5. CHANGES IN PLAN

After three years working together, the members of the center have increased the focus of their research on cell therapy. This can be evaluated by the comparison of the list of projects initially proposed (Table 1) and the projects that are being developed at present (Table 3).

The main aspects of this change are:

1. Most of the studies on structural and functional genomics are now directed at the better characterization of cells used for cell therapy. This includes the comparison of mesenchymal stem cells and $CD34^+$ obtained from different sources. Characterization of gene expression of CD34+ is still fragmentary and, for mesenchymal stem cells, ours are the first wide scale data. Also, we are evaluating the early changes subjacent to the CD34+ differentiation into erythroid and granulocytic-monocytic pathways.

2. The studies of gene polymorphisms that have been an important contribution of our group in the last years (60 articles in the last 10 years) have been focused on the evaluation of their role in the response and outcome of cell therapy (for instance, hematopoietic stem cell transplantation) and susceptibility to diseases treatable by cell therapy.

3. The clinical and applied research increased its participation in the project. Although the main purpose of the center is, as stated in the initial document, to focus on "basic cellular mechanisms (cell differentiation, cell recognition, cell-to-cell interaction, cell mediators, inflammation, coagulation, and apoptosis) and processing (isolation, expansion, selection, purging) which are relevant for cell-based therapy and its relation with gene structure and protein expression", we started to move to the applications of cell therapy, both in animals and in humans. Thus, mesenchymal stem cells are now starting to be tested for their possible therapeutic uses, and the experimental approach with dendritic cells is also starting to move to the clinical application. Moreover, innovative clinical trials with hematopoietic stem cell transplantation are underway or starting, for auto-immune diseases and diabetes mellitus.

4. Project 12 (listed in Table 3) "The use of pulsed autologous dendritic cells for the treatment of human melanoma" is a collaboration with the CEPI Centro Antonio Prudente para o Tratamento do Câncer. It applies part of the basic research related to dendritic and melanoma cells (projects 17, 18, 19, 20, 26) to more "applied" models of animal experiments and tests in humans. A summary of this project is added at the end of this chapter.

Table 1 – The 10 projects initially proposed and their evolution

	Active		
		Expanded or modified	
		Active as proposed	
	Finished or deactivated		
	↓	↓	↓
Immunologic Mechanisms Involved in the Response of Chronic Myelocytic Leukemia to α-Interferon and to Allogeneic Lymphocyte Infusion	✓		
Gangliosides in Hematological Malignancies and Lymphocyte Functions		✓	
Cancer Genome Anatomy Project for Hematological B-cell Malignancies			✓
Combined Quantification of CD79a, TdT, CD34, CD19, and CD10 Densities by Quantimetry in Normal Bone Marrow and Leukemic B-precursors	✓		
Cell Selection, Immunological Manipulation and Recovery in Autologous Transplants of Peripheral Blood Progenitor Cells for Hematopoietic Malignancies			✓
Cell Therapy with Granulocytes	✓		
Culture and *Ex vivo* Expansion of Dendritic Cells from Hematopoietic Stem Cells Obtained from Bone Marrow, Umbilical Cord or Peripheral Blood			✓
Tumor-Associated Antigens (TAA) and Autologous Dendritic Cell-Based Cancer Vaccines		✓	
Mechanisms and Genetic Manipulation of Graft-*versus*-Host and Graft-*versus*-Leukemia Effects in Animal Models of Human Neoplastic Diseases		✓	
The role of leukocyte tissue factor in inflammation	✓		

Table 2 – The present framework of research considering the list of projects indicated in Table 3.

	Included in subproject number ↓
Characterization of the disease and of the patient: identification of features that are related to the neoplastic process or that may interfere with cell therapy	
Morphology of cells, flow cytometry, molecular biology	1, 2, 5, 6, 7, 8, 9, 10, 11, 13, 14, 17, 18, 19, 20, 21, 22, 23, 25, 28
Functional role of gene polymorphisms in therapeutic responses	6, 7, 8
Functional roles of tumor-associated markers in tumor progression	1, 18, 19, 21, 22
Biological computation methods (Bio-informatics)	1, 2, 9, 10, 11, 14, 17, 26
Infectious diseases that may be transmitted or may be treated by cell therapy	
HTLV I/II	8, 25
HIV	8, 25
Identification, isolation and characterization of cells for therapy	
Collection, identification and culture	5, 12, 27, 28
Functional analysis: gene expression profiles	1, 2, 3, 9, 10, 11, 17
Functional analysis: the proteome	1, 2, 16, 18, 26
Manipulation of cells that may increase the efficiency of cell therapy	
Neoplasia	2, 22, 24
Infectious disease	8, 25
The patient submitted to cell-therapy and the cell donor	
The effect of gene polymorphisms on the disease	6, 7, 23
Immunologic mechanisms	3, 4, 18, 19, 20, 21, 22
Hematopoietic and mesenchymal stem cell autograft	5, 9, 10
Cell transfusion	12
Clinical trials of cell therapy	

Melanoma	12
Diabetes mellitus	4
Autoimmune diseases	3
Experimental animal models	
Pathogenesis of diseases	13, 14
Immunologic and/or inflammatory reaction	27
Cell therapy	27, 28

Table 3 – Present list of research projects at the Center for Cell-based Therapy.

1. Functional genomics of B-cell malignancies: the gene expression profiles of chronic lymphocytic leukemias and mantle cell lymphomas
2. The change of gene expression caused by in vitro treatment of malignant cell lines with drugs used for cancer chemotherapy
3. Treatment of immunological diseases by high dose chemotherapy and autologous bone marrow transplantation
4. Treatment of late onset type II diabetes mellitus by bone marrow transplantation
5. Generation, characterization and in vitro manipulations of mesenchymal stem cells aiming at their use for cell therapy
6. The impact of gene polymorphisms on the response to treatment with cell therapy
7. The impact of gene polymorphisms on the susceptibility to hematological diseases
8. The impact of gene polymorphisms on the response to HIV and HTLV infection
9. The functional genomics of cells used for cell therapy: the gene expression profiles of human mesenchymal stem cells obtained from different sites
10. The functional genomics of cells used for cell therapy: the comparison of the gene expression profiles of human $CD34^+$ cells obtained from bone marrow, umbilical cord blood and peripheral blood
11. The early gene expression changes in hematopoiesis: comparison of the erythroid and the granulocytic-monocytic pathways
12. The use of pulsed autologous dendritic cells for the treatment of human melanoma
13. Animal model of dyskeratosis congenita
14. Analysis of the molecular basis of leukemogenesis in the transgenic model of acute promyelocytic leukemia
15. Assessment and treatment of iron overload in β-thalassemia homozygous patients
16. Analysis of the crystal structure of human proteins coded by genes related to oncogenesis
17. Gene expression profiles during melanoma progression
18. Analysis of cell membrane molecules during melanoma progression
19. Gangliosides in hematological malignancies and lymphocyte functions
20. Cancer vaccine for chronic myeloid leukemia
21. Analysis of the expression of adhesion molecules in the leukemic phase of non Hodgkin's lymphomas
22. Analysis of blast adhesion and tethering upon histone deacetylase inhibitor and G-CSF treatment in acute promyelocytic leukemia
23. Analysis of FLT-3 mutations in acute myelogenous leukemia by Single Strand Polymorphism
24. Analysis of the effect of vitamin E isomers in acute promyelocytic leukemia
25. Cloning and expression *in vitro* of HIV and HTLV genes
26. Proteomics analysis of dendritic cell differentiation
27. Development of an animal model for testing cell therapy for lung disorders
28. Development of an animal model for the study of mesenchymal cell differentiation *in vivo*

The use of pulsed autologous dendritic cells for the treatment of human melanoma

Subproject coordinator: Roger Chammas
Investigators: Dimas T Covas, Aparecida M Fontes, Ricardo R Brentani, Debora CP da Silva, Marco A Zago
This project involves a collaboration between the CEPIDS Center for Cell Therapy (Ribeirão Preto) and The Antonio Prudente Center for Cancer Therapy (Hospital do Câncer)

In a recent study from the Center for Cell Therapy, it was shown that patients bearing advanced stage melanomas had a smaller proportion of dendritic cell (DC) precursors, as compared to healthy individuals. However, *ex vivo* differentiation and maturation of DC from these patients were normal. In keeping with this, a melanoma vaccination protocol in a similar group of patients, conducted by Debora CP da Silva (PhD thesis, FMUSP and Hospital do Câncer) had unsatisfactory results, likely associated with the decreased immune functions of the patients enrolled in the clinical protocol. Different lines of evidence indicate that patients with advanced stage tumors are indeed immunossuppressed. The immunization protocol in this first clinical trial at the Hospital do Cancer was based on the use of autologous melanoma cells used as immunogens in the presence of either BCG and GM-CSF, associated with cyclofosfamide.The notion that there may be a deficiency in dendritic cell differentiation in vivo prompted us to design a second protocol, based on the use of these cells as an adjuvant to the vaccination protocol evaluated at the Hospital do Câncer.

In this protocol, 30 stage III or IV melanoma patients will be enrolled. After tumor excision, melanoma cells will be cultured. Three weeks after surgery, a first delayed hipersensitivity test will be done, using lysed autologous melanoma cells. A punch biopsy of the intradermal reaction on day 2 will be analyzed. Peripheral blood will be drawn for lymphocyte and DC precursors isolation. DC will be differentiated in vitro, pulsed with irradiated autologous cells (apoptotic cells), maturated in vitro and then used as a cellular vaccine (intradermal injection). A total of 6 doses of this cellular vaccine will be administered. Immune function will be followed during the vaccination protocol. The tests for immune function will include: (1) analysis of the intradermorreaction triggered by lysed melanoma cells by routine histopathology and immunhistochemistry for qualification of the cellular infiltrate before and after at least 4 doses of the cellular vaccine; (2) production of specific anti-melanoma antibodies; (3) production of IFN-γ by lymphocytes cocultured with DC pulsed with irradiated melanoma cells; (4) integrity of IFN-c signaling cascade in melanoma cells. These parameters will be compared to the clinical follow-up of the immunized patients.

This protocol will also serve for *in vitro* testing of the role of tumor associated gangliosides in the immunization against melanoma. Depending on these results a second trial will be then suggested.

CEPID PROJECT – PROCESS 98/14247-6

TECHNICAL RESERVE

Amount granted for three years**:** R$ 322.251,35

Amount spent in the third year R$ 65.212,81

NATIONAL MARKET PERMANENT MATERIAL

QUANT	*DESCRIPTION OF THE MATERIAL PURCHASED*	*VALUE R$*
02	Scale, model MS6/1	1.870,00
01	Color table scanner, model Scanjet	650,00
01	Sonicator, 2.9 liters, Time MAX 301 - Model USC 1400 Bivolts	870.00
02	Micropipette adjustable from 0.5 to 10 µl	1.734,00
02	***Micropipette adjustable from 2 to 20 µl***	1.602,00
02	Micropipette adjustable from 20 to 200 µl	1.602,00
01	***Transponder reader ISO FDX-B animaltag model KT34 – Voltage: 110 volts***	1.053,15
02	***Transparent upper reservoir with an electrode – accessory of the ABI instrument model 377***	3.339,99
	Total:	**12.721,14**

NATIONAL CONSUMABLE MATERIAL

Other material	R$ 27.025,02

THIRD PARTY SERVICES

Description	*Value R$*
Preparation of the release and editing of the 7th Science Journal	900,00
Registration of Marcia Ferraresi de Araujo at the International Symposium on New Clinical and Molecular Approaches to Cancer	80,00
Binding of teaching material for the summer course of 2003	180,00
Maintenance of the Megabase sequencer	6.510,25
Maintenance of the VDS Image Master equipment	4.948,70

Serologic exams (virology) of transgenic mice performed in the Charles River Laboratory	1.615,68
Preparation of the Patient Manual for the Bone Marrow Transplant Unit	1.300,00
Preparation of the 7th edition of the Science Journal	896,00
Salary of a Laboratory Assistant and of Informatics Technicians	19.360,38
TOTAL	**35.791,01**

HOTEL EXPENSES

Description	*Value R$*
Per diem for Rodrigo Ratier, Lecture on June 28, 2003	150,00
Per diem for Dr Francisco Miragaia Peruzzo, Lecture on June14, 2003	100,00
Per diem for Diogo Meyer, Shelton Inn Hotel	53,30
Per diem for Dr. Welligton Azevedo, Residence Flat	92,40
Per diem for Dr. Mark Walters, Residence Flat	95,60
Per diem for Dr. Vanderson Rocha, Stream Palace Hotel	71,50
Per diem for Dra Margarida Maria Silveira, Lecture on May 29, 2003	150,00
Per diem for Prof. Mauricio Zaporoli, Lecture on April 23,l 2003	150,00
Prof. Maria Socorro Pombo de Oliveira's stay at the Shelton Inn Hotel	51,50
Per diem for Carolina Falcão Motoki	150,00

TOTAL: 1.259,42

AIRPLANE TICKETS

Air ticket for Dr. Dr. Mark Walters

3.991,17

Air ticket for Prof. José Eduardo Kreiger, speaker at the Joint Seminar, November 13, 2003

632,40

Air ticket for Sérgio Coutinho, speaker at the Joint Seminar, December 4, 2002

957,06

Air ticket for Prof. Roseli Figaro, speaker for the Educational Project, April 26, 2003

586,86

Air ticket for Prof. Magda, speaker at the Joint Seminar, May 22, 2003

653,50

Air ticket for Dr. Celso Massumoto, speaker at the Joint Seminar, June 26, 2003

725,81

TOTAL:

7.546,80

OTHER RESOURCES

		R$
1. FINEP	Process nº 64.00.0487.00	***1.040.330,00***
2. PADCT	Process nº 62.0019/99-9	202.000,00
3. FINEP	Process nº 0659/02	300.000,00
4. PRONEX – CNPq	Process nº 66.1132/1998-6	746.500,00
5. FAPESP	Process nº 00/01854-3	99.000,00
	Total:	2.288.830,00