Synopsis of N. G. Bazan contributions to Medicine.

Nicolas G. Bazan's research has opened neuroscience conceptual in-roads in biology, neuroscience and medicine during the years that led him to uncover mediators of neural cell integrity, signaling mechanisms, and to define novel molecular principles of cell survival and neuroprotection. His contributions, relative to that of others, can be highlighted as unique and pioneering. From the beginning of his scientific career, he has contributed innovative concepts and discovered molecular principles of cell survival. He was the first to uncover arachidonic acid (AA) and docosahexaenoic acid (DHA) brain release upon stimulation by ischemia or seizures at rates comparable to those of maximal hormonal lipolityic activation. At the time, medical sciences were captivated by the discovery of prostaglandins and other eicosanoids from AA and the elucidation of their functions by B. Samuelsson, S. Bergstrom and J. Vane. Bazan took a different focus and approach and began aiming to understand the significance and consequences of brain DHA release. During this time, Bazan became aware of initial studies that observed beneficial health effects of diets rich in omega-3 fatty acids. Thus, he began conceptualizing and pondering on the biology of the omega-3 family member DHA, which is prominently concentrated in the central nervous system (CNS), and he formulated hypotheses and tested them under various conditions, as described below. Many unexpected outcomes have since evolved, including his findings on the phospholipid-mediator, platelet-activating factor (PAF). Bazan approached this issue also from a different angle because PAF metabolism involves DHA and AA release. Therefore he was the first to look at the significance of these events and found that PAF modulates hippocampal excitatory synaptic transmission (Clark et al, Neuron, 1992, PMID: 1334422) and presynaptic glutamate release, and that it is a retrograde messenger of long-term potentiation (Kato et al, Nature, 1994, PMID: 8114914), enhancing memory formation (Izquierdo et al, Proc Natl Acad Sci U S A, 1995, PMID: 7761446). He then began connecting the initial findings with synaptic signaling and brain function, and in the 1980's Bazan developed the concept and initial exploration of bioactive DHA derivatives that he suggested calling docosanoids (22C, in contrast to the 20C eicosanoids from AA). In fact, he showed that the retina generated enzyme-derived DHA products (Bazan et al, Biochem Biophys Res Commun, 1984, PMID: 6240268).

While studying DHA brain release due to stimulation early on, Bazan began using the retina as a natural-made brain slice since its differentiated neuron, the photoreceptor cell, is enriched in DHA and its neuronal circuitry makes it an integral part of the central nervous system (CNS). Then he stumbled on new mechanisms regarding how DHA is acquired to reach such a unique endowment in the retina and brain. Major leaders in the field of neurosciences and vision sciences, such as Christine A. Curcio (Univ. of Alabama), Robert E. Anderson (Univ. of Oklahoma), Danielle Piomelli (Univ. California, Irvine) and Steven Fliesler (Univ. of Rochester) supported and confirmed Bazan's findings (*e.g.*, Astaratita et al, PLoS One, 2010, PMID: 20838618). Based on these discoveries, Bazan identified the liver-to-retina (and brain) "long loop" for DHA supply (Scott and Bazan, Proc Natl Acad Sci U S A, 1989, PMID: 2523075) and a retinal pigment epithelium/photoreceptor intercellular "short loop" for DHA retention in photoreceptors (Bazan et al, J Biol Chem, 1985, PMID: 3932343; Gordon and Bazan, J NeuroscI, 1990, PMID: 2142959; Rodrigues de Turco et al, J Neurosci, 1991, PMID: 1834810). This recycling is similar to that seen in retinoids, and he postulated it to be critical for photoreceptor survival; hence, its breakage leads to retinal degeneration. Bazan also found that Acadian Louisiana Usher's Syndrome patients (born deaf, then blind due to retinitis pigmentosa) have DHA shortage in the blood, implicating the long loop in retinal degeneration (Bazan et al, Biochem Biophys Res Commun, 1986, PMID: 3004440). This observation, among others from his lab, led him to further explore the role of DHA in photoreceptor degeneration and to extrapolate to Alzheimer's disease. These findings were confirmed by others (Astaratita et al, PLoS One, 2010, PMID: 20838618). For more than a decade, his quest focused on the specific

molecular mechanisms engaged. Thus, Bazan and collaborators led the discovery of a specific transmembrane protein (adiponectin receptor 1; AdipoR1) for DHA uptake/retention in retinal pigment epithelial (RPE) cells and photoreceptors necessary for cell functional integrity (Rice et al, Nat Commun, 2015, PMID: 25736573). This AdipoR1-protein, although it has seven transmembrane domains, is not a G-protein, and thus he demonstrated that its cognate ligand, adiponectin, is not involved. Therefore, the new function is that AdipoR1 represents a key molecular switch for DHA uptake, retention and conversion into a photoreceptor-specific molecular species of phosphatidylcholine that are decreased in age-related macular degeneration. In fact, when Bazan genetically ablated the protein, retinal degeneration ensued (Rice et al, Nat Commun, 2015, PMID: 25736573). Bazan's thinking and work has influenced others. As an example of an additional impact of Dr. Bazan's discoveries, it was reported that a mutation in AdipoR1 causes nonsyndromic autosomal dominant retinitis pigmentosa, a finding that was based on Dr. Bazan's work on AdipoR1 (Zhang et al, Hum Genet, 2016, PMID: 27655171).

Bazan then demonstrated in 1984 that DHA is the precursor of docosanoids (Bazan et al, Biochem Biophys Res Commun, 1984, PMID: 6240268) and predicted that they are endowed with pro-homeostatic cell survival properties. Here again, comparatively to others, he was ahead by a decade. Dr. Charles Serhan (Harvard Medical School), a recent collaborator with Dr. Bazan, published related work on docosanoids/DHA-derived mediators in the 2000's. This is the case as well, comparatively, regarding others in the field, such as Drs. Robert C. Murphy (Univ. Colorado, Denver) and Gerard Lambeau (Univ. of Nice, France). Dr. Bazan contributed to the discovery of the synthesis and bioactivity of neuroprotectin D1 (NPD1; 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid) in 2003-2004. They uncovered that NPD1 arrests apoptosis in RPE cells at the pre-mitochondrial level and is neuroprotective in brain ischemia-reperfusion (Marcheselli et al, J Biol Chem, 2003, PMID: 12923200) and in cellular models of Alzheimer's disease (AD, Lukiw et al, J Clin Invest, 2005, PMID: 16151530). Thus, Bazan and colleagues coined the name "neuroprotectin D1" for this first-identified docosanoid and showed that NPD1 (Mukherjee et al, Proc Natl Acad Sci U S A, 2004, PMID: 15152078) is a stress/injury response mediator made on demand that counteracts disruptions of cellular homeostasis, and it is an active participant in a well-concerted process that effectively modulates neuroinflammation. Esterified-DHA from phospholipids is cleaved by phospholipase A2 (PLA2), releasing DHA followed by NPD1 synthesis. Bazan also showed that 15-lipoxygenase-1 (15-LOX-1) catalyzes DHA enzymatic lipoxygenation (Calandria et al, J Biol Chem, 2009, PMID: 19403949) and conversion into NPD1, and that neurotrophins stimulate this process (e.g., BDNF, NGF, PEDF) (Mukherjee et al, Proc Natl Acad Sci U S A, 2007, PMID: 17670936). They found enhanced cytosolic PLA2 expression and decreased free-DHA in short post-mortem sampled CA1 hippocampal region of early stages of Alzheimer's disease with a concomitant 25-fold decrease in NPD1 (Lukiw et al, J Clin Invest, 2005, PMID: 16151530). Then he showed that NPD1 promotes down-regulation of pro-inflammatory genes and pro-apoptotic Bcl-2 proteins, and that it also enhanced abundance of anti-apoptotic proteins to counteract Aβ-mediated neurotoxicity. Among the molecular targets that he found for this bioactive lipid is the triggering of de-phosphorylation of Bcl-xL in a PP2A-dependent fashion during oxidative stress, which induces cell survival (Lukiw et al, J Clin Invest, 2005, PMID: 16151530).

Following the early observation of ischemia-mediated release of DHA, Bazan and his colleagues demonstrated that DHA induces cell survival in ischemic-stroke (Belayev et al, Stroke, 2005, PMID: 15569878; Belayev et al, Transl Stroke Res, 2012, PMCID: PMC3284672), modulates neuroinflammation, and activates long-term restoration of synaptic circuits in models of epileptogenesis (Musto et al, PLoS One, 2015, PMID: 25617763; Musto et al, Sci Rep, 2016, PMID: 27444269). Thus, DHA release has well defined beneficial effects. Bazan also

demonstrated that DHA bioactivity is elicited through its conversion into docosanoids (mainly NPD1), which halt the generation of cell death signals. He and his colleagues then found increased NPD1 synthesis, as a consequence of DHA administration, in a middle cerebral artery occlusion (MCAo) stroke model that, in turn, prompts selective neuronal cREL translocation followed by BIRC3 gene expression, resulting in remarkable neurological recovery (Calandria et al, Cell Death Differ, 2015, PMID: 25633199). Thus, cREL was translocated into the nucleus to a greater extent in DHA-treated animals, suggesting that NPD1 produced by the conversion of systemically-administered DHA acts through cREL-mediated BIRC3 transcriptional activation to exert its neuroprotective bioactivity. This series of studies also included the use of a cellular model, where Bazan found that when the cREL protein abundance increases, it leads to survival and a decrease in p65/RelA, in response to NPD1 (Calandria et al, Cell Death Differ, 2015, PMID: 25633199). These findings helped to further unravel the endogenous signaling that sustains cellular integrity, thus providing a new understanding of the mechanisms that could lead to precise therapeutic approaches for neuroprotection.

Bazan and his colleagues recently discovered a new family of lipid messengers, which they coined “elovanoids” (ELVs, Jun et al, Sci Rep, 2017, PMID: 28706274; Battacherjee et al, Sci. Adv, 2017, in press). Elovanoids are set apart from all other lipid messengers. Known lipid mediators, such as prostaglandins, leukotrienes, lipoxins, resolvins and docosanoids, are derived from 18-, 20-, and 22-carbon-length fatty acid precursors. Elovanoids, on the other hand, have structures derived from 32- or 34-carbon precursors, with different physicochemical and biological properties. Bazan reported the complete structures and stereochemistry of the novel elovanoids ELV-N32 (derived from 32:6,n-3) and ELV-N34 (derived from 34:6,n-3), the complete R/S configuration, and the Z/E geometry of the double bonds as generated in retinal cells and neurons. Dr. Bazan furthermore showed that ELVs are cell-specific mediators necessary for neuroprotective signaling for cell integrity.

In addition, Bazan has designed and developed several molecules for clinical application, (covered by patents assigned to LSU Health New Orleans) including non-narcotic, non-toxic analgesics for a variety of conditions, including: neuropathic pain; novel anti-inflammatories; compounds effective for slowing down invasiveness of glioblastoma multiforme; genetically-engineered transdifferentiated fibroblasts for neurons and genetically-engineered adipose tissue cells for neurodegenerative diseases (Alzheimer’s, Parkinson’s, etc.); stroke and traumatic brain injury; and a variety of eye and brain diseases.

Dr. Bazan’s ongoing quest in the fields of biology and medicine is, in a way, reflected as a response to one major challenge to civilization: the growing incidence in the loss of sight and cognition due to increased life expectancy and to other factors (Bazan, Mol Neurobiol, 2014, PMID: 25236258). His ideas are synergized with a rise in the occurrence of photoreceptor- and neuronal-survival failure, as reflected mainly by age-related macular degeneration (AMD) and Alzheimer's disease (AD). The development of the nervous system is driven by neuronal apoptotic cell death and, thereafter, for the lifespan, neurons are post-mitotic. In neurodegenerative diseases, apoptosis and other forms of cell death lead to selective neuronal loss. Although age is the main risk factor, not everyone develops these diseases during aging. Thus Bazan posed the following questions: why can the latency period last for decades without disease manifestation, for example, in inherited familial forms of AD and in retinal degenerations including AMD?; and does a cell-specific initial response/s counteract the consequences of mutation/polymorphism expression? (Bazan, Mol Neurobiol, 2014, PMID: 25236258). There are many factors involved, including developmentally-expressed genes, since most of the inherited neurodegenerative diseases remain asymptomatic during development and maturation of the nervous system. Bazan clearly began deciphering the molecular logic that sustain neuronal

survival by uncovering molecular principles (transcriptional signatures) governed by the docosanoid NPD1 and likely by other key mediators, such as the newly-discovered elovanoids. Bazan is already untangling issues to be explored, including the decision-making process involved in storage specificity/retrieval of lipid mediators and the molecular sensors in early stages of neurodegenerations.

His laboratory has led to the uncovering of new gene regulation and necessary proteins for cell survival in RPE cells and these molecular events provide unified responses amenable to be harnessed to slow down the onset and early progression of neurodegeneration. Thus, Dr. Bazan uncovered a different signal bifurcation mechanism that aims to sustain cell integrity (Jun et al, Sci Rep, 2017, PMID: 28706274. Overall, Dr. Bazan's findings reveal a novel pro-homeostatic and neuroprotective lipid-signaling mechanism that aims to sustain neural cell integrity.