



## Statement of Impact

The Center for Cell-based Therapy (CTC) is composed of a group of researchers interested in understanding the biology of pluripotent and somatic stem cells in normal or pathological conditions, as well as in the development of new technologies for application in the clinical practice. To achieve these goals, CTC consists of an interdisciplinary group of physicians, biomedical professionals, biologists, pharmacists, veterinarians, engineers, among others. The gathering and cooperation of their expertise enabled the establishment of solid research lines with major scientific, clinical, and social impact.

In this edition, CTC focused its interest on the clinical development of hematopoietic and stromal mesenchymal stem cells for using in the treatment of (1) autoimmune diseases; (2) sickle cell disease; (3) bone marrow failure; (4) graft-versus-host disease; and (5) acute leukemia. Systemic sclerosis and sickle cell disease are severe chronic degenerative and inflammatory diseases whose current treatments are palliative and prolonged, not preventing irreversible cumulative organ dysfunctions, compromising the quality of life and decreasing survival.

**Autoimmune diseases.** CTC transplanted 98 patients with multiple sclerosis (MS), enrolled in clinical trials of the Center, in collaboration with international researchers from Université Paris-Diderot (France) and Northwestern University (Chicago, USA). Our data showed that hematopoietic stem cell transplantation is associated with improvement of neurological deficit, in addition to improvement of quality of life. Likewise, the Center demonstrated that autologous hematopoietic stem cell transplantation improves pulmonary function in systemic sclerosis, although it also showed that pretransplantation cardiac involvement is a determining factor for treatment success.

**Sickle cell disease.** It is the most common monogenic disease in Brazil, for which allogeneic hematopoietic stem cell transplantation is the only opportunity for cure. This morpho-physiological alteration in blood promotes chronic degeneration of the nervous system, lungs, kidneys, heart, among other organs, dramatically reducing life expectancy and significantly limiting quality of life. CTC is the only center in Brazil to perform transplantation against sickle cell diseases; it has already enrolled 45 patients in its clinical trial, mainly devoted to transplant adult patients, different from other international centers dedicated to transplanting children. Our outcomes demonstrated that related donor myeloablative transplantation for sickle cell disease in adults is feasible and safe, with high survival rate, reduction of organ damage caused by sickle cell disease, improvement of quality of life, and low graft-versus-host disease rate.

**Bone marrow failure.** CTC leads new cellular and non-cellular approaches for the treatment of both acquired and inherited hematopoietic stem cell failures with more than 250 patients followed and treated at the Center. Making use of CTC technology for the clinical use of mesenchymal cells to treat immune processes, we added these cells, in a clinical trial, to the immunosuppressive treatment of patients with immune aplastic anemia. We demonstrated that in these cases the addition of mesenchymal cells does not result in greater therapeutic response, perhaps because they do not migrate satisfactorily to the bone marrow of these patients. We also showed for the first time, in collaboration with the National Institutes of Health, that in genetic bone marrow failures the treatment with male hormone might modulate in vivo telomere length of human cells, what results in clinical improvement.

**Graft-versus-host disease.** GVHD is a potentially severe complication of the allogeneic hematopoietic stem cell transplantation resulting from genetic disparities between graft (transplanted cells) and recipient (patient). In a clinical trial enrolling more than 40 patients, we demonstrated that the infusion of stromal mesenchymal cells from other person is a safe treatment for GVHD and it can be effective. The use of these cells is a viable alternative to costly immunobiologicals with the same indication. In collaboration with the Université Paris-Diderot, CTC demonstrated in the analysis of over 450 patients subjected to allogeneic bone marrow transplantation that telomere length is an independent predictor of transplantation-related mortality.

**Acute leukemia.** CTC leads the International Consortium on Acute Promyelocytic Leukemia (IC-APL) and the International Consortium on Acute Leukemia (IC-AL), a current consolidated group of the American Society of Hematology (ASH), promoting cooperation among Brazil, Chile, Peru, Uruguay, and Paraguay. By analyzing the treatment of more than 220 APL patients, CTC was able to identify new biomarkers that may predict better or worse response to the current treatment, what might early guide towards treatment intensification.

## Publication of scientific impact:

1. Gluckman E, Cappelli B, Bernaudin F, Labopin M, Volt F, Carreras J, Pinto Simões B et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. *Blood*. 2017;129(11):1548-1556. Citations: 46 (Most cited article on ISI)
2. Burt RK, Oliveira MC, Shah SJ, Moraes DA, Simoes B et al. Cardiac involvement and treatment-related mortality after non-myeloablative hematopoietic stem cell transplantation with unselected autologous peripheral blood for patients with systemic sclerosis: a retrospective analysis. *Lancet*. 2013;381(9872):1116-24. Citations: 53
3. Burt RK, Balabanov R, Han X, Sharrack B, Morgan A, Quigley K, Yaung K, Helenowski IB, Jovanovic B, Spahovic D, Arnautovic I, Lee DC, Benefield BC, Fütterer S, Oliveira MC, Burman J. Association of nonmyeloablative hematopoietic stem cell transplantation with neurological disability in patients with relapsing-remitting multiple sclerosis. *JAMA*. 2015;313(3):275-84. Citations: 54
4. Clé DV, Santana-Lemos B et al. Intravenous infusion of allogeneic mesenchymal stromal cells in refractory or relapsed aplastic anemia. *Cytotherapy*. 2015;17(12):1696-705. Citations: 6
5. Xhaard A, Cunha R et al. Clinical profile, biological markers, and comorbidity index as predictors of transplant-related mortality after allo-HSCT. *Blood Adv*. 2017 Aug 2;1(18):1409-1413. Citations: 1
6. Townsley DM, Dumitriu B, Liu D, Biancotto A, Weinstein B, Chen C, Hardy N, Mihalek AD, Lingala S, Kim YJ, Yao J, Jones E, Gochuico BR, Heller T, Wu CO, Calado RT, Scheinberg P, Young NS. Danazol Treatment for Telomere Diseases. *N Engl J Med*. 2016 May 19;374(20):1922-31. Citations: 66 (Most cited article on ISI)
7. Dotoli GM, De Santis GC et al. Mesenchymal stromal cell infusion to treat steroid-refractory acute GvHD III/IV after hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2017;52(6):859-862. Citations: 7
8. Lucena-Araujo AR, Pereira-Martins DA et al. Clinical impact of BAALC expression in high-risk acute promyelocytic leukemia. *Blood Adv*. 2017;1(21):1807-1814.
9. Lucena-Araujo AR, Kim HT, Thomé C et al. High  $\Delta Np73/TP73$  ratio is associated with poor prognosis in acute promyelocytic leukemia. *Blood*. 2015;126(20):2302-6. Citations: 7
10. Rego EM, Kim HT, Ruiz-Argüelles GJ, Undurraga MS et al. Improving acute promyelocytic leukemia (APL) outcome in developing countries through networking, results of the International Consortium on APL. *Blood*. 2013;121(11):1935-43. Citations: 37

# Scientific Impact

The development of clinical trials involving samples of patients with hematopoietic stem cell or other stem cell diseases offers CTC the unique opportunity to conduct scientific investigation of the mechanisms of disease and response to these patients' treatment.

**Autoimmune diseases.** CTC researchers characterized the immunomodulatory properties of stromal mesenchymal cells derived from patients with autoimmune diseases, they also determined immune reconstitution mechanisms after autologous hematopoietic stem cell transplantation.

**Adult stem cell failure.** In partnership with the National Institutes of Health, CTC derived (iPS) induced pluripotent stem cells from patients with bone marrow failure and mutations in genes involved in telomere repair. We showed defects in telomere elongation during cell reprogramming to the pluripotency state; moreover we showed how environmental factors can modulate the process. Additionally, we identified new genes involved in the etiology of hematopoietic stem cell failure. CTC identified that telomere shortening and telomerase deficiency, apart from inducing proliferative senescence, promote extensive metabolic alterations in murine hepatocytes, provoking blockade in Krebs cycle and hepatocyte incapacity to respond properly to external metabolic variations.

**Sickle cell disease.** The Center derived induced pluripotent stem cells (iPS) from patients with sickle cell anemia to modulate the disease and serve as a platform for the development of gene correction therapies. Equally, CTC researchers were able to modulate the inflammatory response induced by intravascular hemolysis through nitric oxide in murine model, indicating the role of this molecule in the pathophysiology of sickle cell disease.

**Cancer stem cells.** For the last five years, CTC researchers have investigated molecular pathways of cancer stem cells, both in solid tumors and in onco-hematology, involved in maintenance of stemness, epigenetic control, epithelium-mesenchymal transition, and differentiation control. These pathways enable better understanding of oncogenesis mechanism, as well as identifying new possible therapeutic targets. With attention to one accomplishment, the Center identified long non-coding RNA Hotair as necessary for epithelium-mesenchymal transition in cancer, mechanism responsible for the formation of metastases. It also demonstrated that all trans retinoic acid (ATRA), used to treat acute promyelocytic leukemia, induced the degradation of Pin1 isomerase, vital to oncogenic signaling pathways, suggesting that ATRA could also be effective in the treatment of other cancer types. CTC researchers showed as well that in myeloid neoplasias, somatic mutations in genes involved in epigenetic regulation have impact on the response to treatment with DNA methyltransferase inhibitors. These data might be helpful to determine a more precise treatment to patients.

## Publications of scientific impact:

1. de Lima KA, de Oliveira GL, Yachite JN, Pinheiro DG, de Azevedo JT, Silva WA Jr, Covas DT, Couri CE, Simões BP, Voltarelli JC, Oliveira MC, Malmegrim KC. Transcriptional profiling reveals intrinsic mRNA alterations in multipotent mesenchymal stromal cells isolated from bone marrow of newly-diagnosed type 1 diabetes patients. *Stem Cell Res Ther.* 2016;7(1):92. Citations: 4
2. Arruda LCM, Malmegrim KCR, Lima-Júnior JR, Clave E, Dias JBE, Moraes DA, Douay C, Fournier I, Moins-Teisserenc H, Alberdi AJ, Covas DT, Simões BP, Lansiaux P, Toubert A, Oliveira MC. Immune rebound associates with a favorable clinical response to autologous HSCT in systemic sclerosis patients. *Blood Adv.* 2018;2(2):126-141. Citations: 3
3. Winkler T, Hong SG, Decker JE, Morgan MJ, Wu C, Hughes WM 5th, Yang Y, Wangsa D, Padilla-Nash HM, Ried T, Young NS, Dunbar CE, Calado RT. Defective telomere elongation and hematopoiesis from telomerase-mutant aplastic anemia iPSCs. *J Clin Invest.* 2013;123(5):1952-63. Citations: 42
4. Marsh JCW, Gutierrez-Rodriguez F, et al. Heterozygous RTTEL1 variants in bone marrow failure and myeloid neoplasms. *Blood Adv.* 2018;2(1):36-48. Citations: 2
5. Alves-Paiva RM, Kajigaya S, Feng X, Chen J, Desierto M, Wong S, Townsley DM, Donaires FS, Bertola A, Gao B, Young NS, Calado RT. Telomerase enzyme deficiency promotes metabolic dysfunction in murine hepatocytes upon dietary stress. *Liver Int.* 2018;38(1):144-154.
6. Almeida CB, Souza LE, Leonardo FC, Costa FT, Werneck CC, Covas DT, Costa FF, Conran N. Acute hemolytic vascular inflammatory processes are prevented by nitric oxide replacement or a single dose of hydroxyurea. *Blood.* 2015;126(6):711-20. Citations: 30
7. Junqueira Reis LC, Picanço-Castro V, Paes BCMF, Pereira OA, Gerdes Gyuricza I, de Araújo FT, Morato-Marques M, Moreira LF, Costa EBO, Dos Santos TPM, Covas DT, Pereira Carramaschi LDV, Russo EMS. Induced Pluripotent Stem Cell for the Study and Treatment of Sickle Cell Anemia. *Stem Cells Int.* 2017;2017:7492914.
8. Arruda LC, Lorenzi JC, Sousa AP, Zanette DL, Palma PV, Panepucci RA, Brum DS, Barreira AA, Covas DT, Simões BP, Silva WA Jr, Oliveira MC, Malmegrim KC. Autologous hematopoietic SCT normalizes miR-16, -155 and -142-3p expression in multiple sclerosis patients. *Bone Marrow Transplant.* 2015 Mar;50(3):380-9. Citations: 27
9. Gutierrez-Rodriguez F, Santana-Lemos BA, Scheucher PS, Alves-Paiva RM, Calado RT. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. *PLoS One.* 2014 Nov 19;9(11):e113747. Citations: 29
10. Yachite JN, Calari-Oliveira C, Davanzo MR et al. Dynamic changes of the Th17/Tc17 and regulatory T cell populations interfere in the experimental autoimmune diabetes pathogenesis. *Immunobiology.* 2013 Mar;218(3):338-52. Citations: 23

# Social Impact

The social relevance of the work developed in CTC can be assessed from two branches: education and health care.

In the educational branch, the House of Science has profoundly influenced science education in elementary and high school students of Ribeirão Preto and region, approaching the University to the public education network. This initiative has brought students and teachers to laboratories inside Hemocentro RP and the USP campus in Ribeirão Preto (<http://www.casadaciencia.com.br>).

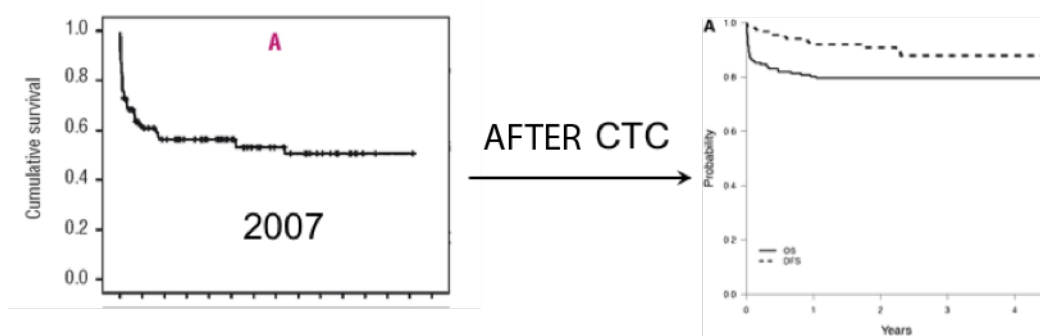
Among the programs “Adopt a Scientist”, “Vacation with Science”, and “Little Scientist”, there have been more than 800 directly involved students of basic and high education since 2013. Equally important, these students’ work motivated the creation of the page on Facebook, with over 7 thousand followers; the House of Science channel on YouTube (<https://www.youtube.com/CasadaCienciaHRP>) with more than 200 videos, live broadcasts, and over 230,000 views, the House of Science website, with more than 200,000 accesses, and the Cellularium with over 23,000 visitors from 11 surrounding cities.

In terms of health care, CTC work has a direct impact on changing how patients have been treated by introducing cell therapy. In systemic sclerosis, a severe disease with mortality of 50% in 5 years, CTC studies showed that the autologous hematopoietic stem cell transplantation is superior and cheaper than treatments available today. The survival in 5 years is almost 90% and there is interruption to disease progression. These findings change the way of treating patients at specific stages of systemic sclerosis. In patients with multiple sclerosis, transplantation creates the opportunity to interrupt conventional immunosuppressive treatment, which often demands hospital visits, hospitalization, and intravenous

infusions. The prolonged use of immunomodulators to treat the disease is associated with the incidence of severe opportunistic infectious diseases. The multicenter randomized trial in which we participated showed that transplantation promotes better disease control, with reactivation in the first 3 years in 6% of patients versus 60% of patients treated with conventional medication (results divulged in the Annual Meeting of EBMT in Lisbon, in March 2018, and submitted to publication).

Sickle cell anemia is the most prevalent monogenic disease in Brazil, causing chronically irreversible damages to several organs, such as brain, lungs, heart, and kidneys. CTC is the only center in Brazil to perform allogeneic bone marrow transplantation, the sole curative therapeutic modality available today, that has changed the quality of life and reduced or even reverted the damage to many organs. Its impact is not measurable yet in terms of cost reduction to the health system, improvement of patient’s quality of life and productivity, apart from the psychological impact in a common genetic disease in the country and most prevalent in less favored regions, as the Northeast. The use of the transplantation for sickle disease developed in CTC can change the reality of care to these patients in Brazil in the upcoming years.

The work of CTC researchers also changed the natural history of promyelocytic leukemia in the country and abroad, elevating from 50% to 80% survival of these patients in 2 years, it may be the study that has mostly modified the treatment response to cancer in Brazil (Figure 1). This work has trained and gathered groups in seven centers in Brazil, transferring technology that has direct impact on the SUS (Unified Health System) care.



**Figure 1. Change in the survival of patients with acute promyelocytic leukemia after the treatment protocol developed and led by CTC.**

# Technological Impact

Among the activities developed at the CTC level, we underline the ones related to target activities, it means, therapies that use cells. Keeping in mind that the clinical impact has been already a target for a discourse, in this topic, we will cover the pioneer use of methodologies developed by the group that are concrete proofs of technology transfer to society. Before writing about the methodologies, we emphasize that, in the medical areas, products and medications follow a strict regulation that demands rigorous clinical trials and compatible quality patterns. CTC has been working consistently and determinedly on that.

We can give some examples: 1) production of GMP (pathogen free) cells; 2) development of methodology to produce Xenofree cells (free from components derived from animals); 3) culture of cells on demand scale ( $5 \times 10^7$ ); 4) development of a battery of tests aiming at the safety of both infused cells and blood derivatives used in their production and in cryopreservation and stock; 5) development of equipment and methodologies for sending cells to different hospitals for safe and effective use.

To the best of our knowledge, CTC is the first and only center to offer this service in the country to date and it

has been providing these products for large health care facilities as HC, Hospital do Servidor Público, Hospital do Câncer de Barretos, Hospital Albert Einstein, among many others.

The set of these technologies is dealt as a technological package, developed in house or in association with technology-based companies (such as WTA Med), that will be part of a set of rules required by health regulatory agency at the approval for routine clinical use.

In essence, our efforts are focused on the development of innovative products in a term compatible with other therapeutic products to the medical area, aiming at their effective application.

Besides the methodologies of cell infusion-based therapies, there is another set of therapies based on products of recombinant cells linked to coagulation. These products include coagulation factors VII, VIII, and IX, which already have approved/filed patent registers and have been validated regarding their function/activity and now they are at a scale of pilot tests together with Instituto Butantã. Once the technical viability of the products is confirmed, a business plan will be developed to define possible commercialization strategies.