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FEATURE

"Centro Infantil Boldrini" is considered a Brazilian Reference in Child Oncology

The "Centro Infantil de Investigações Hematológicas Dr. Domingos A. Boldrini", usually simply referred to as 'Boldrini', is a philanthropic teaching hospital affiliated to the University of Campinas (Unicamp) that offers high technology for diagnosis and a complete infrastructure of care. Different lines of research permeate its daily routine, stimulating new discoveries in pediatric oncology and hematology.

The story of the foundation of Boldrini in the city of Campinas, SP, Brazil, merges with the life story of physician Silvia Regina Brandalise, graduated in medicine from the Federal University of São Paulo (UNIFESP) in 1967, where she did her residency in pediatrics. In 1975, she completed her doctorate in pediatrics at the Faculty of Medical Sciences of the University of Campinas (FCM-Unicamp).

Back in the 1970s, Dr. Brandalise was touched by the cases of two children diagnosed with leukemia. At the time, the treatment of children diagnosed with cancer was transferred to doctors who specialized in adult oncology. However, the cases of these two children changed Dr. Brandalise's professional trajectory, as she migrated to pediatric oncology and created what would become the Boldrini children's center.

In 1978, with the support of the Lady's Club of Campinas, an outpatient clinic was created in a rented house. Two years later, together with the Brazilian Society of Hematology and Hemotherapy (SBHH), Dr. Brandalise launched the first protocol for the treatment of children's acute lymphoid leukemia (ALL) at a national level. In 1986, a 1,500 m² building was donated by the Robert Bosch Institute of Brazil. This property was expanded to 35,000 m² with resources donated by companies and individuals from the city of Campinas and region. Thus, the outpatient clinic became a hospital.

Currently, Boldrini comprises a hospital, a building for radiotherapy, nuclear medicine and imaging, the Boldrini Lucy Montoro Rehabilitation Center, the Ronald McDonald Pediatric Institute of Pediatrics, a

Fundraising Center, a Volunteer Center, the Boldrini Station, and the Institute of Cellular and Molecular Engineering.

When the subject is humane, fair, and effective care, Dr. Brandalise is cited as a professional reference in the fight for Brazilian science and health.

"A great challenge was to bring to Brazil, in 1980, the first asparaginase, an enzyme used as an oncological therapy for acute leukemias. At the time, the Ministry of Health did not approve the purchase of this enzyme. The alternative I found, with the help of a tourism agency in Campinas, was to ask travelers going abroad to bring me a bottle of this medicine. Thanks to the solidarity of these people, we were able to get enough vials to treat the children with acute lymphoid leukemia. None of the children who were under our care were deprived of this medication," explained Dr. Brandalise.

It is also important to point out that an increase in the cure rate of cancer in children and adolescents has been observed in centers specializing in pediatric oncology, such as Boldrini, which today reaches around 80% cure. "In the last four decades, the survival rates of children with cancer have increased significantly, especially in developed countries. Protocol VIII from St. Jude Children's Research Hospital, a pediatric treatment and research facility located in Memphis, Tennessee, USA, inspired us to create the Brazilian Childhood Leukemia Treatment Group (GBTLI) and the first GBTLI LLA-80 protocol. This initiative raised the survival rates of Brazilian children from less than 5% to 40%. Survival rates have increased with subsequent protocols; today at Boldrini, they are around 80%. We simply follow international models, in line with our reality," clarified Dr. Brandalise.

Boldrini Center Differentials

- To be the only hospital in Brazil to perform retinal mapping for the early diagnosis of retinoblastoma in all children. A municipal law guarantees the universality of this test for all babies born in the region of Campinas.
- Provide comprehensive and multi-professional care, which decisively contributes to the success of the treatment. Patients with reduced mobility or disabling physical, motor, and sensorimotor disabilities, for example, can have their treatment performed at the Boldrini Lucy Montoro Rehabilitation Center.
- Ensure the continuous presence of a companion, with the support of volunteers, which accelerates the patient's recovery.
- Have the Ayrton Senna toy library, where there is entertainment, art workshops and theatrical performances, and a pedagogical support room that allows young people to continue their studies.
- Offer complementary therapies of proven effectiveness, such as Reiki and acupuncture.
- Provide a hotel service in partnership with the Ingo Hoffmann Institute, the Ronald McDonald House/ APACC, and the David Rowe Association.
- Make it possible for children undergoing treatment and their families to wait at the Boldrini station for their return journey home, where comfort, food, rest, leisure, and craft workshops are offered.

Boldrini Research Center (CPB)

The first and only research center in Brazil to be exclusively dedicated to pediatric cancer research, the Boldrini Research Center (CPB) connects scientists, physicians, and universities to boost research into new oncology treatments and leads the good results in pediatric cancer treatment in Brazil.

Inaugurated in November 2018, with funds donated by the Labor Public Ministry, the CPB’s main purpose is to contribute with a great high-tech platform, focused on new knowledge related to child and adolescent cancer, providing the development and discovery of new drugs and strategies to fight cancer, as well as the incorporation of modern target therapies for the pediatric scenario.

The CPB has nine study groups that work in the most advanced key areas in scientific research on cancer: immunotherapy; therapeutic monoclonal antibodies; circulating tumor DNA; central nervous system tumors; minimal residual disease; new drugs; environmental factors and pediatric cancer; informatics; and mass spectrometry. In addition, CPB has one of the largest pediatric tumor cell biobanks in Latin America, which was built along the trajectory of Boldrini. This biobank will provide the basis for the discovery of new chemotherapeutic and immunotherapeutic drugs for the treatment of cancer in children and adolescents.

Another important aspect of the CPB is its integration with national and international universities and research centers, which means a great foundation for the scientific and technological development of Brazil. The CPB is in constant collaboration with researchers from major national institutions such as Unicamp, LNBio, CNPEM, UFSC, INCA/MS, and international institutions such as COG (USA), Instituto de Medicina Molecular (Lisbon), Hôpital Robert Debré (Paris), Institut Pasteur (Paris), St. Jude Children’s Research Hospital (Memphis, USA), Broad Institute (Boston, USA), National Institutes of Health (Frederick, USA), and The University of Queensland (St. Lucia, Australia).

CPB’s main research lines

- Development of new drugs for the treatment of cancer, in partnership with major research institutions.
- Development of technologies and therapeutic approaches to overcome drug resistance in refractory cancer.
- Implantation of genetic markers for tumors of the nervous system, envisioning a 70% cure for Brazilian children. Today, the chance of a cure is below 30% in Brazil.
- Creation of *in vitro* models for studies that help to understand how tumors develop.
- Creation of systems for the production of biopharmaceuticals (medicines of biological origin), with pre-clinical and clinical tests.
- Development of immunotherapy aimed at pediatric cancer, with a high probability of clinical implementation in Brazil in the next 3 years.

BrJAC spoke with Dr. José Andrés Yunes, a researcher at Boldrini since 1997, and also a volunteer collaborating researcher at Unicamp. Dr. Yunes holds a degree in Agronomic Engineering from the Federal University of Santa Catarina (1988) and a PhD in Genetics and Molecular Biology from the University of Campinas (1997).

BrJAC: What is the research you are developing at Boldrini?
Dr. Yunes: At the moment, we are working on a large project, funded by the Ministry of Health, which consists of establishing a method to make a kind of antibiogram of different drugs against the leukemic cells of each patient. We hope that the test will help to identify which drug can be used in the case of a leukemia that is responding less than expected to conventionally-used drugs. The treatment of leukemia is mainly based on the use of chemotherapy. About ten different chemotherapy drugs are used. However, sometimes leukemia does not respond very well to treatment. Thus, the goal is to test between 120 and 150 chemotherapeutics approved by the FDA for use in oncology, mostly for other types of cancer (*i.e.*, not used for leukemia). It may be that one of these chemotherapeutic agents proves to be effective against leukemia that is resistant to conventional treatment, and can be used in the patient in question. It is a patient-to-patient approach, where we will seek to know, on a case-by-case basis, the sensitivity profile to chemotherapy. However, we will also carry out genetic analyses of each case of leukemia to later study whether some genetic alterations are associated with sensitivity to a specific drug, so that in the future, eventually, we may narrow down the number of drugs included in the screening to those that are more likely to have an effect, depending on the mutations found.

BrJAC: How is this work developed?
Dr. Yunes: We need to overcome several challenges, starting with setting up an infrastructure in the laboratory, such as a pipeline for gases and an adequate electrical network for the installation of the instruments that will be used. For example, we had to build a laminar air flow cabin to house a pipettor robot. However, the laminar flow cabinet motor shakes and affects the operation of the pipettor. We are currently looking for a solution to this issue. Another example of an issue that we must resolve is the new instruments that have a defect in the XY locomotion engine and therefore need to be replaced by the supplier. All these issues happen at the same time that we work in the search for the best composition of the culture medium where we will place the leukemic cells to grow and test the effect of the drugs. We have already tested more than 20 different culture media, as well as three different types of collagens and three types of stromal cells. These components are added to cell culture to support the leukemic cells and better mimic the composition of the bone marrow microenvironment where leukemic cells normally reside in the patient's body.

BrJAC: Would you briefly explain what the test under development in your research consists of?
Dr. Yunes: The test, once implanted, will consist of seeding 3,000 leukemic cells, collagen matrix, and stromal cells in all the 'wells' of a tray containing 384 wells. Each tray will allow testing of 31 drugs in duplicate and at five different concentrations. This tray is placed on an instrument that maintains a constant temperature at 37 °C and takes pictures of the wells every 30 minutes for 4 days. At the end, the photos are analyzed sequentially, as in a film, which allows us to see if each of the photographed cells has moved or not. Dead cells remain static, and live cells move. This image analysis then allows us to count how many live cells were present in each of the 384 wells over the 4 days of the experiment. If the drug is effective against leukemia, the number of living cells rapidly declines, otherwise it remains stable. With this information, we will discover the sensitivity profile of the 31 tested drugs. We currently have 3 image analysis instruments, so we can test 93 drugs in 4 days.

BrJAC: What will be the benefits of this research in the short, medium, and long term?
Dr. Yunes: This drug screening system was developed by a doctor who graduated from Boldrini and currently works in the United States with multiple myeloma, a type of leukemia that affects the elderly. In myeloma, the system is already being used in clinical practice, with success. Therefore, we are hopeful that it could also be very useful in acute leukemia in children in the short term, and in the medium term, we hope that this personalized drug test will serve as a basis for designing a clinical study, to be developed by the medical team at Boldrini. Clinical tests are essential to demonstrate the effectiveness or otherwise of the proposed approach. In the long term, we hope to do a similar test for other pediatric cancers, including solid tumors.

BrJAC: For you, what is it like to participate in something that can save many lives?
Dr. Yunes: We work in an organized, meticulous, critical, and very hard way; that is, our work requires a cold analysis of situations, which does not seem to give rise to more tender feelings. However, pausing to consider this question, I cannot deny that, internally, there remains a feeling similar to tenderness. Yes, our days are rewarding.

BrJAC also talked to Dr. Fabio Cesar Gozzo, Professor at the Institute of Chemistry at Unicamp and collaborating researcher at the CPB. Dr. Gozzo has a degree in Chemistry (1995), a master’s degree (1996), and a doctorate (2000) from Unicamp.

BrJAC: What is the research you are developing at Boldrini?
Dr. Gozzo: Our group is developing new approaches to mapping interactions between proteins that are important both for cancer initiation and for identifying new therapeutic targets for treatment.
Although we now have extensive knowledge about key proteins for the initiation and development of cancer, it is estimated that about 80% of protein-protein interactions in cancer are still unknown.
One of the great ways, both for a more complete understanding of cancer and for the development of new treatments, is to understand how the interactions between proteins that carry cellular signaling information happen.

BrJAC: How is this work developed?
Dr. Gozzo: Our work is intense and necessarily multidisciplinary. Our group, which specializes in mass spectrometry, develops the analytical approach using mass spectrometry as the main tool. This encompasses the synthesis of new compounds that allow the chemical labeling of proteins to establish the network of interactions, to the development of new strategies for data acquisition. Another important branch of this work is data processing, which is carried out in collaboration with Dr. Paulo Carvalho (Instituto Carlos Chagas do Paraná, PR, Brazil), a specialist in computing applied to mass spectrometry. Finally, there is the fundamental participation of researchers from the Boldrini Research Center, who contribute from the provision of biological material to the discussion of experimental results. The vast knowledge in pediatric cancer of the Boldrini researchers serves as a guideline for defining the research steps taken by our group.

BrJAC: What analytical techniques are used? What instruments are used?
Dr. Gozzo: Because it is complex and multidisciplinary work, we use traditional molecular biology techniques through to advanced mass spectrometry experiments. We have two mass spectrometers: a Q-Exactive (Thermo Fisher Scientific) and a Xevo TQS micro (Waters Corp).

BrJAC: What will be the benefits of this research in the short, medium, and long term?
Dr. Gozzo: As mentioned earlier, it is estimated that about 80% of interactions between proteins are unknown. Knowledge of these interaction networks helps, in the short term, to understand the mechanisms by which cancer starts and develops. Once we understand how these interactions drive cell signaling and which interactions are key to the cancer development processes, we can develop ligands to modulate these interactions and thus pave the way for a new treatment in the next step. This is the basis, for example, of several immunotherapies, where an antibody binds to a specific protein and blocks the interaction with other proteins, thus interrupting the signals vital for the development and survival of tumor cells.

BrJAC: For you, what is it like to participate in something that can save many lives?
Dr. Gozzo: Of all the work I have done in my research career, the development of current research with researchers at the Boldrini Research Center is, without a doubt, the most important work I have ever done. Knowing that I can use all my knowledge acquired in 30 years in mass spectrometry in a project that can, in a real and concrete way, have a great impact on the lives of many children with cancer, is the most motivating factor that any researcher can have. There is no greater motivation than seeing a positive experimental result and knowing that this is another step taken on the road to a future additional treatment for pediatric cancer.

Source: Boldrini