How Asfotase Alpha, a treatment for congenital hypophosphatasia was developed by Canadian researcher Philippe Crine

Professor Philippe Crine was a great biochemist, pioneer, and visionary, who invented a treatement for hypophosphatasia (HPP), an infantile debilitating illness that can be lethal. Unfortunately, Dr. Crine's life ended prematurely within weeks because of a fulgurant cancer in 2023, but his discovery continues to save lives, and his legacy endures in those who knew him.

HPP is characterized by poorly mineralized soft bones that cause multiple fractures and inflict life-long handicaps on patients. Severe forms of HPP cause early death after birth. In their memorial comments to the Crine Family, the SoftBones Association, a resource for HPP patients and their families, mentions: «Dr. Crine rewrote HPP history, transforming it from a story of sorrow and tragedy to one of hope and full of life».

Born in Belgium, Pr Crine emigrated to Canada in 1968 for his graduate studies at the University of Montréal under the mentorship of Pr. Walter Verly, then director of the Department of Biochemistry. After his Ph.D., he moved to Brandeis University in Boston in 1974 for postgraduate studies on enzymatic DNA repair. In 1975, his scientific curiosity was attracted by the discovery of enkephalin/endorphin neuropeptides and he joined M.C.'s laboratory at the Institute of Clinical Research of Montreal (IRCM) where an endorphin chapter was in the making. With his sense of humor, Philippe mentioned later that his choice was timely when he read the Research News in Science on endogenous opiates (*1*). Philippe successfully took on the challenge as he was the first to show how a neuropeptide is made from its presumed precursor cleaved at motifs containing pairs of basic amino acids. Using radioactive micro-sequencing, he demonstrated that beta-endorphin is biosynthesized in endocrine cells from its precursor ProOpioMelanoCortine (POMC) following cleavage at pairs of basic amino acids, setting the guiding principle for the maturation of other neuropeptides. After 3 years of «endorphinisation» at the IRCM, Pr Crine joined the Department of Biochemistry at the Université de Montréal in 1978 where he spent his entire and prolific academic career, later acting as chair of the department (1989-1997) and then vice-dean of research at the Faculty of Medicine (1999-2002). At the helm of the department, he recruited highly productive scientists ending up doubling his department research grants (*2*). According to previous graduate students, he was a mentor with great empathy, while keeping the cap on scientific rigor and a strict evaluation of experimental methods and results. «Philippe always met his students with his «signature» big smile while challenging them with data interpretation and the design of the next series of experiments. He did it with engaging words and great enthusiasm*».*

His approach, anchored on scientific rigor and challenges together with empathic mentorship to the young generation, led to the discovery of the Asfotase Alpha. In a nutshell, while Pr Crine carried his academic duties as chair of the department, he built his own research program to become a highly respected academic scientist in the fields of molecular enzymology and protein engineering. Two decades later, he developed with colleagues a revolutionary therapy for congenital HPP (*3,4*), a rare juvenile illness caused by mutations in the common alkaline phosphatase (ALP) (*4*). Philippe Crine teamed up with Guy Boileau and Denis Gravel to create a start-up company, BioMep. Under his

leadership, BioMep produced a soluble form of alkaline phosphatase and expanded to become Enobia in 2003 which invented a hybrid protein that targets the enzyme to bone tissue, as described below.

«One morning in February 2005 I received the telephone call that would be the most significant of my medical career. It was from Philippe Crine» recalls Michael P. Whyte, MD, Emeritus Professor of Medicine, Washington University in St. Louis, USA. «He explained that Enobia had created a recombinant protein having the potential to treat HHP, an inborn-error-of-metabolism characterized by deficient ALP (*3*). ALP activity must be increased in the skeleton so that it can hydrolyze the accumulated ALP natural substrate and potent inhibitor of biomineralization, inorganic pyrophosphate. This might permit hydroxyapatite crystals to form and grow and then enter the skeletal matrix. ALP is bound to the surface of the plasma membrane of osteoblasts and their matrix vesicles to enable bone mineralization. By the late 1980s we had confirmed that HPP was caused by loss-of-function mutations of the *ALPL* gene that encodes the tissue-nonspecific ("bone") isoenzyme of ALP (*5*). Dr. Crine shared how they had developed a recombinant tissue-nonspecific ALP to which they had added a deca-aspartate motif that would target the enzyme to hydroxyapatite crystals. Fascinating! … We had a knockout mouse model for HPP in which this protein, ENB-0040, could be tested. In 2008, we published success treating the mice (*3*). Clinical trials began that same year, first for life-threatening HPP in infants and young children. The remarkable success was published first in the *New England Journal of Medicine* in 2012 (*4*). ENB-0040 would be called "asfotase alfa". Soon after, benefits followed for young children compromised by the muscle weakness and skeletal disease of HPP (*6*). In 2015, one decade after Dr. Crine called me, asfotase alfa was approved multinationally (*7-9*). It was Philippe Crine, the gifted and affable scientist, who envisioned and successfully organized this work. My colleagues say to me that they dream for such success in developing a medical treatment sometime during their medical careers. It was Philippe Crine who made this dream come true for patients and their families suffering from hypophosphatasia.»

«I was already working to capacity as a metabolic doctor and Head of the Dept of Paediatrics and Child Health at the University of Manitoba in Winnipeg». Dr Cheryl Rockman-Greenberg, a leading member of the Global HPP Consortium for Functional Testing of Genetic Variants in the ALPL gene, recalls how Enobia Pharma reached out to her by phone in 2005. «They had learned that HPP was overrepresented in the Mennonite population in Manitoba (and other Mennonite communities in Canada). The frequency of very severe life-threatening HPP in our population was 1/2500 live births vs 1/100, 000 in the general population… The prospect of turning HPP an untreatable disorder with 100% mortality in the perinatal and infantile forms in our population into a treatable one was beyond exciting and of course I said yes… After phase 1 studies on the safety of asfotase alfa (Strensiq®) (then known as ENB- 0040), we worked closely with Philippe and others … to start the process for Phase 2 clinical trials at our WInnipeg site (*4,6,8*). As it turns out, the next baby who needed such help was an infant, Baby Amy, from Northern Ireland. In October of 2008, she was airlifted to Winnipeg by Lear Jet. I was at the airport to greet the family. Baby Amy stay with us for 6 months before being able to return home to Northern Ireland. She is now a thriving 16-year-old young lady.»

This is how, in 2006, a team of scientists at the University of Montreal invented the Asfotase Alpha, the first enzyme replacement therapy for a bone metabolic disorder, a therapeutic agent that dramatically cures young patients with HPP. In 2011, Alexion Pharmaceuticals, which specialized in rare diseases (*10*) acquired Enobia for more than $700 millions, one of the major success stories in biotechnology in Montréal. Asfotase Alpha is now commercialized by AstraZeneca under the name Strensiq with sales of over one billion dollars per year.

Philippe showed us touching videos in which we see newborn babies fighting to survive under respirators who, after treatment, can freely breathe and develop normally; other kids moving in wheelchairs who, once treated, can finally have normal physical activities in playgrounds. This constitutes an apotheosis rarely attained in biomedical research. Mrs. Barbara Fowler, President of the American Soft Bones Foundation (*11*) gave a vibrant tribute to Pr Crine. «Amy shares the story about how Strensiq saved her life. Two years ago, she said she wanted to die. She started therapy about a year ago and says today - her life is forever changed. Her twin sister is equally thankful because she has also been positively impacted. In her words, "Dr. Crine will not be forgotten. I will be forever grateful for his gift to us all." These are just a few stories – of literally hundreds more – that I was able to collect from the outpouring of sympathy and sorrow that emanated from our patients upon learning of his passing. I could go on and on. When sharing these stories with Dr. Crine, he would be brought to tears. He was such a gentle and compassionate human being. He would respond by saying, "These stories keep me going and make me want to do more. You are my true heroes and an incredible source of inspiration in my work and my life." … We owe everything to this incredible man and are eternally grateful for his genius mind and generous heart. To his family, thank you for sharing your husband/father with us and our HPP family. His legacy and the impact of his work will live on for generations to come.»

Annie Mear, Philippe's wife, remembers that « One month before his death, Philippe insisted on giving the opening conference at a symposium organized by Effervescence Montréal for young scientists who wanted to have their own start-ups…Even cancer cannot stop him from sharing his knowledge. He enjoyed life in a phenomenal way » (*12*). Still active until recently, Philippe was a highly sought-after consultant for young pharma in search of innovative therapeutics.

The scientific world will remember Philippe as an inspiring teacher who shared with students the pursuit of excellence, the eagerness to discover, and the reflex for innovation. In addition to a dedicated academic career at the Université de Montréal, Philippe will be remembered as a pioneer in biomedical sciences and biopharmaceuticals for his invention of a mind-blowing therapeutic agent that cures a debilitating and often lethal disease in infants and for the creation of a start-up that squarely sets priorities on patients needs. He is a role model of an academic professor who put his expertise and his scientific talent at the service of medicine and society.

PHILIPPE CRINE IS A HERO FOR HUNDREDS OF CHILDREN WHO ARE CURED FROM HPP.

Michel Bouvier, Ph.D., Professor, Department of Biochemistry and Molecular Medicine, Université de Montréal

Trang Hoang, Ph.D. Professor, Department of Pharmacology and Physiologiy Univesité de Montréal

Michel Chrétien, FRS, M.D. Professeur Emeritus, IRCM and Université de Montréal.

References:

1. JL Marx. Neurobiology: Researchers High on Endogenous Opiates | *Science*. **193**: 1227-1229 (1976). DOI:10.1126/science.193.4259.1227
2. https://biochimie.umontreal.ca/departement/historique/

3. JL Millán, S Narisawa, I Lemire, TP Loisel, G Boileau, P Leonard, S Gramatikova, R Terkeltaub, NP Camacho, MD McKee, P Crine, MP Whyte: Enzyme Replacement Therapy for Murine Hypophosphatasia. J Bone Miner Res. **23**, 777-87 (2008).
4. MP Whyte, CR Greenberg, NJ Salman, MB Bober, WH McAlister, B Van Sickle, D Wenkert, TS Edgar, ML Bauer, M Hamdan, JH Simmons, N Bishop, RE Lutz, M McGinn, S Craig, JN Moore, JW Taylor, RH Cleveland, WR Cranley, R Lim, TD Thacher, JE Mayhew, M Downs, JL Millan, A Skrinar, P Crine, H Landy: Enzyme Replacement Therapy In Life-Threatening Hypophosphatasia. *N Engl J Med* **366**, 904-13 (2012).
5. Weiss MJ, Cole DE, Ray K, Whyte MP, Lafferty MA, Mulivor RA, Harris H. A missense mutation in the human liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia, *Proc Natl Acad Sci U S A*.;**85**(20):7666-9 (1988)
6. MP Whyte, CR Greenberg, K Ozono, R Riese, S Moseley, A Melian, D Thompson, C Hofmann: Asfotase Alfa Treatment Improves Survival For Perinatal And Infantile Hypophosphatasia. *J Clin Endocrinol*, **101**: 334-42 (2016).
7. MP Whyte; JH Simmons, S Moseley, KP Fujita, N Bishop, NJ Salman, J Taylor, D Phillips, M McGinn, WH McAlister: Asfotase Alfa For Infants And Young Children With Hypophosphatasia: 7 Year Outcomes Of A Single-Arm, Open-Label, Phase 2 Extension Trial. *Lancet Diab Endocrinol* **7**: 93-105 (2019).
8. MP Whyte, KL Madson, D Phillips, A Reeves, WH McAlister, A Yakimoski, K Mack, K Hamilton, K Kagan, A Melian, D Thompson, S Moseley, T Odrljin, CR Greenberg: Asfotase Alfa Therapy For Children With Hypophosphatasia. *JCI Insight* **1**:e85971; 1-10 (2016).
9. MP Whyte: Hypophosphatasia: Enzyme Replacement Therapy Bring New Opportunities and New Challenges. J Bone Min Res **32**: 667-675 (2017).
10. Kendall Powel Top firms prioritize transformative technologies, patients | *Science* | AAAS, Focus on careers, 574-582 (30 OCT 2015)
11. https://softbonescanada.ca/ and https://softbones.org/
12. Annie Mear, https://www.linkedin.com/feed/update/urn:li:activity:7082391428198215680/