



Activity Report 2013/2014

Acknowledgment

Dear supporter,

Since 2005, the Pelé Little Prince Research Institute has pursued new methods for the diagnosis and treatment of children's disease, contributing to the reduction of child-adolescent mortality. The creation of the Institute was made possible by the support of Edson Arantes do Nascimento, Pelé.

Making advancements in health care for children and adolescents is the motivation for the Little Prince Complex. The Goals for Life project raises funds for the Institute, providing support for cutting-edge research in the treatment of children and adolescents.

The Institute features 17 researchers and more than 80 ongoing projects. These projects broaden the horizons of our mission and result in scientific advancement and innovation through new methods of diagnosis and treatment, more efficient techniques that are less invasive, less costly, and the expansion of possibilities for care and treatment.

The deep knowledge of our professionals and researchers leads to a common purpose: to generate and democratize health knowledge for other regions of the country and the world, contributing to the early diagnosis, prevention, and cure of diseases in various locations, thus ensuring better health and quality of life for patients. With the dedication of our staff, the confidence of the people we serve, and the commitment of our partners, the Little Prince Complex is proud to serve as the leading pediatric service in Brazil, a major training center for health professionals, and a cradle of scientific innovation.

We are proud to see that throughout our history many hands and many people have taken our cause to their hearts, contributing so that more fruits of this labor can be collected and shared. The following pages will show how these valuable contributions, in collaboration with others, help to raise treatment and cure rates through research developments, bringing better health and hope to thousands of boys and girls in Brazil and in the world.

Thank you for caring and for recognizing and believing in our work.



Ety Cristina Forte Carneiro
Pelé Little Prince Research Institute General Director
Little Prince Hospital Chief Executive Officer



José Álvaro da Silva Carneiro
Corporative Director of Little Prince Complex

Institutional Presentation

About us

Little Prince Complex is a nonprofit philanthropic organization focused on promoting the health of children and adolescents. Founded in 1919, with the support of the Hospital Association for the Protection of Children Dr. Raul Carneiro, the institution is committed to improving the health of thousands of children and adolescents in Brazil.

Comprised of the Little Prince Hospital, Little Prince College, and the Pelé Little Prince Research Institute, the Little Prince Complex operates in the areas of health care, teaching, and research. With this triple focus and the synergies resulting from the combined effort, we are constantly improving the health services offered to society.

To maintain a level of excellence, we seek to educate professionals, who are trained and prepared to provide qualified and humanized care to patients. Education derives new perspectives from scientific research, which enable professionals to disseminate information, innovate, and improve medical practices.

The results obtained in the laboratory are brought directly to patients, providing new diagnostic methods, better techniques and more assertive treatments. They also serve as the basis for new medical protocols and new public policy.

The three units of the Little Prince Complex work in an integrated and complementary manner to promote good health, education, and science. Our teams include doctors, nurses, psychologists, physiotherapists, administrators, biomedical experts, pharmacists, biologists, social workers, educators, speech therapists, mathematicians, physicists, and engineers. In meeting the demands from situations in clinical practice, they work to expand the scientific knowledge and improve and develop diagnostic techniques and treatment for our children. Our professionals seek continuing education and scientific updates, promoting the diversification of specialties, the modernization of diagnostic tools, and the humanization of services.

Virtuous circle

Our three units come together in a virtuous circle, allowing all the knowledge generated within the complex to be improved, shared, analyzed, researched, studied and put into practice by professionals and spread throughout Brazil by educational programs as well as by medical education in the home. Thus, we train new professionals for the market; disseminate discoveries to other parts of the world; establish new health procedures as result of research; and develop more accurate diagnoses and healing techniques, thus providing better quality of life for our children and adolescents, giving hope to their families, and reducing child mortality.



Little Prince Complex in numbers 2014

63%

of the patients are from the Brazilian Unified Health System (SUS)

2,056

employees

300

physicians

84

professors in the undergraduate, graduate specialization, master's, and doctoral programs

17

researchers

96 years

of work

1,151

volunteers



Little Prince Hospital in numbers 2014

The largest hospital dedicated exclusively to children in Brazil.

32

medical specialties (from birth to 18-year-olds)

390 leitos

beds, **62** of them ICU's

19,061

surgeries

313,859

outpatient clinic appointments

23,624

in-patients

169

transplants (bone tissue, organs, and bone marrow)



Little Prince College in numbers 2014

Training new qualified professionals, prepared to offer humanized care to patients, and ready to conquer the labor market.

5

undergraduate programs (Biomedicine, Nursing, Pharmacy, Medicine, and Psychology)

100

openings per year in the undergraduate medical program

5

graduate courses (specialization, master's, and doctoral degrees)

115

scientific papers published by graduate students

1,105

students



Pelé Little Prince Research Institute in numbers 2014

Focusing on innovation, we put science at the forefront of new diagnostic methods, new treatments and the prevention of complex diseases.

17

researchers

7

research lines

80

ongoing projects

29

scientific papers published

61

national and international partner institutions



Brazil at the cutting edge of knowledge in the area of child and adolescent health



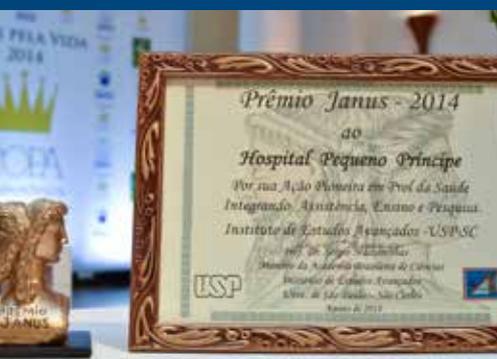
The health of children and teenagers, from the point of view of health policy, is perhaps the most relevant issue to be taken into account, since it serves to secure the most important element for any society: the quality of its human resources. It is well-known, moreover, that without the triad of teaching, research, and outreach, no institution in any area of science, technology or innovation can realize complete achievement if it lacks the synergies of inter- and trans-disciplinary knowledge required in today's globalized world.

There is another requirement in addition to those above: the absolute necessity of international cooperation in the field of medicine. To some it may seem difficult for a developing country to have an institution with such a complex profile. However, supported by its 96-year tradition, Little Prince Hospital made the exceptional decision to build an institution of great complexity that will be a health care model not only in Brazil and Latin America but globally, by bringing together teaching, research, and support with the most modern techniques available, covering molecular biology, psychopharmacology, genomics, bioinformatics and imaging, with equipment such as MRI scanners and positron emission tomography (PET). In short, these major investments will make Curitiba, Brazil, the home of Little Prince Complex, a true mecca of health research at the frontier of science in the twenty-first century.

An expansion of this size for Little Prince Complex requires strategic management, high quality human and structural resources, human and infrastructure, as well as solid experience with high-level missions and relevance on the international scene. These factors are fully realized at the Pelé Little Prince Research Institute in Curitiba. It may sound like a dream, but it is real. As the Nobel Prize winner Abdus Salam said, "Ideals and Realities are the essence of progress for the evolution of humanity!" Congratulations Curitiba, congratulations Brazil!

Prof. Dr. Sérgio Mascarenhas

Emeritus Researcher of CNPq-MCT; Member of the National Order of Scientific Merit – Grã-Cruz; Member of the Brazilian Academy of Sciences; Member of the Trieste Science Academy and the Latin American Academy of Sciences; Award Conrado Wesse – General Sciences; honorary president of the Brazilian Society for the Progress of Science, and Visiting Researcher at Harvard Medical School, as well as the Children's Hospital in Boston.



Research for better health and life

With a highly qualified staff, Pelé Little Prince Research Institute has made important contributions to saving and improving the lives of children and adolescents through seven different research lines. The research is conducted in conjunction with the graduate programs of the Little Prince College, through the master's and doctoral programs. In 2013 and 2014, more than 80 research projects were underway and more than 49 scientific articles were published in specialized journals, in addition to development of three patents.

The studies have been developed along the following seven research lines:

1. COMPLEX AND ONCOGENETIC DISEASES

The study of diseases caused by a combination of genetic, environmental, and lifestyle factors. We also provide counseling and oncological genetic risk estimates.

2. EPIDEMIOLOGICAL, CLINICAL AND EDUCATIONAL STUDIES

Projects related to determining factors for health/disease processes and research on preventive interventions, therapies and rehabilitation for certain diseases. This also includes studies on health education, highlighting the methodological dimensions and curriculum development of undergraduate and postgraduate healthcare students.

3. IMAGE, RADIOLOGICAL, AND RADIOTHERAPY PROTECTION

Studies on imaging for diagnostic, radiation protection equipment, and radiopharmaceuticals.

4. NEUROSCIENCE

Studies in neuroscience applied to neurology, mental health, and psychiatry.

5. MICROBIOLOGY AND INFECTIOUS DISEASES

Studies involving the diagnosis, epidemiology, and applied research on diseases related to microorganisms (viruses, bacteria, fungi, and protozoa).

6. CELLULAR AND PHARMACOLOGICAL THERAPY

Studies on cellular therapy and the impact of pharmacological agents on treatments.

7. MOLECULAR MEDICINE AND BIOINFORMATICS

Studies of molecular biology and genomics to investigate molecular anomalies and to characterize and quantify biological processes, whether normal or not.



Research Areas and Main Studies - 2014

I. COMPLEX AND ONCOGENETIC DISEASES

The study of diseases caused by a combination of genetic, environmental, and lifestyle factors. We also provide counseling and oncological genetic risk estimates.

- Genetic and molecular alterations in adrenocortical tumors and cells carrying the TP53 mutation R337H.
- Anticipation and environmental influences on cancer frequency in families with the TP53 mutation R337H.
- Study of DNA methylation in people with the TP53 mutation R337H.
- Development of nanostructured compounds for the diagnosis and treatment of childhood adrenocortical tumors.
- Micro-RNAs and immunohistological markers in childhood adrenocortical tumors.
- Development of diagnostic methods for identification of human childhood adrenocortical tumors from animal models.
- Early diagnosis of childhood adrenocortical tumors.
- Toxic effects of nanostructured compounds in normal cells and cells from adrenocortical tumors.
- Study of copy number variation profile on chromosome from adrenocortical tumor cells from children carrying the TP53 mutation R337H.
- Analysis of the interaction between progranulin mahogunin in modulating amyloid aggregates deposition in vitro.
- Prevalence of viral genomes in hepatocellular carcinoma.
- Studies of molecular pathways in childhood central nervous system tumors.
- Antibody synthesis and development of nanostructured tests for neuroblastoma markers.
- Implementation of molecular cytogenetic methods for diagnosis of acute leukemia in children.
- Genomic biomarkers to evaluate the progression of chronic lymphocytic leukemia.
- Use of ELINOR effect for fast detection of gene fusions in leukemias.
- Geomedicine: Environment influence on leukemia prevalence in the United States and Paraná.
- Use of telemedicine to improve health care of children with cancer living in remote areas.



Research and its effects on care: finding cures to save lives

COMPLEX AND ONCOGENETIC DISEASES

MORE PRECISION IN LEUKEMIA DIAGNOSIS

Glendha Wilczek was only one year old when she arrived at the emergency room of Little Prince Hospital in February 2014 on the recommendation of her pediatrician. It was a Friday. At the hospital, she underwent tests and was admitted. By Sunday, she had a diagnosis: leukemia.

To determine the best treatment, Glendha was tested using **FISH** technology (fluorescence in situ hybridization) to provide with greater accuracy in diagnosis and indication of treatment options.

"I think the exam is great because the goal is to improve the patient's life," said Glendha's mother. "The more kids have access to it, the greater the probability of cure, the faster the diagnosis and the guiding for more specific treatments. I find it amazing," she continued.

Glendha underwent treatment at Little Prince Hospital, including a bone marrow transplant in January 2015. Her older sister was the donor. Today, two years and eight months old, Glendha makes weekly visits to the hospital for follow-up and testing.

96%

WAS THE REDUCTION IN THE COST OF THE FISH EXAM OBTAINED BASED ON A RESEARCH PROJECT CONDUCTED AT THE PELÉ LITTLE PRINCE RESEARCH INSTITUTE.

COST SAVINGS = MORE LIFE

A research project conducted at Pelé Little Prince Research Institute led to a reduction in the cost of the FISH exam from R\$300.00 to only R\$12.00, effectively democratizing the access to it. As a result, all patients with leukemia treated at the Little Prince Hospital and at other hospitals can benefit from the procedure and receive accurate diagnoses and guidance for treatment.



FISH

FLUORESCENCE IN-SITU HYBRIDIZATION

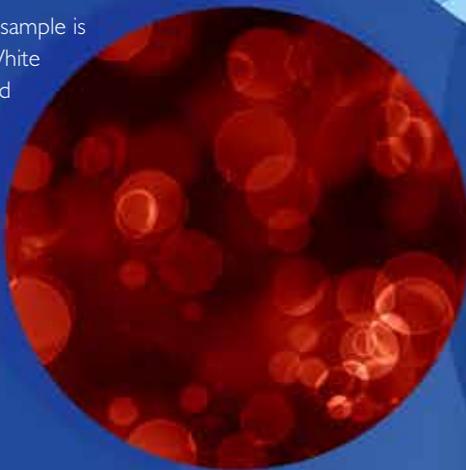
1. Biological sample (blood or bone marrow)

Blood or bone marrow are withdrawn from the patient.



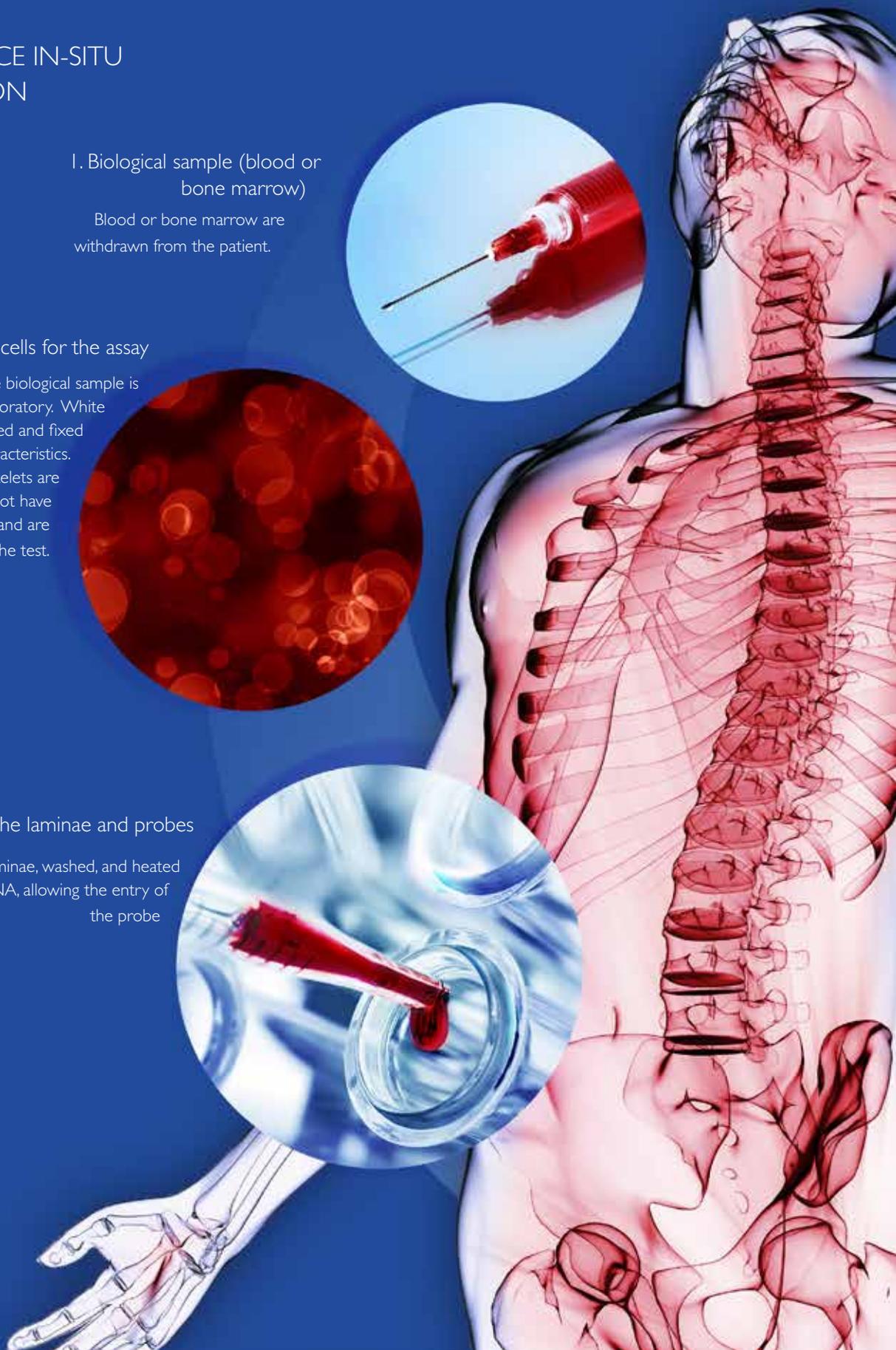
2. Preparation of cells for the assay

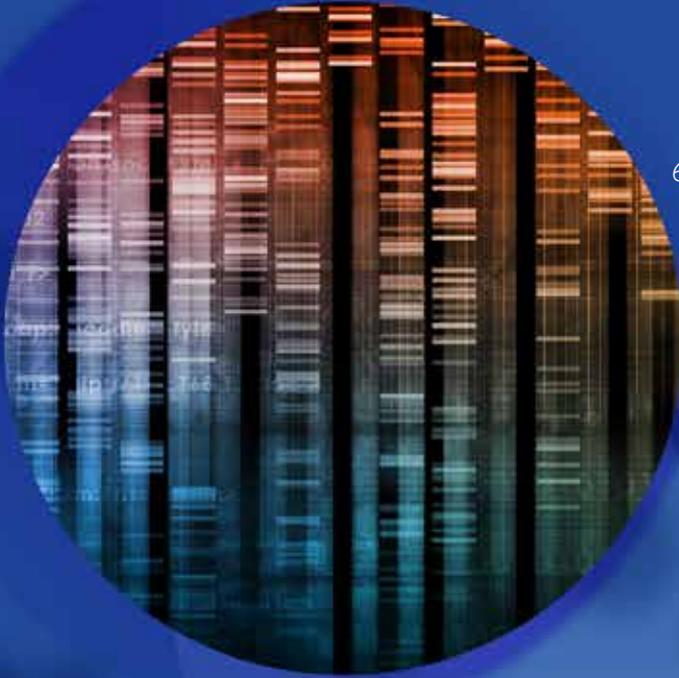
When necessary, the biological sample is cultivated in the laboratory. White blood cells are separated and fixed to preserve their characteristics. Red blood cells and platelets are discarded as they do not have nuclei, do not have DNA, and are thus not required for the test.



3. Preparation of the laminae and probes

Cells are spread in laminae, washed, and heated to open up the DNA, allowing the entry of the probe





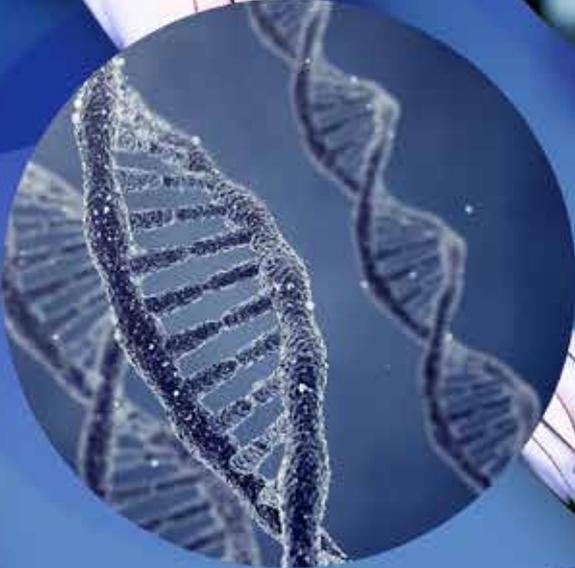
6. Analysis of Results under the microscope

Fluorescent signals are analyzed under the microscope. Based on the number and position of different color signals, it is possible to know whether the sample is normal or presents an abnormality.



5. Wash

Excess probe is removed.



4. Hybridization of the probe

A droplet of the probe is placed on the slide, covered with cover glass and left for a few hours, so that the probe and the DNA hybridize.

Research Areas and Main Studies - 2014

2. EPIDEMIOLOGICAL, CLINICAL, AND EDUCATIONAL STUDIES

Projects related to factors that determine health/disease processes and research on preventive interventions, therapies and rehabilitation for certain diseases.

- Epidemiological, cytogenetic, and molecular alterations analysis in cases of acute leukemia in children of the western region of Paraná.
- Analysis of the process of implementation of the health system in schools in the context of the city health system.
- Assessment of quality of life, functional ability and fatigue of patients at different stages of liver post-transplantation in Little Prince Hospital.
- Construction and validation of tools for assessing general skills in health related courses.
- Development and evaluation of an online serious game for training students and health professionals in the management of insulin for treating diabetes mellitus.
- Comparative study of antibiotic therapy protocols in pediatric liver transplantation: risk factors for infection and pharmacoeconomics in Little Prince Hospital.
- Risk factors associated with the occurrence of liver cancer in Paraná.
- Frequency of genomic abnormalities in pediatric patients with acute lymphoblastic leukemia being treated in Curitiba.
- Comparison of different growth charts for nutritional follow up of patients with neuropathies at Little Prince Hospital.
- Environmental and socioeconomic profiles and their influence on the incidence and mortality rates of leukemia in the state of Paraná and the United States.
- Epidemiological profile of exogenous poisoning in children and adolescents ages 0 to 19 reported in epidemiological surveillance in the city of Curitiba, Paraná (2007/2011).
- Use of auriculotherapy in the control of orthopedic postoperative pain in hospitalized children and adolescents.
- Development of an online system to elaborate family trees for multicenter studies.

3. IMAGE, RADIOLOGICAL, AND RADIOTHERAPY PROTECTION

Studies on imaging for diagnostic, radiation protection equipment and radiopharmacological compounds.

- Evaluation of x-ray practices in pediatric radiology in Paraná aimed at the optimization of radiological protection.
- Production of better quality images for diagnosis using smaller doses of radiation for the patient.
- Equipment quality control.
- New methods and procedures of computed tomography based on the use of proton beams.

Research and its effects on care: finding cures to save lives

IMAGING, RADIATION PROTECTION AND RADIATION THERAPY

LOWER DOSES OF RADIATION ON IMAGING TESTS

Imaging tests are essential for the treatment of hundreds of pediatric patients every year. Many protocols indicate two daily x-ray examinations for weeks. However, the scientific literature has associated radiation exposure to an increased risk of developing cancer, and children tend to be more vulnerable.

A study developed by the Pelé Little Prince Research Institute focuses on pediatric radiology and develops new standards to reduce the radiation doses in x-ray examinations for patients from birth to 15 years old. Vanessa da Silva Oliveira, age 16, for example, is one of hundreds of children undergoing x-ray examinations every year. Her first imaging studies took place before she was one year old. Diagnosed with a congenital heart defect, she received specialized treatment at Little Prince Hospital, which is 400 km away from her city, Marialva.

Being in a state-of-the-art children's hospital makes all the difference in treatment, according to Vanessa's mother, Maria Antonia da Silva. "I do not know how many x-ray examinations she had," she said, "there were many, very many, but knowing that the equipment is programmed to preserve the life and the health of my daughter and other patients gives me peace."

77.247

**X-RAY EXAMINATIONS PERFORMED BY
LITTLE PRINCE HOSPITAL IN 2014.**

IMPORTANCE OF THE STUDY

Children are more vulnerable to radiation and have a longer life ahead of them in which to develop diseases, such as cancer, which can be caused by radiation over the long term. When submitted to tests too early during an important period in the development of their organ systems, children are at greater risk for these consequences than adults.

CONGENITAL HEART DISEASE

Congenital heart disease is a malformation in a baby's heart, which occurs during pregnancy. According to the World Health Organization, 8 to 10 out of every 1000 newborns have congenital heart disease.



Research Areas and Main Studies - 2014

4. NEUROSCIENCE

Neuroscience research applied to neurology, mental health, psychiatry, and learning process.

- Analysis of communication ability in children with cochlear implants, using the “Functioning After Pediatric Cochlear Implantation (FAPCI)” for impact assessment.
- Use of clinical and psychometric scales to diagnose children with ADHD (Attention Deficit Disorder / Hyperactivity Disorder) and Bipolar Disorder.
- The impact of prior psychoeducational training on adherence to treatment and computerized attention training (CAT) in children with Attention Deficit Disorder / Hyperactivity Disorder.
- Study of changes in dopamine genes in children with ADHD.



Research and its effects on care: finding cures to save lives

NEUROSCIENCE

METHOD FOR EVALUATION OF VERBAL COMMUNICATION AFTER COCHLEAR IMPLANT

Since the 1970s, the cochlear implant has been used and improved upon. At Little Prince Hospital, cochlear implantation has been performed since 2007, the only pediatric hospital in Brazil to offer the procedure through the Brazilian Unified Health System (SUS). The first SUS cochlear implantation took place in 2011. Gabrielly Victoria da Silva was only 3 years old. Her mother, Nelcimara, waited anxiously for the procedure. “When they told me that the only hope for successful treatment was the cochlear implant, I went for it,” she recalls.

They travelled the 400 km from their town of Iretama to Curitiba on the day of the surgery. The mother will never forget the date—January 14. “With the implant, Victoria won once more,” she said, “we could start talking and it was a dream come true to hear her saying ‘mommy’.”

The monitoring of post-implant patients revealed the need for an instrument to objectively measure the development of their verbal communication. Thus the Pelé Little Prince Research Institute conducted a study to this end.

Based on the US-developed FAPCI method (Functioning After Pediatric Cochlear Implant)—the only existing validated psychometric instrument for such measurements—the Institute created a

Brazilian version (FAPCI-BP) that has been adopted and validated for use. This version can be used to evaluate verbal communication performance in children with or without cochlear implants.

Out of every thousand babies born in Brazil, two to seven have hearing impairment. Deafness hinders the development of verbal communication, and life becomes a big silence.

COCHLEAR IMPLANT

The Cochlear Implant consists in inserting an electronic receiving device into the inner ear. Behind the ear, a battery-operated microphone processes the sounds.



50TH

COCHLEAR IMPLANT IN A PATIENT WITH SUS BENEFITS WAS PERFORMED IN JUNE 2015.

Research Areas and Main Studies - 2014

5. MICROBIOLOGY AND INFECTIOUS DISEASES

Studies involving the diagnosis, epidemiology, and applied research on diseases related to microorganisms (viruses, bacteria, fungi, and protozoa).

- Analysis of resistance to antifungal agents and molecular characterization of *Candida* spp detected in pediatric patients with suspected septicemia.
- Epidemiological analysis of tuberculosis in the state of Paraná, with emphasis in the cities of Curitiba, Guarapuava, and Paranaguá.
- Retrospective evaluation of cerebrospinal fluid samples from neonates from January 1996 to June 2011.
- Genotypic characterization of HIV-1 and correlation with neurocognitive abnormalities.
- Molecular characterization of antimicrobial resistance in *Aeromonas* spp and antimicrobial residues in effluent samples collected in health services, the Little Prince Hospital, and domestic effluents.
- Molecular characterization of Enterobacteriaceae antimicrobial resistance and antimicrobial residues in wastewater samples collected from health services, the Little Prince Hospital, and domestic effluents.
- Comparison of phenotypic, molecular and mass spectrometry methods for the identification of coagulase negative staphylococci (CoNS) in biological samples isolated from patients hospitalized at Little Prince Hospital.
- Pancreatitis associated protein (PAP) concentration for Cystic Fibrosis neonatal screening.
- Study of mechanisms of resistance to carbapenems in biological samples of Enterobacteriaceae species.
- Genetic and molecular characterization of CFTR gene in patients with cystic fibrosis in a tertiary hospital in Curitiba.
- Risk factors for invasive candidiasis in pediatric patients in Intensive Care Units (ICU) of the Little Prince Hospital.
- Identification of genomic variants of the *Burkholderia cepacia* complex in children with cystic fibrosis in the State of Paraná.



Research and its effects on care: finding cures to save lives

MICROBIOLOGY AND INFECTIOUS DISEASES

NEW FUNGUS FOUND IN BRAZIL

Treatment of critically ill children in the intensive care unit (ICU) and children receiving chemotherapy for cancer include invasive procedures such as the use of central venous catheter and parenteral nutrition. If on the one hand this is crucial for the treatment of these children, on the other it increases the risk of fungal infections, which can be fatal.

A study of invasive infection by the fungus *Candida*—carried out with ICU patients in Little Prince Hospital—identified a fungus that caused serious infections and had not been isolated in Brazil before. A delay in the diagnosis of fungal infection brings risks to the patient when it reaches the bloodstream. Prior to this study, no one had reported this microorganism in Brazil.

The fungus was identified in children undergoing complex surgeries, premature infants or those immunocompromised. The results of this study led to the development of other projects, including a study to identify the risk factors of these infections in order to prevent them.

7,07

DAYS IS THE AVERAGE STAY OF A PATIENT IN THE ICU.

PIONEERING IN THE IDENTIFICATION OF FUNGI

The results of the study identifying this fungus in Brazil were published in the *Journal of Clinical Microbiology*, a leading scientific journal in the Microbiology field, and has led to other studies in progress. Among them, new research at the Pelé Little Prince Research Institute aims to identify the risk factors of these infections in order to prevent them.



Research Areas and Main Studies - 2014

6. CELLULAR AND PHARMACOLOGICAL THERAPIES

Studies on the impact of cellular therapy and pharmacological compounds for the treatment of diseases.

- Use of autologous biomaterial in the treatment of periodontal diseases: a pre-clinical study.

Acaricidal activity of *Ocimum gratissimum* essential oil (Labiatae).

- Evaluation of tissue regeneration in second-grade burn treated with micro and nanostructured matrix with incorporation of mesenchymal stem cells.
- Evaluation of potential use of nanostructured gel as a vehicle for the implantation of mononuclear cells in the treatment of spine trauma.
- Evaluation of mesenchymal stem cells versus mononuclear cells implant for spine trauma: a pre clinical study.
- Evaluation of the effects of adipocyte derived precursor Schwann cells trasplant for sural nerve lesions: an experimental study in animal models.
- Combination of platforms for discovering new therapeutic options for the treatment of adrenocortical tumor: natural compounds and nanobiotechnology.
- Development of a compound based on biopolymers presenting a new formulation of L-DOPA for the treatment of Parkinson's disease: a preclinical study.
- Development of a nanostructured implant containing adipose tissue mesenchymal stem cell derived dopaminergic cells for the treatment of Parkinson's disease: an in vitro study.
- Development and validation of infrared and CLAE-EM/EM based method for simultaneous quantification of metformin and vildagliptin pills.
- CLAE-FLU and CLAE-MS/MS based method for simultaneous quantification of drugs used for ADHD treatment for bioavailability, pharmacokinetics and treatment follow-up

studies.

- Methods for Vancomycin serum concentration in neonatal ICU.
- Effects of cellular therapy for experimental autoimmune encephalopathy.
- Effects of cellular therapy for unilateral cerebral ischemia: a pre clinical model for Alzheimer's disease.
- Comparison of CD271 versus CD90, CD105, CD34, CD45, and CD79 markers in mesenchymal cells from umbilical cord blood.
- Studying exopolysaccharide isolated from *Curvularia brachyspora*: optimization, characterization and biological activity.
- Immunophenotyping in the process of differentiation of adipocyte derived mesenchymal cells into Schwann cells.
- Modified technique for evaluation of angiogenesis using chorioallantoic membrane (CAM)—eggs for evaluation the action of *Chamomilla recutita* L., Asteraceae extracts
- Optimization and characterization of *Asperillus terreus* exopolysaccharide and its clinical application.
- Optimization and characterization of *Paecilomyces variotti* exopolysaccharide and its clinical application.
- Development of micro and nanostructured polysaccharide for biological dressings.

7. MOLECULAR MEDICINE AND BIOINFORMATICS

Studies of molecular biology and genomics to investigate molecular anomalies and to characterize and quantify biological processes, whether normal or not.

- Characterization of gene expression of multipotent cells located in human hair follicles.
- Phenotypic and molecular characterization of coagulase negative *Staphylococcus* isolated in biological samples from patients being treated at Little Prince Hospital.
- Genetic and molecular characterization of CFTR gene in patients with cystic fibrosis in a tertiary hospital in Curitiba.
- Genetic and molecular characterization of patients with oculocutaneous albinism and its correlation with ocular abnormalities.

Research and its effects on care: finding cures to save lives

MOLECULAR MEDICINE AND BIOINFORMATICS

IN SEARCH OF EARLY DIAGNOSIS FOR PRIMARY IMMUNODEFICIENCIES

Ruan Miguel Mendes dos Santos was just 15 days old when he was treated for the first time in Paranaguá, Paraná, due to a blood infection. Within a month of life, he had his first case of pneumonia.

Three months later, he had pneumonia again. “In my town, they did not want to treat him because I had already lost a one-month-and-nine-days-old baby the previous year. He was transferred to another hospital in the region, but they could not find what he had,” his mother, Emanuelle do Rocio Mendes, said. “He was finally sent to Little Prince Hospital in Curitiba.”

Ruan’s case was handled by medical researcher Carolina Prando at the Pelé Little Prince Research Institute. He was submitted to specific tests and was diagnosed with a primary immunodeficiency, Chronic Granulomatous Disease. Today, he is a one-year-old and is treated at the outpatient clinic for Primary Immunodeficiencies, an important part of the research work of the Institute.

“Little Prince saved Ruan,” says his mother. The Institute found a genetic abnormality running in the family. Ruan’s mother had a nephew with the same illness and three cousins died before they were two years old with clinical symptoms similar to those of Ruan.

200

GENES HAVE BEEN IDENTIFIED AS PRIMARY IMMUNODEFICIENCY CAUSING GENES, AND THERE ARE MORE THAN 200 CLINICAL FORMS OF PRIMARY IMMUNODEFICIENCY.

PRIMARY IMMUNODEFICIENCY

Primary immunodeficiencies are genetic disorders characterized by recurrent and severe infections. A change in genes that code for immune system compounds causes the immune system not to function in the way that it should. There are more than 200 primary immunodeficiencies. The most serious forms lead to death in the first year of life if not diagnosed and treated.

EXAMS AND ADVICE

The study on primary immunodeficiencies at the Institute began in 2013. Since then, 20 patients had their diagnoses confirmed and another 100 are under investigation. Each case is evaluated in the clinic. Patients undergo testing for functional and genetic modifications. By knowing which genes are involved in immunodeficiency, it is possible to offer precise genetic counseling and treatment advice.

200 genes have been identified as primary immunodeficiency causing genes, and there are more than 200 clinical forms of primary immunodeficiency.



Science and care: an unbreakable pair

If we have the largest pediatric hospital in Brazil, with clinical staff and the leading-edge technology to make diagnoses and treat patients, and the ability to disseminate this knowledge through residency programs and colleges, why invest in basic research? How do experiments in the laboratory, which sometimes seem far from reality, help save the lives of our children?

One of the most important revolutions in medicine, the discovery of penicillin in the 1930s, is the result of basic research work in the laboratory. Sir Alexander Fleming, a Scottish biologist, pharmacologist and botanist, came to realize that infections killed more than the enemy artillery in the First World War. From the war, he returned to his laboratory in order to find a substance that could control the development of serious bacterial infection. Thanks to his discovery and subsequent research in the area of microbiology and chemistry, today thousands

of children, youth and adults are able to receive treatment for various infections.

In the mid-1970s, a survey showed that 62% of scientific articles considered basic science research to be paramount to the development of complex techniques such as open heart surgery, vascular surgery, cardiac pacemakers, treatment of hypertension and tuberculosis, and prevention of polio. We cannot fight cancer, for example, without knowledge of the control of cell proliferation and changes that occur at the molecular and genetic level. Only through research can we develop new prevention methods, diagnostics and treatments.

Even today we need to break the paradigm that basic research is located far away from the clinic. Little Prince Complex is fundamental in this process. Looking at Little Prince Hospital, we see the need to elucidate the diagnosis for

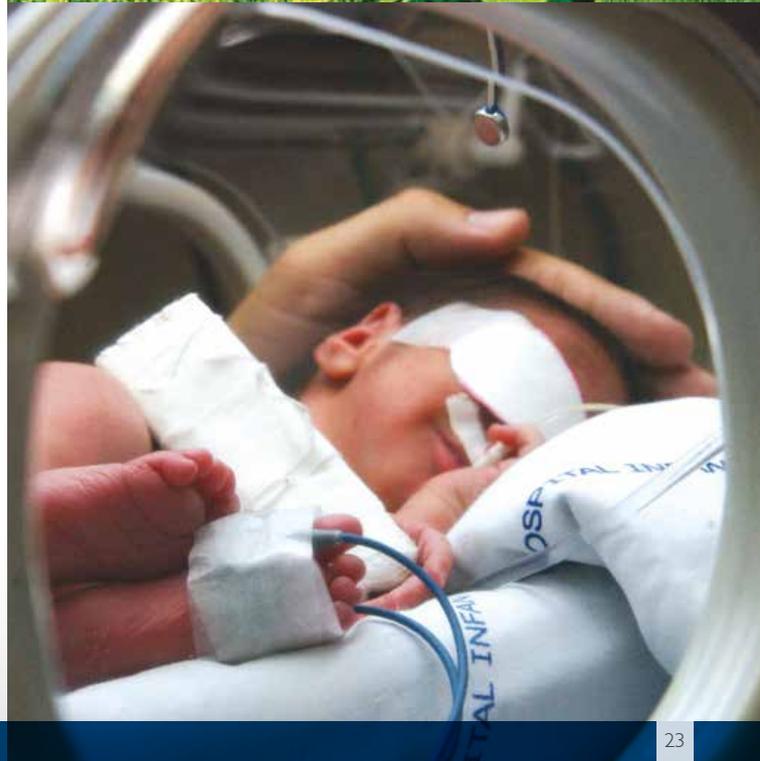
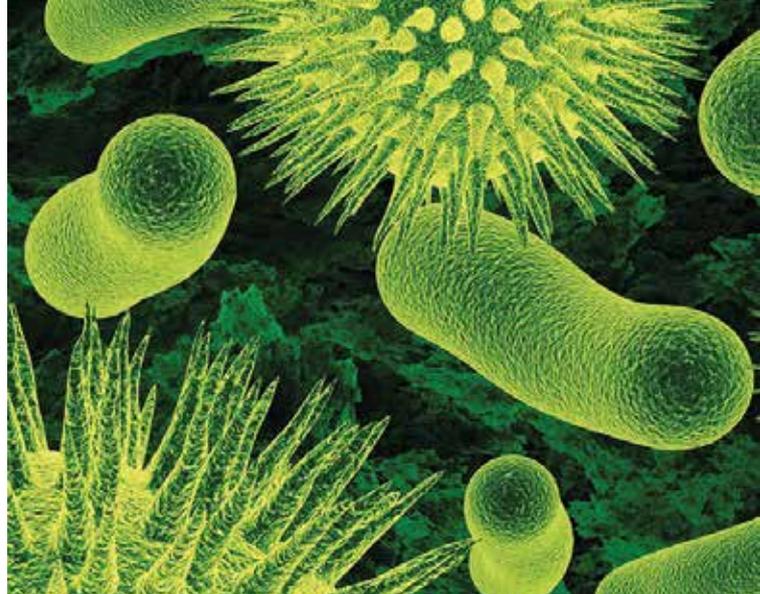




children in critical conditions, or families affected over generations by the same disease, the need of changing ineffective treatment options, to treat complications in organ and bone marrow transplants. As a result, the Pelé Little Prince Research Institute has developed studies that focus on these needs, bringing results that directly benefit children, young people and their families.

At this point in time, the Pelé Little Prince Research Institute is focused on the Biobank and the Genomics and Bioinformatics Unit.

Our supporters have already made part of this dream possible, as we were able to start furnishing our units with the most advanced equipment in the world. The first projects developed in the Genomics and Bioinformatics Unit point to important discoveries that will benefit our children immediately. To continue this research and apply its results in saving lives, we count on the support and social investment of our partners.



Network of institutional partnerships

A science network for the health of children and adolescents, this is how we define the work carried out by the Pelé Little Prince Research Institute in partnership with institutions in Brazil and abroad.

By working together with these organizations, we have better opportunities to find new forms of diagnosis and treatment for complex childhood and adolescence diseases. Thus, boys and girls in Brazil and all over the world are directly benefited.

INTERNATIONAL

- University of Toronto, Institute of Medical Science, Canada
- Hospital for Sick Children, Division of Clinical and Metabolic Genetics, United States
- University of British Columbia, Department of Pathology, Canada
- British Columbia Cancer Agency, Department of Pathology and Laboratory Medicine, Canada
- Centre for Lymphoid Cancer British Columbia Cancer Research Centre, Canada
- Vancouver General Hospital, Department of Hematology and Cytogenetics Laboratory, Canada
- St. Jude Children's Research Hospital de Memphis, United States
- University of Nice, France
- Molecular and Cellular Pharmacology Institute of Valbonne – Inivesità the University of Perugia, Italy
- Loma Linda University Medical Center, United States
- International Agency of Atomic Energy, Austria
- University of California, Department of Psychiatry and Behavioral Sciences, United States
- Univale University, Grenoble, France
- Research Center for Plant Macromolecules (CERMAV), France
- Institute George Pompidou, France
- Harvard University, United States
- University of Paris Descartes, France
- Rostock University, Germany
- North American University, United States
- University of British Columbia, Canada
- University of South Florida, United States



NATIONAL

- Universidade Federal do Paraná, Departamento de Fisiologia, Departamento de Química e Departamento de Física, Paraná
- Universidade Tecnológica Federal do Paraná, Paraná
- Universidade Estadual do Oeste do Paraná, Paraná
- Universidade Federal de Pernambuco, Pernambuco
- Universidade Federal do Rio de Janeiro, Rio de Janeiro
- Instituto de Energia Nuclear, Rio de Janeiro
- Universidade Estadual de Campinas, São Paulo
- Instituto de Física de São Carlos, São Paulo
- Instituto de Radioproteção e Dosimetria, Rio de Janeiro
- Instituto de Pesquisa Energética, São Paulo
- Rede ELINOR, Universidade Federal de Pernambuco, Pernambuco
- Rede de Saberes e Práticas, Ensino, Saúde e Desenvolvimento
- Parceria Interinstitucional com a Universidade Estadual de Londrina, Paraná
- Universidade Estadual de Maringá, Paraná
- Universidade Federal de Alagoas, Alagoas
- Universidade Federal de Santa Catarina, Santa Catarina
- Secretaria de Estado da Saúde do Paraná, Paraná
- Secretaria Municipal da Saúde de Paranaguá, Paraná
- Serviço de Infectologia do Departamento de Pediatria do Hospital de Clínicas de Curitiba, Paraná
- Hospital Israelita Albert Einstein, São Paulo
- Clinirad/Hospital Angelina Caron, Paraná
- Clínicas Oncológicas Integradas, Rio de Janeiro
- Mac Gayver's Manutenções e Equipamentos Hospitalares, Paraná
- Hospital Erasto Gaertner, Paraná
- Comissão Nacional de Energia Nuclear, Centro de Desenvolvimento de Tecnologia Nuclear (CDTN), Minas Gerais
- Programa de Qualidade em Radioterapia (PQRT/INCA), Rio de Janeiro
- Minerais do Paraná, Paraná
- Centro de Estudos em Biofarmácia, Paraná
- Centro de Patologia de Curitiba, Paraná
- Departamento de Anatomia Patológica do Hospital A C Camargo, São Paulo
- Fundação Ecumênica de Proteção ao Excepcional, Paraná
- Instituto Carlos Chagas (FioCruz Paraná), Paraná
- Pontifícia Universidade Católica do Paraná, Paraná
- Prefeitura Municipal de Curitiba, Paraná
- Secretaria de Estado da Educação do Paraná, Paraná
- Secretaria de Estado da Saúde do Paraná, Paraná
- Serviço de Anatomia Patológica do Hospital de Clínicas de Curitiba, Paraná
- Universidade Estadual de Londrina, Paraná
- Universidade Estadual do Oeste do Paraná, Paraná
- Universidade Federal de São Paulo, São Paulo



The Little Prince Complex establishes partnerships on diverse levels of activity that can be for institutional support, collaboration, or technical partnership.

Goals for Life Gala: taste, knowledge, and solidarity



GOLS PELA VIDA

The Goals for Life Gala is the expansion of the Gastronomic Cup Goals for Life. These series of events bring together high gastronomy and entertainment in perfect harmony with the support of children's health. They have been held since 2011, providing much needed resources for Little Prince Complex.

The funds raised at the events are earmarked for Pelé Little Prince Research Institute and benefit thousands of boys and girls every year.

So far five events have featured the renowned French chef Claude Troisgros. They were held in **Curitiba (2011)**, **São Paulo (2012)**, **Paris (2012)**, **New York (2013)**, and **Rio de Janeiro (2014)**.



Fundraising and proceeds from the New York (2013) and Rio de Janeiro (2014) events

GENOMICS AND BIOBANK UNIT

The first phase of the Genomics and Bioinformatics Unit of the Pelé Little Prince Research Institute has been implemented and now features next-generation DNA sequencing equipment with capacity to sequence up to 25,000 genes in a single test. This technology allows us to not only identify variations in genes related to existing diseases, but also to discover new genes and new functions they perform in the human body.

The impact of using this technology transcends several medical specialties, particularly those in which the deficiency of a particular molecule is the triggering agent of clinical symptoms. The next-generation sequencing technique also offers the capacity for unprecedented research on the genomic features of cancer.

The bioinformatics network consists of high-processing computers and skilled operating staff. They convert the data generated by the sequencer into meaningful information from a biological point of view, contributing to the development of more accurate algorithms for DNA sequencing analysis and discoveries that will result in new diagnosis and treatment.

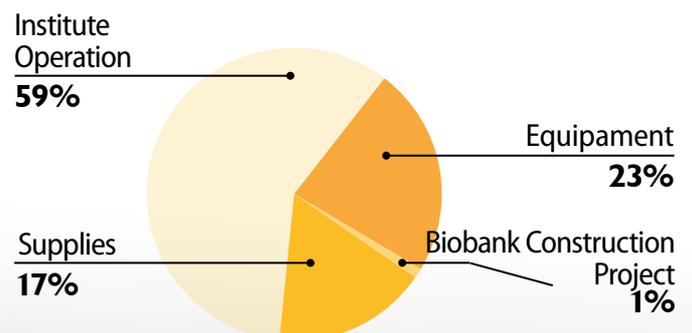
FUNDS RAISED AT GALAS

EVENT	AMOUNT RAISED
New York 2013	R\$ 1,887,590
Rio de Janeiro 2014	R\$ 1,178,835

NEXT STEPS AND EXPECTED RESULTS

- Improve medical intervention and the application of more accurate diagnostic and follow up tests
- Monitor risk situations, allowing early medical intervention;
- Assist in the treatment of children's diseases, providing specific and appropriate treatments for each of them
- Inform parents about risks of recurrence of a family illness in other generations;
- Provide greater possibility of treatment and quality of life for patients;
- Collect and store biological samples, offering a safe storage and organized collection for research
- Optimize the use of biological samples through sample sharing between research groups;
- Participate in the World Biobanks Network, contributing to the sharing of scientific knowledge.

USE OF PROCEEDS



How the resources from the Gastronomic Cup Gala 2013/2014 were used

Contributions to the Gastronomic Cup Goals for Life were essential to the development of the scientific activities of the Pelé Little Prince Research Institute in recent years.

In this section, we describe how proceeds from the events held in New York in 2013 and in Rio de Janeiro in 2014 were used.

2013

Resource used in 2013 – 2014

INSTITUTE OPERATIONS				Expenses (in BRL)	
COSTS	Human Resources / Researchers			913,356	
	Human Resources / Administrative			159,946	
	Research projects direct costs			102,654	
	Research projects indirect costs			239,974	
	Administrative expenses			41,927	
	Subtotal			1,457,857	
SUPPLIES					
	Supplies - Genomic Unit			107,751.77	
	Subtotal			107,751.77	
INVESTMENT	EQUIPMENT - GENOMIC UNIT		Quantity	Unit Price	Total (in BRL)
		Dynamag-2	1	1,017.87	1,017.87
		E-Gel Ibase Safe Imager	1	1,771.56	1,771.56
		Ion Proton System	1	456,060.67	456,060.67
		Ion Torrent Storage Device 20Tb	1	25,589.20	25,589.20
		Sample preparation station	1	31,294.97	31,294.97
		Ion Personal Genome Machine (PGM) System	1	104,097.28	104,097.28
		Subtotal			619,831.55
TOTAL (Costs + Investment)				2,185,440.32	
Funds Raised – Gala 2013				1,887,590.00	
Little Prince Investment				297,850.32	

2014

Resource used in 2014 – 2015

		INSTITUTE OPERATIONS		Realizado (em R\$)		
COSTS		Human Resource		490,981.00		
		Research projects indirect costs		51,500.00		
		Subtotal		542,481.00		
		SUPPLIES				
		Supplies - Institute		459,889.61		
		Subtotal		459,889.61		
		Project Elaboration – Biobank Construction				
		Electric design		1,340.00		
		Water sanitation project		1,900.00		
		Air conditioning project		4,500.00		
	Soil analysis		2,100.00			
	Structural design		8,000.00			
	Architectural design		25,000.00			
	Subtotal		42,840.00			
INVESTMENT		EQUIPMENT – INSTITUTE		Quantity	Unit Cost	Total (in BRL)
		Scientific refrigerators	2	13,000	26,000.00	
		Firewall for link balancing	1	23,284	23,283.89	
		Computers	2	6,000	12,000.00	
		- 86°C freezer with CO2 backup	1	45,000.00	45,000.00	
		-30°C 500 liter freezer	1	17,500.00	17,500.00	
		Refrigerated centrifuge	1	22,400.00	22,400.00	
		Inverted microscope	1	19,000.00	19,000.00	
		Optic microscope	1	6,000.00	6,000.00	
		Centrifuge	1	10,600.00	10,600.00	
	Subtotal				181,783.89	
	TOTAL (Costs + Investment)				1,226,994.50	
	Funds Raised Gala 2014				1,178,375.00	
	Little Prince Investment				48,619.50	

2013 / 2014

Resources used in 2013 - 2015

		RESOURCES USED	Valor Total (em R\$)
SUMMARY		Costs – Institute Operations	2,000,338.00
		Costs – Supplies	567,641.38
		Costs – Project elaboration / Biobank construction	42,840.00
		Investments – Equipment	801,615.44
		TOTAL	3,412,434.82

Goals for Life Gala



SAVE THE DATE

Goals for Life Gala | **October 1, 2015** | In Gotham Hall in New York



GOALS FOR LIFE GALA 2015

The combination of high gastronomy and solidarity with children's health has been central to the support of the Little Prince Complex. The success of the Goals for Life Gala contributes to the development of scientific research projects of the Pelé Little Prince Research Institute.

The annual event began as the Gastronomic Cup Goals for Life, a successful program held between the years 2011 and 2014, and taking place in five stages—Curitiba, São Paulo, Paris, New York, and Rio de Janeiro. The initiative is led by Daniele Giacomazzi Behring, our Ambassador for the Goals for Life program and godmother to all our children. The gala annual dinner and ceremonies are held interchangeably in Brazil and the United States—every two years in New York—and can also be promoted in other countries.

Our invaluable sponsor and curator is French chef Claude Troisgros, who gathers renowned chefs to design the special menus. In 2015, the event will be on October 1, in the traditional and sumptuous Gotham Hall in New York, with dishes specially created by the chefs Claude Troisgros, Daniel Boulud, François Payard, Jérôme Bocuse, and Roberta Sudbrack.

The honorary chairs of the event, in addition to Daniele Giacomazzi Behring, are Amalia Spinardi Thompson Motta and Belinda Badcock Brito.

Our special guests for this special evening are the eternal “king of football,” Pelé, and Dr. John P. Howe, president and CEO of Project Hope (Health Opportunities for People Everywhere).

All proceeds from the Goals for Life Gala will go to development and maintenance of scientific studies conducted by the Pelé Little Prince Research Institute.





LITTLE PRINCE COMPLEX

Rua Desembargador Motta, 1.070 • Curitiba . PR . Brasil • CEP: 80.250-060

phone: + 55 41 3310.1722 +55 41 9962.4387

e-mail: carolina.fossati@hpp.org.br • site: www.pequenoprincipe.org.br