Córdoba, September 16, 2019

Progress report CAEN Category 1C Return Home Grant
ISN-CAEN Supported Activities 2017

Project Title: **Delivery of microRNA-219 mimics by MSC-derived extracellular vesicles to promote remyelination.**

Responsible Investigator:
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Centro de Investigación Medicina Traslacional "Severo Amuchástegui" (CIMETSA)
Instituto Universitario Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina

To the ISN Committee for Aid and Education in Neurochemistry,

I would like to take this opportunity to appreciate and acknowledge the incredible support provided ISN-CAEN and all its members through the Return Home Grant. Receiving this grant has had a huge impact to build my scientific career as an independent researcher in my home country Argentina. The ISN-CAEN Return Home Grant allowed me to establish my independent line of research, establish new collaborations and start training future PhD students. I also would like to recognize the financial opportunities offered by ISN-CAEN to foster young researchers in developing countries.

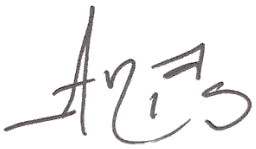
I received my Ph.D. in Biological Chemistry from the Universidad Nacional de Córdoba in 2011. After completion of my doctoral degree I did a postdoctoral training at the Department of Anatomy and Cell Biology at the University of Illinois in Chicago in USA. In January 2018 I received a Repatriation Fellowship from CONICET (National Research Council - Argentina) in the laboratory of Dr Alfredo Cáceres at the Centro de Investigación en Medicina Traslacional "Severo Amuchástegui" (CIMETSA) at the Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC). Since November 2018 I hold an Assistant Researcher position (CIC-CONICET) at CIMETSA-IUCBC.

Currently my laboratory is focused on the pathogenic mechanisms involved in demyelinating diseases of the central nervous system (CNS) and the development of therapeutic approaches to promote the regeneration of myelin. We are working on several aspects of CNS-derived extracellular vesicles as therapeutic agents to induce the endogenous regeneration mechanisms of the CNS. My goals for the future are to enhance the development of the applied (translational) Neuroscience research field in the Latin American region and to contribute not only to the knowledge and development of new therapeutic approaches for demyelinating diseases but also to foster the training of students in this field.

Besides the immense positive impact of the ISN-CAEN Return Home Grant in my scientific career, I also would like to appreciate the continuous support and great mentoring of Dr. Alfredo Cáceres and all the help from the members of his lab. Likewise, thanks to the institution that allowed me to establish my laboratory (CIMETSA-IUCBC) and the Instituto de Investigación Médica Mercedes y Martín Ferreyra

(INIMEC-CONICET-UNC) that gave me access to its facilities and equipment. I am also profoundly grateful for the great support and collaboration of Dr. Pablo López (INIMEC-CONICET-UNC) and all the members of his lab.

At the end of this letter you will find a summary of my financial report and a detailed description of the activities and projects performed during the funded period.



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1 - FINANCIAL REPORT AND RESEARCH ACTIVITIES 2017-2019

Materials and supplies	Total (U$D)
Cell culture plastic, media and reagents	2,400
Media and antibiotics (Byodinamics); Media (NewCell); cell culture plastic (Ap Biotech); FBS (ThermoFicher and Internegocios); LPC, LPS and Collagenase (Sigma); Plates (TNT Laboratories); inserts (Millipore)	
Molecular biology	2,600
Filter tips, PBS and water nuclease free (Byodinamics); microRNA mimics (Dharmacon); Transfection reagent (Nueromics); Label IT siRNA (Mirus)	
Biochemical assays, DLS and EVs isolation	850
Western blot reagents (BioRad); ultracentrifuge tubes and parts (Beckman Coulter - Bioesanco); ECL films (Fissore); DLS use (CIQUIBIC)	
Immunohistochemistry and electron microscopy	950
Antibodies (Cell signaling and Sigma), Confocal use (CEMINCO), EM (INTA)	
General laboratory reagents	900
General laboratory reagents (Todo Droga); Plastic cylinders (Amazon); Pippet calibration (Bioingeniería)	
Costs for national meetings	300
Sociedad de Biología de Córdoba (SBC) and Sociedad Argentina de Neurociencias (SAN)	
TOTAL	**8,000**

RESEARCH PROJECT AND PRELIMINARY RESULTS

Myelin surrounds the axons of neurons and provides metabolic support and electric insulation essential for nerve impulses. Central nervous system (CNS) demyelinating diseases are characterized by the loss of myelin and oligodendrocytes, the cells that produce and assemble myelin. New oligodendrocytes can be generated and replace lost myelin sheaths (remyelination), but gradually this process fails and results in progressive neurological dysfunction. Current therapies for myelin diseases are disease-modifying drugs that are unable to recover myelin loss and stop disease progression. During my last tenure at University of Illinois in Chicago using different experimental models of demyelination and multiple sclerosis (most common CNS demyelinating disease) we found that miRNA-219 is a potential therapeutic agent that promotes remyelination. Here, we hypothesize that stem cell-derived extracellular vesicles (EVs) could enhance CNS targeting of miRNAs and integrate their intrinsic regenerative effects to promote remyelination. The main goal of this project is to use EVs as delivery system for miRNA-based therapies and further improve their therapeutic potential.

Characterization of hiPSC-derived EVs

EVs were isolated by differential ultracentrifugation (Figure 1) from conditioned media of human induced pluripotent stem cells (hiPSC) and hiPSC under differentiation (d-hiPSC).

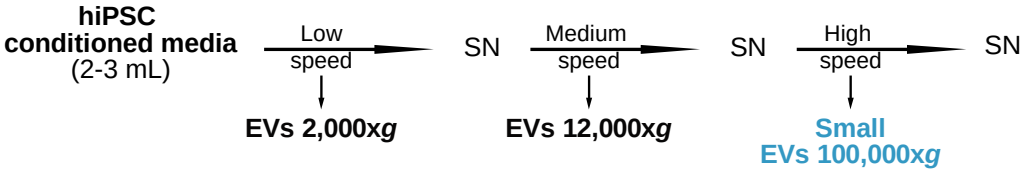

Figure 1| *Schematic diagram showing successive differential ultracentrifugation steps during EVs isolation*. (I) large EVs pelleting at low speed (2,000x*g*); (II) medium-sized EVs pelleting at intermediate speed (12,000x*g*); and (III) EVs pelleting at high speed (100,000x*g*) highly enriched in exosomes. SN: supernatant.

To characterize the structure of small EVs isolated at 100,000x*g* obtained from hiPSC and d-hiPSC conditioned media we analyzed these preparations by electron microscopy (EM). EM analysis showed small EVs with typical morphology and different diameters in both samples (Figure 2A). To further characterize hiPSC-derived small EVs we analyzed their particle size distribution by dynamic light scattering (Figure 2B) and their protein content by bicinchoninic acid assay (Figure 2C). Our results indicate that hiPSC-derived small EVs have a particle size distribution ranged from 50 to 250 nm and significantly different protein levels between hiPSC and d-hiPSC.

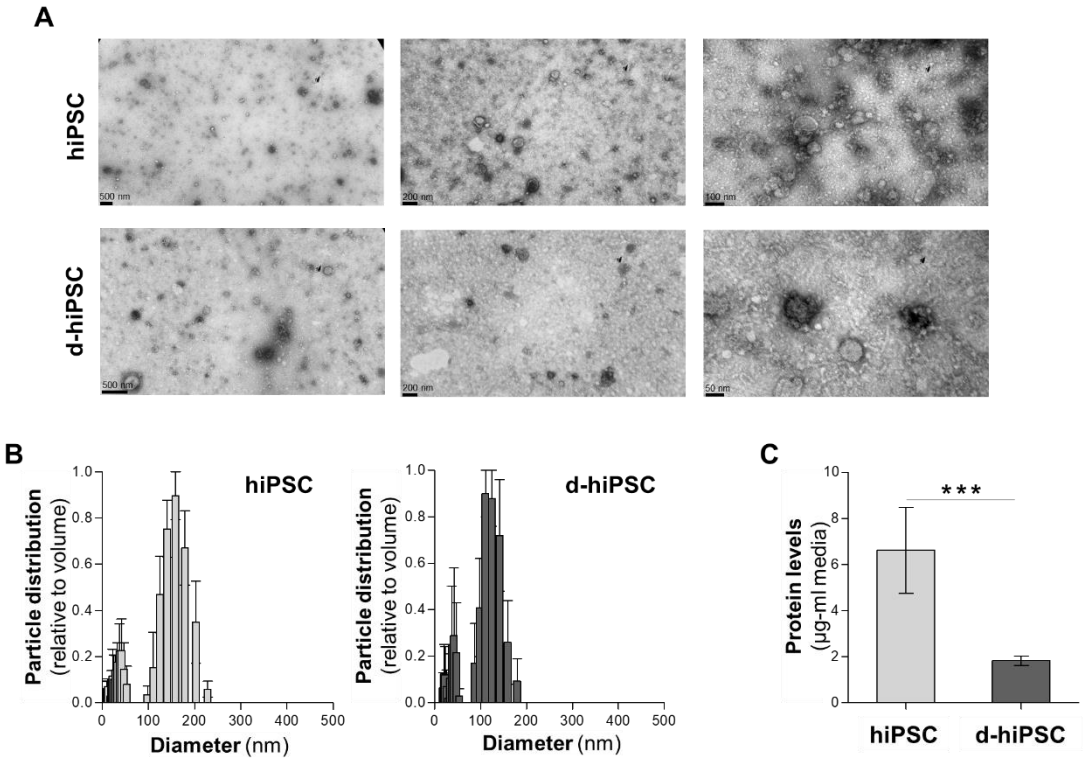

Figure 2| *Characterization of small EVs isolated at 100,000xg from hiPSC and d-hiPSC.* (**A**) Representative EM micrographs of small EVs from hiPSC and d-hiPSC. (**B**) EVs particle distribution by dynamic light scattering of small EVs from hiPSC and d-hiPSC. (**C**) Protein levels in small EVs show significant differences in hiPSC compared with d-hiPSC. Data are presented as mean ± SEM.

Biological activity of hiPSC-derived EVs

To determine whether hiPSC-derived small EVs promote remyelination we used and *ex vivo* model of demyelination induced by lysophosphatidylcholine (LPC) in organotypic cerebellar cultures. These cultures preserve the original architecture of the tissue and are an ideal tool to study normal

myelination, demyelination and remyelination *ex vivo*. Immunohistochemistry and confocal microscopy analysis showed that LPC-induced demyelination was reduced by hiPSC-derived small EVs (Figure 2). These results indicate that EVs secreted by hiPSC have biological activity during demyelination and might promote myelin regeneration.

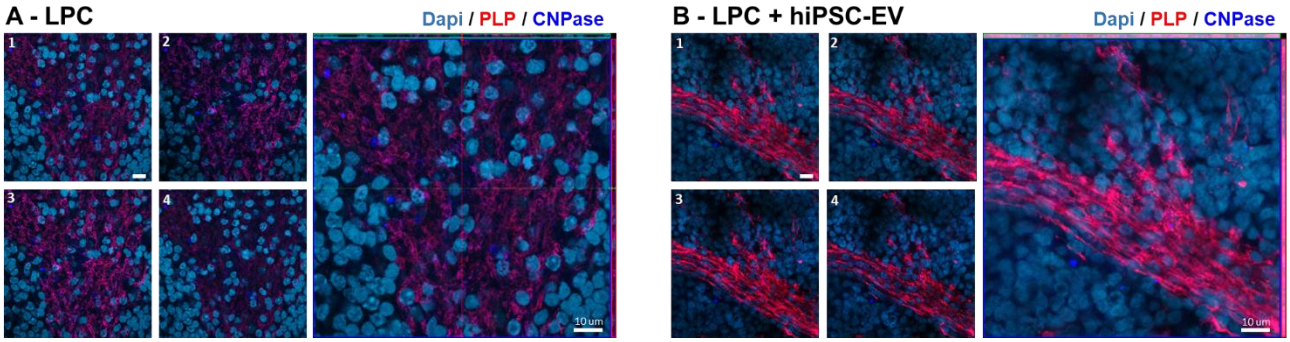

Figure 2| *hiPSC-derived small EVs promote remyelination.* Representative confocal immunofluorescence for myelin markers PLP (red) and CNPase (blue) in cerebellar cultures treated with LPC (**A**) and hiPSC-derived small EVs after LPC (**B**). Scale bar = 10 µm. Nuclei stained with DAPI (light blue).

Currently we are working to establish the cellular and molecular mechanisms involved in hiPSC-derived small EVs effects *ex vivo* and EVs biodistribution in different the CNS cell types. We are very excited about these results and expect to send them for publication at the end of this year.

Research project in collaboration with Dr. Cáceres

The Laboratorio de Investigación en Células Madre (LINCEMA) at the IUCBC was created in 2015 as part of a Translational Research project between Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC), Fundación Instituto Leloir (FIL), Hospital General de Agudos "Eva Perón", CONICET and Universidad de Buenos Aires. This research team project was funded by our former Ministro de Ciencia y tecnología (MinCyT) through the FONARSEC 2015 that promotes the development of Biotechnological Projects on Translational Research (PBIT 2015). One of the objectives of this project was the establishment of hiPSC cultures from healthy patients as well as from patients suffering from familial Alzheimer's (with mutations in Presenilins, PS1 and PS2). Currently we are exploring several aspects of EVs secreted by hiPSC with these mutations. This project is in collaboration with Dr. Alfredo Caceres (INIMEC-IUCBC), Dr. Laura Gastaldi (IUCBC) and Dr. Monica Remedi (INIMIEC-CONICET).

2 - Other Research Activities and Publications

Research Positions

2018-present **Research Assistant (CIC-CONICET)** Centro de Investigación en Medicina Traslacional "Severo Amuchástegui" (CIMETSA). Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina.

2018 **Repatriation Fellowship (CONICET).** Centro de Investigación en Medicina Traslacional "Severo Amuchástegui" (CIMETSA). Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina.

FELLOWSHIPS, GRANTS AND AWARDS

2018 **Outstanding Poster Presentation Award.** American Society of Gene & Cell Therapy (**ASGCT**). ASGCT 21st Annual Meeting, May 2018, Chicago, IL, USA.

2018 **Underrepresented Minority Travel Award.** American Society of Gene & Cell Therapy (**ASGCT**). ASGCT 21st Annual Meeting, May 2018, Chicago, IL, USA.

2018 **Repatriation Fellowship.** National Scientific and Technical Research Council (**CONICET**). Centro de Investigación en Medicina Traslacional "Severo Amuchástegui" (CIMETSA). Instituto Universitario Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina.

2017 **ISN CAEN "Return Home Grant"**. International Society for Neurochemistry (**ISN**) Committee for Aid and Education in Neurochemistry (**CAEN**).

PUBLICATIONS

1. Vivinetto AL, Garcia-Keller G, Palandri A, Falcon C, Castañares C, **Moyano AL**, Rozes Salvador V, Rojas JI, Patrucco L, Monferran C, Cancela L, Cristiano E, Schnaar RL, Lopez PHH. "Myelin-associated glycoprotein activation triggers glutamate uptake by oligodendrocytes and mitigates excitotoxicity". *In preparation.*

2. **Moyano AL***, Steplowski J, Wang H, Son K-N, Rapolti D, Marshall J, Elackattu V, Marshall MS, Hebert AK, Reiter CR, Ulloa V, Pituch K, Givogri MI, Lu QR, Lipton HL and Bongarzone ER. (2018) "microRNA-219 Reduces Viral Load and Pathologic Changes in Theiler's Virus-Induced Demyelinating Disease". ***Mol Ther**. Mar 7;26(3):730-743). *co- corresponding author

3. D'auria L, Reiter C, Ward E, **Moyano AL**, Marshall M, Kool N, Scesa G, Hauck Z, van Breemen R, Givogri MI and Bongarzone ER. (2017) "Psychosine enhances the shedding of membrane microvesicles: implications in demyelination in Krabbe's disease". ***Plos One***. May 22;12(5): e0178103.

Google Scholar: https://scholar.google.com.ar/citations?user=JgK3tSoAAAAJ&hl=en#

Pubmed: https://www.ncbi.nlm.nih.gov/pubmed/?term=%22Moyano+AL%22

PARTICIPATION IN SCIENTIFIC MEETINGS

1. Herrera López M, Remedi M, Gastaldi, L, Ceschin D, Cáceres AO, **Moyano AL**. "hiPSC- and hNPC-derived extracellular vesicles: composition and biological activity". 2019 Reunión Anual de la Sociedad Argentina de Neurociencias, October 2019, Carlos Paz, Argentina.

2. Herrera López M, Remedi M, Gastaldi, L, Cáceres AO, **Moyano AL**. "Characterization of hiPSC-derived extracellular vesicles". XXII Jornadas Científicas de la Sociedad de Biología de Córdoba, August 2019, Córdoba, Argentina.

3. **Moyano AL**. "CNS-targeted delivery of miRNAs with extracellular vesicles to promote remyelination". Max Planck - IIDEFAR Workshop "New insights and advances in Neuroscience and Oncology II" November 2018, Rosario, Argentina. **Oral Communication.**

4. **Moyano AL**. "CNS-targeted delivery by extracellular vesicles to promote remyelination". Colloquium Alexander von Humboldt: "Shaping the Future of German-Argentinian Scientific Cooperation - The Role of Curiosity-Driven Research". October 2018, Buenos Aires, Argentina.

5. **Moyano AL**, Steplowski J, Wang H, Son K-N, Rapolti DI, Marshall J, Elackattu V, Marshall MS, Hebert AK, Reiter C, Ulloa V, Pituch KC, Givogri MI, Lu QR, Lipton HL and Bongarzone ER. "MicroRNA-219 Reduces Viral Load and Pathologic Changes in Theiler's Virus-Induced Demyelinating Disease". ASGCT 21st Annual Meeting, May 2018, Chicago, IL, USA. **Outstanding Poster Presentation Award.**

6. **Moyano AL**, Steplowski J, Wang H, Son K-N, Rapolti DI, Marshall J, Elackattu V, Marshall MS, Hebert AK, Reiter C, Ulloa V, Pituch KC, Givogri MI, Lu QR, Lipton HL and Bongarzone ER. "Intranasal therapy with miRNA-219 reduces viral load and encephalomyelitis in the TMEV model of multiple sclerosis". Role of Glia in health and Disease - ISN/IBRO/FONCYT. October 2017, Buenos Aires, Argentina.

7. **Moyano AL**, Steplowski J, Wang H, Son K-N, Rapolti DI, Marshall J, Elackattu V, Marshall MS, Hebert AK, Reiter C, Ulloa V, Pituch KC, Givogri MI, Lu QR, Lipton HL and Bongarzone ER. "Intranasal therapy with miRNA-219 reduces viral load and encephalomyelitis in the TMEV model of multiple sclerosis". Alexander von Humboldt Kolleg: Current Advances on Neurodegeneration: from Molecular Biology to Translational Medicine. September 2017, Córdoba, Argentina.

POSTGRADUATE COURSES

2019 "**Extracellular vesicles in the CNS: biological and biomedical aspects**". Postgraduate course: Molecular and cellular neuroscience and neurochemistry: experimental strategies for studying the nervous system in health and disease**.** Sociedad Argentina de Neurociencias, Universidad Nacional de Córdoba, Argentina.

TRAINING OF YOUNG SCIENTISTS AND TEACHING

2019-present **Research mentor of undergraduate thesis**: Malena Herrera López. Undergraduate student at the Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Argentina.

2018- present **Assistant Professor.** *Discipline:* "General Chemistry". Instituto Universitario Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina.

ADVOCACY

Currently I am a member of the Comisión contra la Violencia y el Acoso (CVA) from INIMEC-CONICET-UNC (http://www.institutoferreyra.org/page-institucional/cva/). This is a great initiative with the main goal to promote a work environment free of violence and discrimination, trough the dialogue and collective work among members of this institution. CVA is composed by members of INIMEC-CONICET-UNC and at least one representative of CIMETSA (RHCS-2018-1449 -E-UNC-REC). This time that I dedicate to advocacy it is a very important part of my responsibilities as a researcher from CONICET in Argentina. I am grateful to be part of a collective of researchers that advocates against violence, discrimination and gender inequality.