SPECIAL CALL FOR PROPOSALS 2014

MYELIN : FROM LESION TO REPARATION

Project file

DEADLINE: SEPTEMBER 27, 2016

To be eligible, the Proposal must include:
- Participation of at least two research laboratories, including one in France.
- Relevance to the proposed general topics.

WARNING: The coordinator of the project will be the person in contact with ARSEP/AFM for all information and correspondence about the project.

COORDINATOR OF THE PROJECT:

NAME	Anna Maria
Surname	Papini
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TITLE OF THE PROJECT:

Intranasal neuropeptide therapy for myelin regeneration in multiple sclerosis

PARTICIPANTS TO THE PROJECT:

Team 1:

Principal Investigator:

NAME	Anna Maria
Surname	PAPINI
Administrative institution	University of Cergy Pontoise
Research laboratory/unit	Laboraotry of Chemical Biology-Peptlab@UCP platform
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Participants to the programme (Please list in order, scientists/clinicians, engineers, technicians.) The principal investigator certifies that all participants named below are informed about their collaboration in the project.

Name, first name Date of birth	Title and degrees	Institution	Percent of research time on the project
Anna Maria, Papini 02/02/1959	Full Professor, PhD	University of Cergy Pontoise	30%
Maud, Larregola 27/02/1982	Associate Professor, PhD	University of Cergy Pontoise	15%
Elisa, Peroni 02/06/1973	Associate Professor, PhD	University of Cergy Pontoise	15%
Olivier, Monasson 06/01/1981	Engineers, PhD	University of Cergy Pontoise	5%

Biographical sketch of the principal investigator.

Education, training			
Institution and location	Degree	Year	Field of study
University of Florence (Italy)	Italian Laurea in Chemistry	1977-1983	Organic Chemistry
University of Florence (Italy)	Ph.D.	1985-1989	Chemical Biology
University of Florence (Italy)	PostDoc	1990	Dermatochemistry

Positions and Honors.

Since 2013	Director of the Platform PeptLab@UCP of the University of Cergy-Pontoise.
2009-2014	Laureate of the Chaire d'Excellence (ANR-09-CEXC-013-01).
Since 2008	Full Professor and Chair of Organic Chemistry at the University of Cergy-Pontoise (France).
Since 2013	Scientific National Habilitation to the function of Full Professor of Organic Chemistry of the Italian Universities.
Since 2010	Founder and coordinator of the French-Italian Laboratory of Peptide & Protein Chemistry & Biology (www.peptlab.eu).
Since 2004	Co-founder and head of the Interdepartmental Laboratory of Peptide & Protein Chemistry & Biology, Polo Scientifico of the University of Florence associating the Departments of Chemistry, Pharmaceutical Sciences, and Neurosciences of the University of Florence.
2001	Ranked 1st for the position of Professor of Organic Chemistry at the Université de Lyon "Claude

Bernard"(France).
2001-2008 Habilitation to the function of Full Professor of Organic Chemistry of the French Universities.
Since 2002 Associate Professor and Chair of Bioorganic Chemistry of the University of Florence.
1991-2001 Researcher at the Dept. of Organic Chemistry, University of Florence.

Academic Awards and Recognitions
2009-2014 Laureate of the Chaire d'Excellence of the Agence Nationale de la Recherche (unique of chemistry out of 16 attributed in 2009).
2008 Zervas Award 2008 for outstanding contribution to peptide chemistry, 30th EPS, Helsinki.
2008 1st D. Theodoropoulos Memorial Lecture Award for outstanding achievements in Peptide Sciences, 6th Hellenic Forum on Bioactive Peptides, Patras.
2005 Bert Schram Price. Honorable Mention, 19th APS, San Diego, CA, USA.
2005 Best Poster Award "Glyco XVIII", Firenze.
1999 Bruce W. Erickson Award for outstanding achievement in peptide sciences, 16th APS, Minneapolis, Minnesota, USA.

Awards for Technology Transfer Activity
2009 Vespucci Award for founding the startup Toscana Biomarkers.
2008 Frost & Sullivan Excellence in Research Award in the European autoimmune disease diagnostics market presented to AM Papini for the startup Toscana Biomarkers.
2006 Start-Cup for the business plan of the start-up Toscana Biomarkers.
2004 "Vespucci Award, Research and Ethical Innovation" to A.M. Papini as inventor of the patent application of the University of Florence.

Activities for the scientific community
2016 Chairperson of the 2016 Gordon Research Conference "Chemistry & Biology of Peptides" with P. Dawson (The Scripps Research Institute, Departments of Cell Biology and Chemistry, La Jolla). Ventura, CA (USA), 23-28 February, 2016.
2012-2018 Panel Member for ERC Advanced Grant evaluation PE5 Synthetic chemistry and materials.
2014 Vice-chair of the GRC "Peptide Science in the Era of Synthetic Biology" Ventura, CA (USA), 23-28 February, 2014.
Since 2013 Coordinator of the International Affairs of the School of Sciences of the University of Florence.
2011-2020 Treasurer and member of the Board of the European Peptide Society.
February 2011 Member of the AERES Committee for evaluation of the ICM of the University of Reims.
March 2010 Member of the AERES Committee for evaluation of iBiTecc-S Equipe SIMOPRO (CEA, Saclay).
Since 2005 Delegate of Chemistry of the Erasmus Programme of the Faculty of Sciences of the University of Florence.
Since 2001 Founder and Coordinator of the dissemination activities of Chemistry in OpenLab at the University of Florence.

Activities aimed to strengthen technology transfer
Since 2014 Member of the Scientific Advisory of Regulaxis (www.regulaxis.com)
2007-2014 Founder & Chief Scientific Officer of the startup Toscana Biomarkers (www.toscanabiomarkers.com)
Since 2003 Founder and member of the Board of EspiKem, first spinoff of the University of Florence (www.espikem.com)

Selected publications

Papers

1. Epitope mapping of anti-myelin oligodendrocyte glycoprotein (MOG) antibodies in a mouse model of multiple sclerosis: microwave-assisted synthesis of the peptide antigens and ELISA screening.
   Pacini G, Ieronymaki M, Nuti F, Sabatino G, Larregola M, Aharoni R, Papini AM, Rovero P. J Pept Sci. (2016) 22(1), 52-8.
2. Production of peptides as generic drugs: a patent landscape of octreotide.
   Sabatino G, Guryanov I, Rombecchi A, Zanon J, Ricci A, Cabri W, Papini AM, Rovero P. Expert Opin Ther Pat. 2016 [Epub ahead of print]
3. Rett syndrome: An autoimmune disease?
   De Felice C, Leoncini S, Signorini C, Cortelazzo A, Rovero P, Durand T, Ciccoli L, Papini AM, Hayek J. Autoimmun Rev. (2016) 15(4), 411-6.
4. Multi-Stage Mass Spectrometry Analysis of Sugar-Conjugated β-Turn Structures to be Used as Probes in

Autoimmune Diseases.
Giangrande C, Auberger N, Rentier C, Papini AM, Mallet JM, Lavielle S, Vinh J. Journal of The American Society for Mass Spectrometry (2016) 27(4), 735-747.

5. Label-free method for anti-glucopeptide antibody detection in Multiple Sclerosis.
   F. Real Fernández, G. Rossi, F. Lolli, A.M. Papini, and P. Rovero. MethodsX (2015) 2, 141-144. Open Access Article.
6. Interactions between human antibodies and synthetic conformational peptide epitopes: innovative approach for electrochemical detection of biomarkers of multiple sclerosis at platinum electrodes
   W. Bellagha-Chenchaha, C. Sella, F. Real Fernandez, E. Peroni, F. Lolli, C. Amatore, L. Thouin, A.M. Papini. Electrochimica Acta, (2015) 176, 1239–1247.
7. Interaction Study of Phospholipid Membranes with an N-Glucosylated β-Turn Peptide Structure Detecting Autoantibodies Biomarkers of Multiple Sclerosis.
   Becucci L, Benci S, Nuti F, Real-Fernandez F, Vaezi Z, Stella L, Venanzi M, Rovero P, Papini AM. Membranes (Basel). 2015 Sep 30;5(4):576-96.
8. Antibody recognition in multiple sclerosis and Rett syndrome using a collection of linear and cyclic N-glucosylated antigenic probes.
   Real Fernández F, Di Pisa M, Rossi G, Auberger N, Lequin O, Larregola M, Benchohra A, Mansuy C, Chassaing G, Lolli F, Hayek J, Lavielle S, Rovero P, Mallet JM, Papini AM. Biopolymers (2015) Sep;104(5):560-76.
9. SERS study of a tetrapeptide based on histidine and glycine residues, adsorbed on copper/silver colloidal nanoparticles.
   C. Gellini, G. Sabatino, A.M. Papini, and M. Muniz-Miranda. J.Raman Spectrosc. (2014), 45(6), 418-423.
10. Alpha actinin is specifically recognized by multiple sclerosis autoantibodies isolated using an N-glucosylated peptide epitope.
    S. Pandey, I. Dioni, D. Lambardi, F. Real-Fernandez, E. Peroni, G. Pacini, F. Lolli, R. Seraglia, A.M. Papini, P. Rovero. Mol.Cell.Proteomics. (2013) 12(2), 277-282.
11. Immune dysfunction in Rett syndrome patients revealed by high levels of serum anti-N(Glc) IgM antibody fraction.
    A.M. Papini, F. Nuti, F. Real-Fernandez, G. Rossi, C. Tiberi, G. Sabatino, S. Pandey, S. Leoncini, C. Signorini, A. Pecorelli, R. Guerranti, S. Lavielle, L. Ciccoli, P. Rovero, C. De Felice, and J. Hayek. J.Immunol.Res. (2014)
12. Evaluation of new immunological targets in neuromyelitis optica.
    J.-B. Chanson, I. Paolini, N. Collongues, M.C. Alcaro, F. Blanc, F. Barbetti, M. Fleury, E. Peroni, P. Rovero, G. Rudolf, F. Lolli, E. Trifilieff, A.M. Papini, J. de Seze. J.Pept.Sci. (2013) 19(1), 25–32.
13. Divergent and convergent synthesis of polymannosylated dibranched antigenic peptide of the immunodominant epitope MBP(83-99).
    Friligou, F. Rizzolo, F. Nuti, T. Tselios, M. Evangelidou, M. Emmanouil, M. Karamita, J. Matsoukas, M. Chelli, P. Rovero, A.M. Papini. Bioorg.Med.Chem. (2013) 21, 6718-6725.

Review

Novel diagnostic tools and solutions for multiple sclerosis treatment: a patent review (2009 - 2014).
Papini AM, König E. Expert Opin Ther Pat. (2015) 25(8):873-84.

Research Support.

Major Grants Responsibility

France

2016-2018	Lauréate MRSEI-ANR Edition 2015. Project REDIPOC (Reliable Diagnostic Point Of Care Network). 20.000 Euro- PI
2013-2016	Thèse CIFRE in cooperation with REGULAXIS, 90.000 Euro
2015-2018	BIRAC, Indo-French Challenge-oriented Call for proposals 2014 : AMIR-PepKit IVD, R&D of a peptide-based-diagnostic kit for for an early detection of a fatal immune response in acute myocardial infarction patients. 50.000 Euro- Co-Investigator
2011-2013	Fondation Pierre-Gilles de Gennes. Immune-mediated diseases: Glycopeptidomic-based diagnostics to follow-up disease activity. 2 post-doctoral fellows + 90.000 Euro for materials.- Co-Investigator
2009-2014	Chaire d'Excellence (ANR-09-CEXC-013-01). Project PepKit: Development of peptide-based-diagnostic kits for autoimmune diseases. 999.998,00 Euro for PeptLab@UCP platform of the UCP.- PI

Team 2:

Principal Investigator:

NAME	Shlomo
Surname	Yitzchaik
Administrative institution	The Hebrew University
Research laboratory/unit	Institute of Chemistry
Address	Safra Campus, 91904 Jerusalem, Israel
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Participants to the programme

Name, first name Date of birth	Title and degrees	Institution	Percent of research time on the project
Yitzchaik, Shlomo 18/08/1961	Full Professor (PhD)	Chemistry	30%
Gilon, Chaim 28/09/1937	Professor Emeritus (PhD)	Chemistry	20%
Hoffman, Amnon 03/06/1946	Full Professor (PhD)	Pharmacy	20%
Domb, Avi 22/04/1952	Full Professor (PhD)	Pharmacy	20%
Balogh, Dora 20/07/1982	Postdoc (PhD)	Chemistry	100%
Schumacher, Adi 11/02/1980	Postdoc (PhD)	Pharmacy	100%

Biographical sketch of the principal investigator.

Education, training			
Institution and location	Degree	Year	Field of study
Northwestern University, USA	Post-doc.	1992-1995	Chromophoric superlattices
Weizmann Inst. of Science, IL	Ph. D. Chem.	1987-1991	Photochromic mesophases
The Hebrew University, IL	M. Sc. Chem.	1984-1986	Biomedical polymers
The Hebrew University, IL	B. Sc.	1981-1983	Plant Genetics

Positions and Honors.

Date	Degree	University/Faculty	Field

2013 - present	Full Professor	HUJI/Natural Sciences	Chemistry
March – May 2010	Visiting Professor	Chalmers University Göteborg, Sweden	Nanoscience
2006 - 2012	Associate Professor	HUJI/Natural Sciences	Chemistry
2002 - 2005	Senior Lecturer	HUJI/Natural Sciences	Chemistry
1996 - 2001	Lecturer	HUJI/Natural Sciences	Chemistry

AWARDS and HONORS

2010: *The Erasmus Mundus Lecturer – Neuroelectronic interfaces* - The European Commission: Nanotechnology in Modern Society (EMM-nano).
2009: The Gesellschaft Deutscher Chemiker (German Chemical Society) award with the *Richard-Willstätter-Lecture*.
2002: Applied Materials Research Excellence Prize for outstanding achievements in the field of surface chemistry science and technology (IVS, Israel Vacuum Society).
2002: The Kaye Innovation Award at the Hebrew University of Jerusalem for the invention of Molecular Layer Epitaxy (MLE).
2000: The Braun-Roger-Siegel Foundation prize (by the Israel Science Foundation, ISF) for the research achievements in the field of organic nano-transistors.
1999: *The Outstanding Young Scientist Award of the Israel Chemical Society* (ICS) – Awarded for the achievements in materials science, surface chemistry research, and the investigation of the interactions of light with mater and their implementation in novel electro-optic devices.
1999: The John van Geuns Lectureship - on the interaction of light with mater.

Selected publications

1. E. Amit, O. Rofeamor, Y-T. Wang, R. Zhuravel, A.J.F. Reyes, S. Elbaz, D. Rotem, D. Porath, A. Friedler, Yu-Ju Chen, **S. Yitzchaik**
   Integrating proteomics with electrochemistry for identifying kinase biomarkers
   Chem. Sci., **2015**, 6, 4756 – 4766.
2. E. Snir, E. Amit, A. Friedler, **S. Yitzchaik**
   A highly Sensitive Square Wave Voltammetry based Biosensor for Kinase Activity Measurements
   Biopolymers **2015**, 104, 515-520.
3. R. Sfez, E. Natan, Y. Bardavid, M. Ikbal, E. Arbeli, S. Arkin, I. Popov and **S. Yitzchaik**
   Enzyme Mediated Encapsulation of Gold Nanoparticles by Polyaniline Nanoshell
   Journal of Self-Assembly and Molecular Electronics **2015**, 3, 1-16.

Research Support.

2008-2011: EC 7th Framework (STREP)*:* **Brain Storm**. "*On-chip simultaneous intracellular recording and stimulation of electrical and biochemical activities from hundreds of neurons.*" Partners: M. Spira and J. Shappir of the Hebrew Univ., S. Borges of IMEC-Belgium, and P. Timmerman of Pepscan-The Netherlands.

2012-2015: Ministry of Sciences and Technology (MOST), *"Nano-composites for direct light induced cell stimulation"* Partners: Prof. Y. Hanein and Prof. O. Cheshnovsky (TAU) and Prof. U. Banin (HUJ).

2012-2015: Academia Sinica – Hebrew University *"Using nanotechnology for developing kinase inhibitors as anti-cancer leads"* partners: Assaf Friedler [HUJI], Danny Porath [HUJI], and Yu-Ju Chen [AS].

2012-2017: The International Nano-Science and Technology Advisory Board (INAB) *FTA program: "Hybrid Nanomaterials and Formulations for Functional Coatings and Printed Devices"*

2013-2016: EC 7th Framework (STREP)*:* **SYMONE**. "*Synaptic Molecular Networks for Bio-inspired Information Processing*"; G. Wendin (Chalmers, Sweden), D. Vuillaume, (CNRS-IEMN, France), G. Cuniberti, (TUD, Germany), C. Gamrat (CEA, France) M. Calame, (UNIBAS, Switzerland), V. Beiu, UAEU (UAE).

2015-2018: EC 8th Framework (H2020): **RECORD-IT**. "*Reservoir Computing with Real-time Data for future IT*" Z. Konkoli (Chalmers, Sweden), D. Vuillaume (CNRS-IEMN, France), R. Gutierrez (TUD, Germany), C. Gamrat (CEA, France), M. Calame

(UNIBAS, Switzerland), K. Szaciłowski, (AGH, Poland), Z. Crljen (RBI, Croatia).

Team 3:

Principal Investigator:

NAME	Karussis
Surname	Dimitrios
Administrative institution	Hadassah HMO-Hebrew Univrrsity
Research laboratory/unit	Neurology/Neuroimmunology
Address	Ein Karem , Jerusalem
email	dimitriosk@ekmd.huji.ac.il
Phone number	+972-2-6776939
Fax number	+972-2-6246741

Participants to the programme

Name, first name Date of birth	Title and degrees	Institution	Percent of research time on the project
Dr Ibrahim Kassis 23/05/1966	PhD	Hadassah HMO-Hebrew University	50 %
Moriel Ben-Zwi 17/07/1980	PhD Student	Hadassah HMO-Hebrew University	30 %
Dr Panayiota Petrou 01/03/1971	MD	Hadassah HMO-Hebrew University	10 %

Biographical sketch of the principal investigator.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Dimitrios **Karussis**	Full Professor of Neurology, Hebrew University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*			
INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Aristotelion University Medical School Thessaloniki, Greece	M.D.	1980-1986	Medicine
Hadassah University Medical School	Ph.D.	1990-1994	Neurobiology (Immunology)

Positions
1990-1995 – Neurology residency in the Department of Neurology, Hadassah University Hospital.
1995 – Board certification in Neurology
1995-present - Senior Neurologist in the Department of Neurology, Hadassah University Hospital
1998 - **Senior lecturer in Neurology**, The Hebrew University, Faculty of Medicine
2003 - **Associate Professor of Neurology**, The Hebrew University, Faculty of Medicine.
2013 - **Full Professor of Neurology**, The Hebrew University, Faculty of Medicine.
2007 - today - Chairman of Multiple Sclerosis Center, Hadassah
2010-today: **President of the Israeli Neuroimmunological Society**
2015-present**: Director of the Hadassah Unit of Neuroimmunology and cell therapies**
2016**: President of the forthcoming world congress of Neuroimmunology in Jerusalem (International society of Neuroimmunology)**

Activities
2000- today: Member of teaching committees in Neurology in the Faculty of Medicine of the Hebrew University.
1993-today: Member of the American Academy of Neurology
2000-today: Member of the Executive and **Scientific Committee of the European School of Neuroimmunology (ESNI)**
2006-2010: **Member of the Executive Committee of the European ECTRIMS association for multiple sclerosis**
2004-today (various years): Member of the Scientific Committee of the ECTRIMS, EFNS and ISNI
2005-today: **Chairman of the Scientific Committee of the Israeli MS Society**
2008-today: Member of the **International Steering Committee for the use of stem cells in MS**
President of the meeting) the forthcoming International ISNI meeting in Jerusalem, 2016.

Member of editorial boards in medical journals:
2009-today: Member of the Editorial Board of the journal: "International Multiple Sclerosis"
2011-today: Member of the Editorial Board (Associate editor) of the journal: "Journal of Neurological Sciences"
2012-today: Member of the Editorial Board (Associate editor) of the journal: "World Journal of Neurology"

Research Grants and recent projects:
1994-1995: The Chief Scientist of Ministry of Health grant
1998: The Harry Stern Center for research in Alzheimer's disease, grant.
2002: The National Institute of Neuropsychobiology Grant
1995-today: Various Grants from TEVA company, BRAINSTORM, PHARMACIA, BIOGEN and SCHERING for research of immunomodulating treatments in MS (Linomide etc) (total 500,000 USD)
2009: Research fellowship grant from the **European Committee of ECTRIMS**

2010: BrainStorm Therapeutics Grant for the ALS MSC studies: 1,500,000 USD
2012: Gathy Foundation competitive grant for NMO research: 96,000 USD (with Dr Vaknin) (pending)
2012: **ISF (Israel Science Foundation**) Grant: Novel therapeutic modalities for the treatment of Multiple sclerosis (MS): a multi-Disciplinary approach (Total: 2,000,000 IS, for 4 investigators)
2015: BrainStorm Therapeutics Grant for the ALS MSC studies: 1,000,000 USD

International Patents

1. International patent for the use of "Linomide" in multiple sclerosis (US patent: 5,580,882)
2. Patent for use of farnesyl-thiosalycylic acid (FTS) in EAE and multiple sclerosis (pending).
3. Patent for neurospheres produced by mesenchymal stem cells (pending)

Publications (a very short list out of a total of more than 110, peer-reviewed papers)

-

1. **Dimitrios Karussis**. Immunotherapy of multiple sclerosis: The state of the art. ***Biodrugs*** (2013) Apr;27(2):113-48.
2. **Karussis D,** Petrou, P. The spectrum of post-vaccination demyelinating CNS syndromes. ***Autoimmunity Reviews*** (2013), Mar;13(3):215-2.
3. Brill, L., Karni, A., Petrou, P., Ben-Hur, T., Abramsky, O., **Karussis, D**., Vaknin Dembinsky, A. Anti Kir4.1 antibodies in MS and NMO: A biomarker for relapse ? . ***Multiple sclerosis***. (2014). Apr;21(5):572-9.
4. **Karussis, D**., Petrou, P., Vourka-Karussis, U., Kassis, I. Hematopoietic stem cell transplantation in Multiple sclerosis. (Invited Review). ***Expert Reviews in Neurotherapeutics***, 2013, May;13(5):567-78.
5. Vaknin-Dembinsky, A., **Karussis, D**., Petrou, P., Ben-Hur, T., Abramsky, O., and A. Lossos. Tumefactive demyelination following in vitro fertilization treatment. ***J. Neurol. Sci.*** (2015) Jan 15;348(1-2):256-8.
6. Vaknin-Dembinsky, A., **Karussis, D**., Abramsky, O. NMO spectrum of diseases: A paradigm of astrocytes targeting autoimmunity and its implications for MS, NMO and other demyelinating CNS diseases. ***J. Autoimmunity***. (2014). Nov;54:93-9.
7. Charbit H, Benis A, Geyshis B, **Karussis D,** Petrou P, Vaknin-Dembinsky A, Lavon I. Sex-specific prediction of interferon beta therapy response in relapsing-remitting multiple sclerosis. ***J Clin Neurosci***. 2015, Jun;22(6):986-9.
8. Petrou, P, Gothelf, Y, Argov, Z, Gotkine, M, Levi, Y, Kassis, I, Vaknin Dembinsky, A, Ben Hur, T, Offen, D, Abramsky, O, Melamed, E, and **Karussis, D**. Safety and clinical effects of mesenchymal stem cells secreting neurotrophic factors transplantation in patients with Amyotrophic Lateral Sclerosis: results of a phase 1/2 and 2a clinical trial ***JAMA Neurology*** (in press).

SUMMARY

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by loss of motor and sensory function, caused by immune-mediated inflammation, demyelination, and subsequent neuronal/axonal damage [1,2,3]. Current treatments for MS are only partially effective and one of the most active areas of research in MS treatment is remyelinating therapies, which can be categorized either as regenerative therapy (aimed to restore myelin) or neuroprotective therapy (aimed to protect axons and restore nerve conduction).[4]
Currently among the 29 neuroprotective drugs in current clinical development for MS, only 4 of them are drugs aimed to remyelination. In this context a range of neurotrophic and neuroprotective peptides has been suggested to restore neuronal function, improve behavioral deficits and prolong the survival in animal models of neurodegenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis. Among them are the peptides NAP, Humanin and its analog Colivelin. The neuroprotective ability of both peptides has been demonstrated by their intranasal administration to animal models.
Neuropeptides therefore represent a rapidly-expanding class of promising new CNS drugs. Unfortunately, delivery of neuropeptides to the brain has been a major obstacle in their therapeutic development. Nevertheless interestingly, in humans, within the nasal cavity the olfactory epithelium contains olfactory neurons that are the

only nerves in direct contact with the external environment. This unique neuroanatomical relationship presents a means to limit metabolism and bypass the blood brain barrier to enhance access to olfactory neuronal transport mechanisms to target the CNS. Therefore the development of new surface-eroding biodegradable nanoparticle (NP) carriers could provide a means to better exploit this nose-to-brain pathway. To this aim we have developed a method for the preparation of peptide-nanoparticles that allow intranasal controlled delivery and sustained release of peptides to the brain.

In this context, the present proposal is aimed to develop innovative technologies, based on neuroprotective and neurotrophic peptides, for novel regenerative therapies, especially remyelination, as new avenue for treating MS.

A novel and metabolically stable chimeric neuropeptide, called MYRP-1, will be therefore synthesized and its potential for promoting remyelination in the animal models of MS will be tested, utilizing the intranasal way of administration. In parallel an in vivo assay for myelin repair will be set up and NPs of both MYRP-1 and its radiolabeled analog ^{125}I-MYRP-1 will be prepared. Finally the ability of MYRP-1 and NP-MYRP-1 to promote remyelination will be tested in vivo after nasal administration.

SUMMARY IN FRENCH FOR A LAY AUDIENCE

La sclérose en plaques (SEP) est une maladie chronique du système nerveux central caractérisée par une perte des fonctions motrice et sensorielle. Les traitements actuels de la SEP ne sont que partiellement efficaces, surtout pour favoriser la neuroprotection et la remyélinisation, deux besoins qui ne sont pas encore satisfaits. Une série de peptides neurotrophes et neuroprotecteurs (NAP, Humanine et Coliveline) ont montré des propriétés de restauration de la fonction neuronale dans des modèles animaux de maladies neurodégénératives. C'est pourquoi l'objectif de ce projet est le développement de technologies innovantes, basées sur des peptides neuroprotecteurs et neurotrophes, pour des nouvelles thérapies régénératives, en particulier pour la remyélinisation comme nouvel axe de traitement de la SEP. Dans ce cadre, un nouveau neuropeptide chimère métaboliquement stable sera synthétisé et son potentiel à promouvoir la remyélinisation par administration intranasale sera testé dans des modèles animaux de SEP. Ceci sera possible grâce aux nanoparticules cargo innovantes, biodégradables par érosion de surface, développées par l'une des équipes impliquées dans la présente proposition.

SUMMARY IN ENGLISH FOR A LAY AUDIENCE

Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by loss of motor and sensory function. Current treatments for MS are only partially effective in particular in promoting neuroprotection and remyelination which remain yet unmet needs. A range of neurotrophic and neuroprotective peptides (NAP, Humanin and Colivelin) has been suggested to restore neuronal function in animal models of neurodegenerative diseases. Therefore aim of this project is the development of innovative technologies, based on neuroprotective and neurotrophic peptides, for novel regenerative therapies, especially remyelination, as new avenue for treating MS. To this aim a novel and metabolically stable chimeric neuropeptide, will be synthesized and its potential for promoting remyelination in the animal models of MS will be tested utilizing the intranasal way of administration This will be possible thanks to innovative surface-eroding biodegradable nanoparticle carriers developed by one of the team involved into the present proposal.

DESCRIPTION OF THE RESEARCH PROJECT (8 PAGES MAXIMUM)

For clinical trials, add the entire protocol.

Introduction and preliminary results

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by loss of motor and sensory function, caused by immune-mediated inflammation, demyelination, and subsequent neuronal/axonal damage[5,6,7]. Clinically, most MS patients experience recurrent episodes (relapses) of neurological impairment (relapsing-remitting MS). Early neurological dysfunction may resolve spontaneously, partially or completely, but usually the course of the disease becomes chronic and progressive (primary or secondary progressive MS), leading to accumulating motor disability and cognitive deficits.
Histologically, perivenular inflammatory lesions involving infiltrating mononuclear cells are evident in the earlier phases of the disease, resulting in demyelinating plaques which are the hallmark pathological feature of MS[2]. Inflammation leads to damage or loss of oligodendrocytes and demyelination leads to disruption of the conduction of neuronal signals in the affected regions. In the initial stages of MS, compensatory pathways (such as the upregulation of ion-channels in the affected areas) may partially restore conduction and reverse the neurological dysfunction. As the disease progresses, significant axonal loss and eventually neuronal damage occurs[4] and the lost function becomes permanent and non-reversible.
Current treatments for MS are only partially effective, probably due to inadequate control of self-reactive T cells and/or defective remyelinating/regenerating mechanisms, resulting in chronic, accumulating disability and irreversible axonal/neuronal damage[1,2]. Additionally, it seems that the currently used immunotherapies in MS cannot exert significant in-situ immunomodulating effects in the CNS. It has been suggested that in MS two pathogenetic processes run in parallel: an inflammatory one (with clinical relapses, gadolinium-enhancing lesions in the MRI and prompt response to immunomodulation), and a degenerative phase (with less relapses, less inflammatory activity in MRI and poor response to immunotherapies), which is probably the result of chronic neuroinflammation. It is therefore obvious that, in order to improve treatment outcome in MS, innovative approaches are required for more effective in situ immune regulation into the CNS rather than non-selective immunosuppression, as well as therapeutic interventions which may promote neuroprotection and remyelination, which remain yet unmet needs.[8]
In this context within the present proposal we will develop technologies, based on neuroprotective and neurotrophic peptides, for novel regenerative therapies, especially remyelination, as new avenue for treating MS.
At present there is a growing list of new drugs and repurposing of drugs being tested from phase 1 to phase 3 trials.[9] One of the most active areas of research is remyelinating therapies, which can be categorized either as regenerative therapy (aimed to restore myelin) or neuroprotective therapy (aimed to protect axons and restore nerve conduction).[10] Among the 29 neuroprotective drugs in current clinical development for MS, only 4 of them are drugs aimed to remyelination. Two of them are monoclonal antibodies (mAbs) and two are small molecules.
A recent trial testing the mAb blocking Lingo-1 (BIIB033) has shown improvement in the latencies of the visual evoked potentials in patients with optic neuritis, suggesting an improvement of nerve conduction typically associated with remyelination (NCT01721161). Another mAb promoting remyelination is rHIgM22, which was discovered as part of the natural antibody repertoire in humans with remyelinating potential[11] and it is now being tested in clinical trials in MS patients (NCT01803867, NCT02398461). Regarding small molecules, two approved drugs that are being explored for their effect on remyelination (after having shown a remyelinating effect in vitro and animal model) are *Clemastine* and *Guanabenz*[12].
The demonstration that the onset and progression of neurodegenerative diseases in models of transgenic mice, is delayed or improved by the application of neurotrophic factors and peptides derived from neurotrophic factors, has emphasized their importance in neurorestoration. Therefore a range of neurotrophic and neuroprotective peptides has been suggested to restore neuronal function, improve behavioral deficits and prolong the survival in animal models of neurodegenerative diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). Among them are the peptides NAP, Humanin and its analog *Colivelin*. The neuroprotective ability of both peptides was demonstrated by their intranasal administration to animal models.

NAP
The growth factor called activity dependent neurotrophic protein (ANAP) is released by glial cells. It contains within it an eight amino acids peptide (NAPVSIPQ) called NAP. This peptide has highly potent neuroprotective activity. Recent investigations are supporting the rationale for its therapeutic potential range from in-vitro neuroprotection against Aβ, viruses, and oxidative stress to neuroprotection in rodent models of stroke and hypoxia. NAP is thought to modulate the pool of microtubules in neurons, but its exact mechanism of action is not yet clear. Inhibition of programmed cell death and correction of mitochondrial dysfunction also have been proposed as potential mechanisms. NAP was shown to have a brain bioavailability after intranasal administration.[13] The intranasal preparation of NAP, called Davunetide was tested in humans in phase 2/3 on the following indications: Mild Cognitive Impairment, Progressive Supranuclear Palsy, Schizophrenia, and Frontotemporal Dementia. Davunetide failed to get FDA approval for the above indications.[14] To our opinion the FDA failure is due to the metabolic instability of Davunetide.

Humanin

Table 1: names, sequence and in-vitro neuroprotection activity of Humanin and its analogs

Name	Sequence	EC_{50} Neuro Protection
HN	MAPRGASCLLLLT**S**^{14}EIDLPVKRRA	10 µM
HNG17	PAGASRLLLLT**G**^{14}EIDLP	10 nM
Colivelin*	SALLRSIPAPAGASRLLLLTGEIDLP	10 fM

* Colivelin is a synthetic hybrid peptide composed of ADNF (activity-dependent neurotrophic factor) and HNG17.

Colivelin is a highly potent derivative of Humanin (HN) with anti-Alzheimer's disease activity. Nasal Colivelin treatment ameliorates memory impairment related to Alzheimer's disease in AD models.[15] Colivelin prolongs survival of an ALS model mouse by intravenous (IV) injection.[16] Moreover, Humanin cause increased oligodendrogenesis and promotes axonal remyelination and neurological recovery in hypoxic/ischemic brains.[17] Pharmacokinetics and tissue distribution studies of Humanin and its analogues in male rodents after intraperitoneal (ip) injection indicates varying kinetic profiles of HN and analogues. Sustainable levels of circulating HN analogs were measured in plasma.[18] On the other hand, in vivo biodistribution studies of HN and its analogs using ^{125}IHumanin derivatives after IV injection, indicates fast metabolism of HN (T1/2 of 5-7 min.) with increased metabolic stability of HNG17 and Colivelin.[19]

Neuropeptides represent therefore a rapidly-expanding class of promising new CNS drugs. Unfortunately, delivery of neuropeptides to the brain has been a major obstacle in their therapeutic development. Rapid metabolism in all tissue compartments and a lack of blood brain barrier (BBB) penetration are two daunting issues related to neuropeptide bioavailability. Interestingly, in humans, within the nasal cavity the olfactory epithelium contains olfactory neurons that are the only nerves in direct contact with the external environment. This unique neuroanatomical relationship presents a means to limit metabolism and bypass the BBB to enhance access to olfactory neuronal transport mechanisms to target the CNS. Three major barriers to neuropeptide bioavailability exist in this region: presence of tight junctions between sensory neurons and supporting cells, preventing epithelial transport to the submucous space; a mucous layer containing protective proteolytic/hydrolytic enzymes that impart an enzymatic barrier, and mucous layer clearance that influences time-dependent neuropeptide absorption (uptake). Following olfactory neuroepithelium uptake, neuropeptides are susceptible to further degradation during transneuronal transport as they are carried to primary olfactory structures. Next, sufficient sustained neuropeptide release is necessary for a pharmacological effect. The development of new surface-eroding biodegradable nanoparticle (NP) carriers could provide a means of mitigating these impediments to better exploit this nose-to-brain pathway. Team 2 developed a method for the preparation of peptide-nanoparticles that allow intranasal controlled delivery and sustained release of peptides to the brain. The method is shown graphically in Figure 1A and detailed in the appendix 5a. The rate of peptide release from the NP-TRH (Thyrotropin Releasing Hormone) particles in water is shown in Figure 1B.

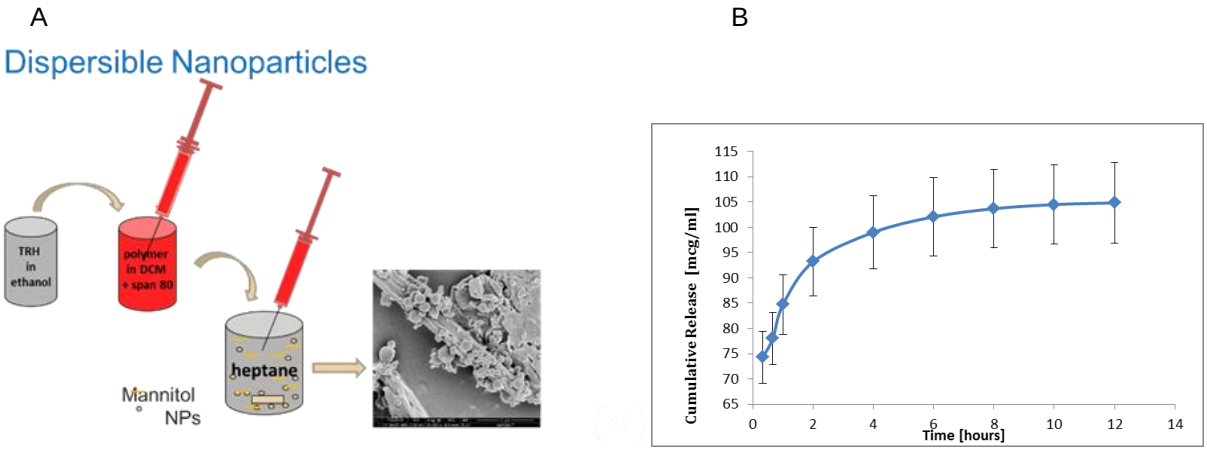


Figure 1: A. *Method for the preparation of NPs for intranasal delivery of peptides;* **B.** *Rate of TRH release from*

prepared PSA (Poly Sebacic Acid) NPs in double distilled water in shaker with 30 rpm at 37°C. (Theoretical loading 50µg/mg NPs; see appendix 5a and 5b).

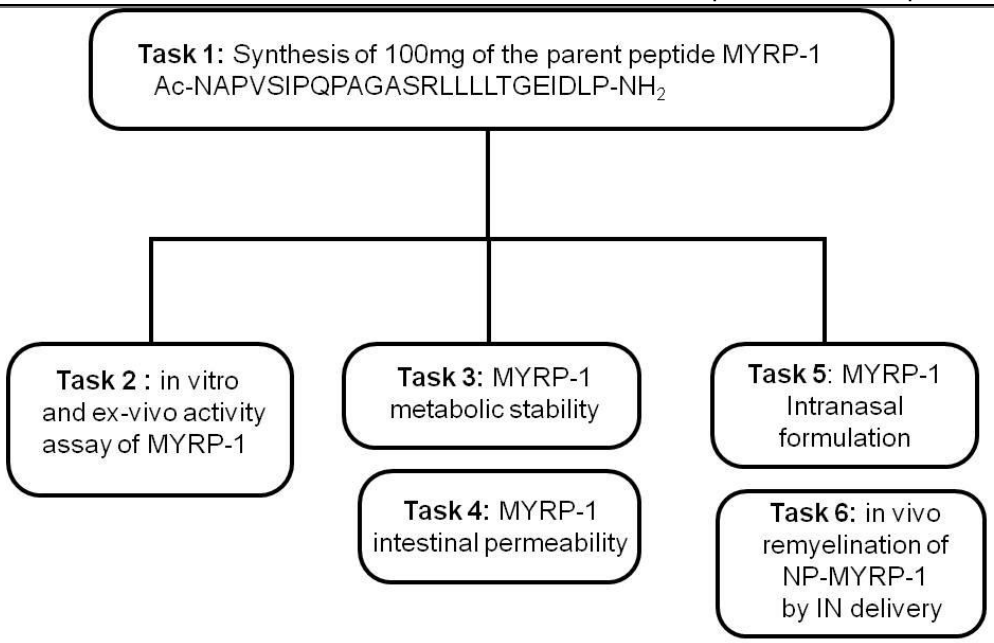
In order to evaluate this delivery approach, a rat model of temporal lobe epilepsy (kindling) has been used to demonstrate intranasal administration of the TRH-loaded nanoparticles (TRH-NPs). As we have demonstrated our TRH-NPs could release sufficient TRH at the seizure focus to impede kindling development (seizureogenesis).[20] Additionally, we utilized intranasal delivery of fluorescent dye-loaded NPs in rats and application of dye-loaded or dye-attached NPs to cortical neurons in culture to demonstrate NP uptake and distribution over time in vivo and in vitro respectively[5] . Moreover, an NP immunostaining method has been developed to directly visualize the tissue density and distribution of TRH-NPs. Taken together; our data provides further proof of concept for intranasal administration of neuropeptide-loaded NPs as a feasible and novel delivery platform for neuropeptide CNS therapeutics.
In summary, the data described by us and others[5] provides proof of concept for intranasal nanoparticle delivery of clinically relevant neuropeptides, where the target receptors are concentrated, particularly in the forebrain, and the therapeutic effect is directly related to CNS function, such as in affective illness, PTSD (posttraumatic stress disorder), PTE (potentially traumatic experiences), and neural recovery. Moreover, our results show that this approach increases transport through the BBB and that release of neuropeptides/drugs at specific sites can be achieved using intranasal delivery of biodegradable neuropeptide-loaded nanoparticles to induce a rapid and sustained therapeutic effect.

Interim conclusions

1.The remyelinating effects of both peptides HNG17 and NAP have not been tested yet in models of demyelination (such as in MS models).[10,21]
2. There is currently no description of any chimeric peptide of NAP and HNG17, published or patented.
3. Metabolic stability plays a crucial role in the conversion of peptides into drugs.
4. Nasal delivery seems to be the optimal method for delivery of peptides to the brain.

Aims

<u>The final aim of this proposal is therefore to develop a novel and metabolically stable chimeric neuropeptide and to test its potential for promoting remyelination in the animal models of MS, utilizing the intranasal way of administration.</u>
Based on the interim conclusions we will synthesize a chimera peptide of NAP and HNNG17 called MYRP-1 (Myelin Repair Peptide 1) having the sequence Ac-YNAPVSIPQPAGASRLLLLTGEIDLO-NH_2. The peptide is blocked in both the amino and carboxy ends to enhance metabolic stability by preventing degradation by amino and carboxy peptidases. The amino acid Tyr is added to allow iodination by ^{125}Iodine essential for the determination of the peptide distribution in the brain. In accord with pharmacologic rational described above MYRP-1 shall need to exhibit "drug like" properties (DLP) that would assure the optimal input into the site of action (biophase) at the CNS following intranasal administration. The required DLP are extended metabolic stability, both at the site of administration and in subsequent biological fluid and tissues. In addition, their membrane permeability properties have to be revealed in order to understand their distribution kinetics into the biophase and other body organs and fluids. In addition, we plan to evaluate their penetration into intracellular site of action and optimize it by chemical modifications. After establishing the metabolic stability of MYRP-1 and its biodistribution in in-vitro and ex-vivo assays we will determine the bio distribution of ^{125}I-MYRP-1 in animals by direct nasal administration. In parallel we will set up an in vivo assay for myelin repair and we will prepare NPs of both MYRP-1 and its radiolabeled analog ^{125}I-MYRP-1. Finally we will test the ability of MYRP-1 and NP-MYRP-1 to promote remyelination in vivo after nasal administration.
The research is summarized in the flow chart below and detailed in the Tasks section and Appendix.



Task 1. Peptide Synthesis (Team 1 and Team 2)
Peptides will be synthesized by the Fmoc-Solid Phase Peptide Synthesis (SPPS) methodology and purified by preparative RP-HPLC. Peptides will be characterized by analytical HPLC and MS. The first part of Task 1 involve small scale (10 mg) SPPS of MYRP-1. This part is essential since MYRP-1 is hydrophobic and contains many Pro residues. Both are known to hamper SPPS using the conventional methodologies. The initial synthesis will be followed by optimization and synthesis of 100 mg of MYRP-1. Detailed strategy is described in Appendix 1. This amount is sufficient for Tasks 2-6. Tasks 2-5 will be performed in parallel. We will also develop the synthesis of ^{125}I-MYRP-1. This analog will be prepared by iodination of MYRP-1 with ^{125}Iodine. ^{125}I-MYRP-1 is essential to determine the bio-distribution of MYRP-1 in the brain.

Task 2. Neuroimmunological characterization (Team 3)
This task involve: (1) ex-vivo immunomodulatory effects of MYRP-1; (2) in vitro remyelination effect of MYRP-1 in demyelination assay. Detailed procedures are described in Appendix 2.

Task 3. Peptide metabolic stability (Team 3)
In this task the metabolic stability of MYRP-1 will be test by various assays including Brush Border Membrane Vesicles (BBMV), serum, liver cytochromes, brain homogenate. The main degradation of the tested peptide evolves from various peptidases. Therefore to understand the metabolic stability of the compound we shall examine its stability following incubation with serum as well as with a rich peptidases medium that exists at the brush border membrane of the small intestine in a vesicular preparation ex-vivo known as BBMV model[22].The stability of the compound against oxidative metabolism will be examined by pooled liver microsomes[23] . To assure stability by other potential metabolic pathways, the compound(s) will be incubated with brain homogenate. It is expected that MYRP-1 will be metabolically stable for the following reasons: (1) both its N and C termini are blocked; (2) the peptide contains four Pro residues that are known to stabilize against degradation by most common intestinal peptidisas; (3) the peptide contain a hydrophobic helical region. All these factors hamper degradation by exo- and endo peptidases. Detailed procedures are described in Appendix 3.

Task 4. Peptide permeability (Team 3)
In this task the cellular permeability properties of MYRP-1 will be tested by a CaCO-2 model. Even though the assay is performed on human intestinal tissue (CaCO-2), it will indicate its ability of distribution in the brain. The permeability studies are routinely performed by Team 3, as described in various publication.[15,24] The active compound concentration both in-vitro and in biologic fluids will be determined by an HPLC-MS analysis. Detailed procedures are described in Appendix 4.

Task 5. Nanofabrication (Team 2)
In this task nanoparticles formulation of MYRP-1 and ^{125}I-MYRP-1 will be prepared for controlled intranasal delivery in humans. The NP-MYRP-1 and NP-^{125}I-MYRP-1 will be prepared following the method described in Appendix 5.

Task 6. Animal assays (Team 3)
In this task the effect of MYRP-1 and NP-MYRP-1 developed in Task 5 will be studied using the following two animal assays: (1) Chronic model of EAE (inflammation and degeneration model); (2) Cuprizone induced demyelination. MYRP-1 and NP-MYRP-1 will be administered intranasal (IN). Detailed procedures are described in Appendix 6. In addition pharmacokinetic studies using NP-MYRP-1 and biodistribution of ^{125}I-MYRP-1 will be performed by comparing their intravenous to IN delivery to animals.

APENDIX

1.**Synthesis, purification and characterization of new peptide MYRP-1**

- Manual parallel SPPS. This time-consuming strategy is necessary to set-up difficult synthesis steps under carefully controlled conditions. In fact, manual SPPS allows an easier monitoring of the reactions.
- Microwave assisted automated SPPS. This strategy enables the fast preparation of peptides, because coupling and deprotection reaction times are strongly reduced. Most importantly, hydrophobic peptide sequences synthesis and/or incorporation of difficult residues are extremely effective compared to the traditional methods. Secondary structure disruption is in fact mediated by microwaves. Moreover, by keeping the synthesis cycles short, repetition of the synthesis can lead to gram scale peptide production in short time.
- Pre-purification steps by gel-filtration and desalting of crude peptides. Pre-purification of crude peptides will be performed on small-scale silica cartridges, or on large scale by automatic solid-phase extractions. This approach will be instrumental to facilitate the chromatographic purification (>98%) of the material to be used in tasks 2-6 .
- Chromatographic purification. A high purity level of the peptide drug candidate for promoting remyelination in multiple sclerosis is fundamental for their in vivo application. Accordingly, semi-preparative reverse-phase chromatographic methods will be set-up using a fully automated instrument in which sample collection is driven by a UV detector.
- Analytical characterization. A thorough analytical characterization of the >98% purified peptides is fundamental. Two techniques will be routinely used both for peptide synthesis monitoring and analytical characterization: reverse-phase chromatography and/or mass spectrometry. Moreover, an efficient UPLC-MS system (Ultra Performance Liquid Chromatography coupled to ESI-MS) will be also used to significantly reduce analysis time for the full characterization of the synthesized peptide mimics.

2a. Proliferation of ex-vivo lymphocytes (immunomodulatory Effects of the peptide)
The immunomodulatory effects of the peptide will be tested by the exposure of draining lymph nodes isolated from C57bl mice to the peptide. Briefly this procedure will be as following: Draining lymph nodes will be excised from C57BL mice on day 10 post MOG-immunization for EAE induction and cultured as single-cell suspensions. Lymph node cell (LNC) proliferation will be assayed in vitro by ^{3}H-thymidine incorporation. All cultures will be carried out in triplicate in 96-well, flat-bottom, and microtiter plates. The assay is performed by seeding 4 x 10^5 cells/well in 0.2 mL of RPMI medium supplemented with 2.5% FCS, 1mM L-glutamine, and antibiotics. Basal ^{3}H-thymidine incorporation is determined in response to purified MOG 35-55 (10 µg/mL) or Phytohaemagglutinin (PHA,1µg/mL). The cultures will be incubated for 48 hours in a humidified atmosphere of 5% carbon dioxide at 37°C and then pulsed for 16 hours with ^{3}H-thymidine (1µCi/well). Cells are harvested on fiberglass filters using a multiharvester and the radioactivity will be counted.

2b. Demyelination assay in vitro
There are various models to study the demyelination / remyelination process. Several culture conditions and cell types and demyelinating agents can be used. The chosen assay in our lab will involve the co-culture of primary motor neuron or PC12 cells with Schwann cells. Co-culture systems composed of both neurons and myelinating glia should provide insights into the interaction between the glial cells and neurons/axons during the destruction and re-building of the myelin sheaths. Previous studies have described such successful co-culture systems of oligodendrocytes and hippocampal neurons, SCs and sensory neurons (from dorsal root ganglia), or oligodendrocytes and retinal ganglion cells. As demyelinating agents we will choose one of the following: Proinflmattory agents (TNF-alpha / IFN-gamma), Ceramide, pyrogallol or Kainate. The myelin formation will be tested by Immunofluorescence and electronic microscopy.

3. Enzymatic degradation of peptides
The enzymatic reaction is performed similar to what is described in (Ovadia 2010)[24]: 2mM stock solutions of the tested compounds are diluted with serum or purified brush border membrane vesicles (BBMVs) solution to a final concentration of 0.5 mM. During incubation at 37ºC samples are taken for a period of 90 minutes. The enzymatic reaction is stopped by adding 1:1 v/v of ice cold acetonitrile and centrifuge (4000g, 10 min) before analysis.

The BBMVs are prepared from combined duodenum, jejunum, and upper ileum (male Wistar rats) by a Ca++ precipitation method.[25] Purification of the BBMVs is assayed using GGT, LAP and alkaline phosphatase as membrane enzyme markers.

4. **Assessment of the permeability properties of peptides (exemplified by backbone-cyclic hexapeptide named 4x4MyDI)**

The investigation of the permeability of 4x4MyDI through CaCO-2 monolayer includes using two main control groups, Atenolol and Metoprolol that show passive para-cellular and trans-cellular permeability, respectively. The CaCO-2 model allows investigating the mechanism of absorption, so the permeability from apical to basolateral membranes (AB) can be measured to evaluate the total permeability. The permeability from basolateral to apical membranes (BA) can also be measured and compared to AB P-apparent (P_{app}) to determine if the absorption is though passive diffusion, active transporters or if there is involvement of efflux systems. As shown in figure 2, the P_{app} value of 4x4MyDI -AB is higher than metoprolol so the permeability of 4x4MyDI is surprisingly high in comparison to metoprolol, suggesting trans-cellular permeability mechanism. Moreover, the BA P_{app} value is also high, suggesting the involvement of efflux system that transports 4x4MyDI from the cytosol through the apical membrane to the lumen.

The Parallel Artificial Membrane Permeability Assay (PAMPA) is another permeability model that is used for investigating the permeability mechanism of new molecules. This model does not include cells, so it is used to evaluate the permeability of molecules through a lipid layer by passive diffusion. The results shown in Figure 2 emphasize that 4x4MyDI does not diffuse well through a lipid layer, in comparison to metoprolol (standard compound for passive diffusion). This result suggests that the mechanism of permeability of 4x4MyDI involves transport system that exists in cellular models only.

A B

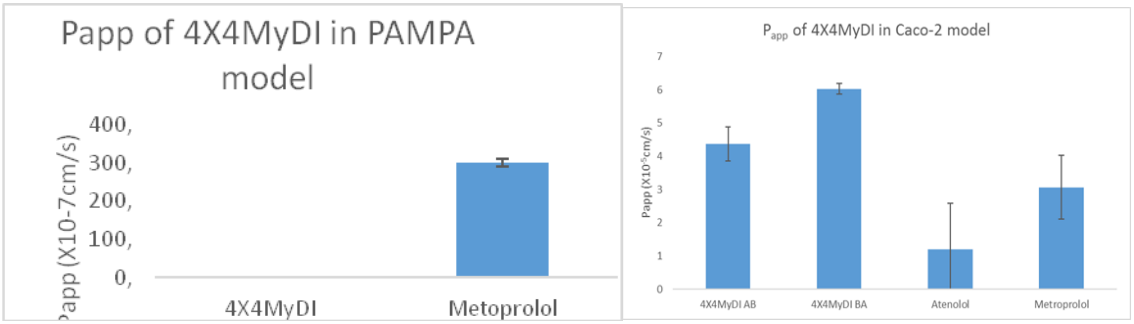
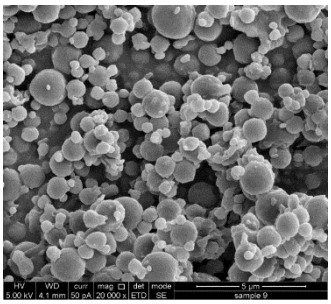


Figure 2: A. P_{app} of 4x4MyDI in the PAMPA model in comparison to metoprolol. B. P_{app} of several compounds in the CaCO-2 model: 4X4MyDI (AB and BA), Atenolol and Metoprolol

5a. NPs Formation

Nanoparticles loaded with peptide are prepared by dissolving the polymer and peptide in a mixture of solvents (dichloromethane and ethanol or DMF or DMSO) and adding the solution in stirred excess anti-solvent (for both the polymer and the peptide) containing a surfactant (Tween or Span) and dispersed mannitol particles (1-10 micron size). After 30 min. of mixing, mixing is stopped to allow precipitation of the dispersed mannitol where the peptide loaded nanoparticles stick to the mannitol surface. The isolated precipitate is allowed to try to yield a white powder that upon addition to water forms a nano-dispersion while mannitol is dissolved. The release rate of the peptide from nanoparticles is determined by dispersing the nanoparticles in physiological media (phosphate buffer pH7.4 at 37°C) and at certain time point, collecting samples of the media and determine peptide content by HPLC. In a typical experiment, poly(sebacic anhydride) (190 mg, prepared from anhydride melt condensation of sebacic acid) was dissolved in methylene chloride (2 mL). TRH (10 mg) was dissolved in 0.4 ml ethanol and then mixed with polymer solution. The polymer/TRH solution was rapidly poured into heptane as anti-solvent (200 ml) containing 500mg of lyophilized mannitol and 10 mg Span 20. The formed nanoparticles precipitate and stick onto the mannitol particles. The mixing was stopped to allow precipitation of the mannitol-nanoparticle powder and isolated by filtration or centrifugation. The particles were characterized by SEM, particles size analysis and TRH release and polymer degradation. Characterization of TRH nanoparticles by using scanning electron microscopy (SEM). TRH loaded PSA and P(CPP-SA) NPs were placed on carbon tape, sputter-coated with platinum/palladium (Pt/Pd) to a thickness of about 10 nm using a sputtering deposition machine (Polarone E5100). Imaged using high resolution SEM, Sirion, (FEI Company, Netherlands) at an acceleration voltage of 30 KV. Results obtained showed most of particles of particle size between 200-500 nm.



Bigger particles present will be removed in further optimization.

5b. In vitro release of peptide from peptide NPs (e.g. TRH-NPs)
The aim of this experiment is to determine release pattern of TRH from NP prepared by the antisolvent method described in Appendix 5a. Release study is carried out by dispersing 2 mg peptide loaded NP in 1 ml of double distilled water (DDW) in shaker with 30 rpm at 37°C (n=2). Sampling was carried out by separating NP by filtering release medium and new samples were used for next time point. Sampling point were 0, 1, 2, 4, and 6h. Using prepared calibration curve peptide concentration in release samples was calculated. The results are shown in figure 1B.

6a. Chronic model of EAE (inflammation and degeneration model)
C57BL/6 mice are immunized with MOG35-55 peptide, to induce an immune–mediated –myelin targeting- process, leading to CNS demyelination and axonal injury, and manifesting clinically with paralytic disease and chronic neurologic disability. This neuroinflammatory process is characterized histopathologically by perivascular and meningeal infiltrates of macrophages / microglia and T cells. More specifically, chronic EAE will be induced in 6- to 7-week-old female C57BL6 mice (of weight apx. 20gr) by immunization with an emulsion containing of purified myelin oligodendrocytes glycoprotein (MOG) 35-55 peptide and an equal volume of complete Freund adjuvant containing H37Ra . Animals with EAE will be examined for neurological symptoms according to the EAE clinical severity scale: 0 = asymptomatic; 1 = partial loss of tail tonicity; 2 = tail paralysis; 3 = hind limb weakness; 4 = hind limb paralysis; 5 = 4-limb paralysis; 6 = death.

The histopathological manifestations of the disease (EAE) will be evaluated and quantified in the experimental animals
The inflammatory process and axonal pathology of chronic EAE will be quantified using axial frozen sections / Paraffin sections at predetermined levels. Sections will be stained with the modified Bielschowsky technique for simultaneous evaluation of the axonal pathology and inflammation. Brain, corpus callosum, cerebellar, and spinal cord sections will be evaluated under (20 x) magnification of optical fields. An examiner blinded to treatment and clinical severity will count the total number of perivascular mononuclear infiltrates in hematoxylin-eosin sections, estimate the axonal loss (modified Bielschowsky stain) and the demyelination grade (Luxol fast blue stain). To grade inflammation, the numbers of perivascular infiltrations are counted. For axonal loss we use a scale of: 0 = normal axonal density; 1 = focused mild to moderate axonal loss; 2 = scattered mild to moderate axonal loss; 3 = focused severe axonal loss; and 4 = scattered severe axonal loss. To grade demyelination we use a scale of: 0=no demyelination; 1=a few scattered naked axons; 2=small groups of naked axons; 3=large groups of naked axons; 4=confluent foci of demyelination and 5=widespread demyelination. The effect of MYRP-1 and NP-MYRP-1 will be determined after periodical IN administration. Dosing will be determined based on the results of the in vitro and ex vivo experiments described in Appendix 2.

6b. Cuprizone induced Demyelination
Young adult male C57Bl/6 mice at 6-8 weeks old are susceptible to demyelination induced by a 4-6 week feeding with 0.2% Cuprizone . Demyelination occurs globally throughout the white matter of the CNS, but it is most easily detected in the area of the corpus callosum. After demyelination occurs the remyelination effect of MYRP-1 and NP-MYRP-1 will be determined after periodical IN administration. Dosing will be determined based on the results of the in vitro and ex vivo experiments described in Appendix 2.

FUNDING REQUESTED FOR THE PROJECT

Funding requested:

	Team 1	Team 2	Team 3	Total
Laboratory expenses	60,5k€	35k€	20k€	115.5
Equipment	5k€	15k€	20k€	40
Salaries (precise the type of contract, the position and the duration)	34,5k€ (Engineer, 1 year contract)	50k€ (2 Post-docs, 1 year contract each)	60k€ (MD,1 year contract; PhD student 1 year contract)	144.5

Other funding sources for the project (including those submitted).

Principal Investigator of selected projects will be asked to send an intermediary report at the end of the Year 1 and a final report at the end of the project (Year 2) to ARSEP/AFM.

Signature of the coordinator of the project:

Anna Maria Papini

Data collected are processed by Fondation ARSEP in a computer database, for management purposes. Some of the project data (the name of the applicant, address of laboratory, the title of the project, its summary, the duration of the project and the ARSEP Foundation funding amount) will be the subject of automatic data processing by the Fondation d'Aide pour la Recherche sur la Sclérose en Plaques (Fondation ARSEP). A public web database will include these data to provide the general public with information regarding the progress of fundamental and clinical research related to multiple sclerosis.

Pursuant to the French law "Informatique et Libertés" of 6 Jan. 1978, you have the right to rectify the personal data retained about you. In case you wish to exercise these rights, please address requests to the secretary of the Scientific Committee of ARSEP Foundation (scientific@arsep.org) .You have also the right to object, on legitimate grounds, to the processing of data related to you.

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SPECIAL CALL FOR PROPOSALS 2016

MYELIN : FROM LESION TO REPARATION

Signature of the Co-investigator(s)

After considering the application: *"title of the project",* I, the undersigned Dr. Pablo Kizelsztein……, give my consent for the participation of the team untitled "…Intranasal neuropeptide therapy for myelin regeneration in multiple sclerosis" localized: Institute of Chemistry, Faculty of Medicine, School of Pharmacy, Hebrew University, Jerusalem, Israel

Date : 19.9.2016 **Done at : Jerusalem**

Signature :

Dr. Pablo Kizelszte
Director
The Authority for Research & Developme
The Hebrew University of Jerusalem

SPECIAL CALL FOR PROPOSALS 2016

MYELIN : FROM LESION TO REPARATION

Signature of the Co-investigator(s)

After considering the application: *"Intranasal neuropeptide therapy for myelin regeneration in multiple sclerosis"*, I, the undersigned M. François Germinet, give my consent for the participation of the team untitled "Laboratory of Chemical Biology (LCB-PeptLab)" localized at the University of Cergy Pontoise, 5 mail Gay-Lussac, Neuville sur Oise, France

Date : **16/09/2016** **Done at : Cergy**

Signature :

UNIVERSITÉ de CERGY-PONTOISE
33, boulevard du Port
95011 CERGY-PONTOISE Cedex
Tel. 01 34 25 60 00

SPECIAL CALL FOR RESEARCH PROPOSALS 2016

MYELIN : FROM LESION TO REPARATION

Administrative file

DEADLINE: SEPTEMBER 27, 2016

To be eligible, the Proposal must include:
- Participation of at least two research laboratories, including one in France.
- Relevance to the proposed general topics.

COORDINATOR OF THE PROJECT:

NAME	Anna Maria
Surname	Papini
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TITLE OF THE PROJECT:

Intranasal neuropeptide therapy for myelin regeneration in multiple sclerosis

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5)Professeur William Lubell
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FUNDING REQUESTED FOR THE PROJECT

	Team 1	Team 2	Team 3
Name of the person to contact	HOEFKENS Isabelle	Dr. KIZELSZTEIN Pablo	*Dr. LEMBERG Hadas Ph.D LLB*
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VALORISATION PATENT POLICY

All inventions or discoveries resulting from a research project supported by ARSEP, in whole or in part, with industrial or financial applications must be reported promptly to the Central Office of the ARSEP Foundation.

ETHICAL ISSUES

If ethical approval is required for a clinical trial or a research project, the scientist in charge must obtain the appropriate authorisation according to the organism involved and the local laws of the country concerned.

ANIMAL EXPERIMENTATION

Animal experimentation related to the project described in this funding request will be carried out in agreement with current rules and regulations.

	Team 1	Team 2	Team 3

Laboratory's agreement number			20156 & 20713
Date & signature of the person in charge			22.9.2016 Dr. Ibrahim Kassis

Dr. Hadas Lemberg, Ph.D LLB
Deputy Director,
Research & Development Division

SIGNATURE OF THE CO-INVESTIGATOR(S)

TEAM 1

After considering the application: **"Intranasal neuropeptide therapy for myelin regeneration in multiple sclerosis"**, I, the undersigned **M. François Germinet**,

give my consent for the participation of the team untitled "**Laboratory of Chemical Biology (LCB-PeptLab)**" localized at the **University of Cergy Pontoise, 5 mail Gay-Lussac, Neuville sur Oise, France**

Date 16/09/2016 **Done at Cergy**

Signature

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TEAM 2

After considering the application: **"Intranasal neuropeptide therapy for myelin regeneration in multiple sclerosis"**, I, the undersigned M Dr. Pablo Kizelsztein

give my consent for the participation of the team untitled " **Institute of Chemistry, Safra Campus** - localized at the **Hebrew university, Jerusalem 91904 Israel**

Date 22.9.2016 **Done at** Jerusalem

Signature

Dr. Pablo Kizelsztein
Director
The Authority for Research & Development
The Hebrew University of Jerusalem

TEAM 3

After considering the application: **"Intranasal neuropeptide therapy for myelin regeneration in multiple sclerosis"**, I, the undersigned M...Dr. Hadas Lemberg

give my consent for the participation of the team untitled **" Department of Neurology, Multiple sclerosis unit "** localized at **Hadassah Jerusalem Medical Center, Jerusalem Israel**

Date 22.9.2016 **Done at** Jerusalem

Signature

Dr. Hadas Lemberg, Ph.D LLB
Deputy Director,
Research & Development Division
הסתדרות מדיצנית הדסה
Hadassah medical organization