Greek Research Findings: the Battle Against Multiple Sclerosis

European Multiple Sclerosis Platform
Greek Multiple Sclerosis Society
Hellenic Federation of Persons with Multiple Sclerosis
Hilton Hotel, Athens
May 19-20, 2017
A New Generation of Drugs in the Treatment of Multiple Sclerosis

Achievements in advancing the de novo design and synthesis of peptide mimetics, and the development of related technologies for Chemistry, Biology, Medicine and Society.

From Basic Research to the Clinic
Science Park of Patras, Greece
where preclinical Myelin Peptide work is carried out
Victoria University, Australia
Western Health: Sunshine Hospital
where clinical trial patient recruitment
will be undertaken (Phase I/II trial)
Professor Vasso Apostolopoulos
University of Patras, Greece
Chemistry, Medicine, Pharmacy, Biology
Preclinical studies for the Myelin Peptides
Design, Synthesis, Biology, Pharmacology
Pasteur Institute, Athens, Greece
Director Lesley Probert, PhD
in vivo studies for Myelin Peptides and Conjugates
In vivo and in vitro studies of Myelin peptides and Conjugates towards clinical trial.
Modeling peptides towards mimetics drugs
(Angiotensin, Myelin, GnRH, TRAPs)
Investors
Spin off Company, Eldrug, Patras Science Park
Drug Discovery, Design, Synthesis, Development
TECHNOLOGY

STEPS IN DRUG DESIGN

1. THE PEPTIDE (The Tool)

2. CONFORMATION (The Ligand-Receptor Interaction)

3. THE CYCLIC PEPTIDE (The Prodrug)

4. MIMETIC (The Drug)

PEPTIDE MIMETICS
A NEW GENERATION OF DRUGS

ANGIOTENSIN II
(Hypertension and Cardiovascular Diseases)
Discovery of Elsartan

MYELIN PEPTIDES
(MBP, PLP, MOG)
(Multiple Sclerosis)
Discovery of Linear and Cyclic Myelin Immunomodulators

THROMBIN RECEPTOR ACTIVATING PEPTIDES
(TRAP)
(Angiogenesis and Cancer)
Cyclic and non-peptide TRAP Mimetics

GONADOTROPIN RELEASING HORMONE (GnRH)
(Fertility and Cancer)
Cyclic Leuprolide Analogues

Design and Pharmacology of Peptide Mimetics
Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the central nervous system (CNS).

MS attacks the myelinated axons in the CNS, destroying the myelin and the axons to varying degrees.

Myelin is the target of an autoimmune attack by T and B lymphocytes of the immune system.

Certain proteins in the myelin sheath such as MBP, PLP and MOG are recognized by leucocytes and are attacked.

The course of MS is highly varied and unpredictable.
Multiple Sclerosis and the development of therapeutic vaccines

- MS is characterized by the destruction of Myelin in CNS.

- Myelin is a white matter that surrounds the nerves which carry brain signals across the body.

- Certain proteins in the myelin sheath such as MBP, PLP and MOG are recognized by leucocytes and attacked.

- Current drugs against MS are not effective as symptoms deteriorate by time.

- The development of immunotherapeutic vaccines with analogues of MBP, PLP, MOG seems a promising new therapeutic approach.
NEW THERAPEUTIC APPROACHES FOR MS TREATMENT

Synthesis of peptide analogues with increased competitive biological action

The use of modified peptides derived from myelin sheath

Mannan

Mannose polysaccharide

Carrier of peptides to mannose receptors on macrophages and dendritic cells
Peptide Conjugate with Mannan

Immunotherapy of Multiple Sclerosis

<table>
<thead>
<tr>
<th>(KG)$<em>5$-MOG$</em>{35-55}$</th>
<th>Molecular mass</th>
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Mannan from Saccharomyces Cerevisiae
Peptide conjugation with the polysaccharide mannan targets the conjugate to mannose receptors on dendritic cells.

https://www.hindawi.com/journals/jdd/2013/869718/
THE ATTACK OF IMMUNE SYSTEM ON MYELIN SHEATH AND ITS DESTRUCTION
MULTIPLE SCLEROSIS CLINICAL SUBTYPES
Magnetic Tomography: The First Diagnostic Tool

Healthy brain

Brain with damage (lesions or plaques) caused by MS

Plaques
Nuclear Magnetic Resonance (NMR)
CYCLIC PEPTIDE MBP82-98

$^1\text{H}$ 1D NMR spectrum

$^1\text{H}-^1\text{H}$ NOESY spectrum of cyclic MBP82-98

$^1\text{H}-^1\text{H}$ TOCSY spectrum of cyclic MBP82-98.
Molecular Modelling

Interactions of myelin peptide with receptor

Design of potent myelin peptides

Crystallography – NMR

A) Linear MOG (yellow)

B) Cyclic MOG (orange)
PEPTIDE SYNTHESIS

Solid Phase Peptide Synthesis: Linear and Cyclic Peptide

Barlos Resin
University of Patras, CBL

Synthetic procedure for cyclic analogues:
cyclo(91-99)[Ala\textsuperscript{96}]MBP\textsubscript{87-99} and cyclo(87-99)
[Arg\textsuperscript{91},Ala\textsuperscript{96}]MBP\textsubscript{87-99}

Elmyelin
Research in Multiple Sclerosis

Elmyelin: Immunotherapy of Multiple Sclerosis

ELMYELIN VS COPAXONE

ELDRUG: Estimated Potential
TEVA: 4,3 € Bn/annum
ANTIGEN TARGETING to Dentritic Cells
ELMYELIN
POTENTIAL THERAPEUTIC DRUG FOR MULTIPLE SCLEROSIS

Elmyelin: suppression of disease

DEVELOPMENT

Myelin Patent 1: Immunotherapy of Multiple Sclerosis (PCT/IB2008/003493)
Myelin Patent 2: Immunotherapy of Multiple Sclerosis Using Mannosylated Peptides as Therapeutic Agents (PCT/IB2009/000382)
SUBMISSION FILE FOR APPROVAL OF CLINICAL TRIALS TO FDA, EMEA, TGA

Investigator’s brochure, IB
- Documentation that supports the proposed study with bibliography
- Physicochemical and pharmacodynamic properties, Chemical synthesis
- Preclinical results that prove the effectiveness and safety in laboratory animals

Investigator’s brochure, IB
- Preclinical results that prove the effectiveness based on blood samples from patients with MS
- Toxicology studies in laboratory animals
PRECLINICAL RESULTS FOR ELMYENIN

- Chemical synthesis, Specifications of final product, Methods for analysis
- Testing cell populations of blood with Elmyelin
- Analysis of blood samples from patients with MS and determination of immune response (Th1/Th2) (in vitro)
- Protection of laboratory animals from EAE induction
- Immunization of transgenic mice with Elmyelin
- Toxicology studies in two laboratory animal species
NEW APPROACHES FOR THE IMMUNOTHERAPY OF MS

**Target:** A cocktail of myelin epitopes
A dendrimer as a new potential immunotherapeutic vaccine (MOG, MBP)

**Target I:** the development of a cocktail with myelin epitopes (MBP, MOG, PLP) conjugated with the polysaccharide mannan.

**Target II:** Preclinical evaluation of the cocktail in *In vitro* and *In vivo* experiments (determination of cytokines, antibodies, immune response, EAE model).

**Target III:** Preclinical toxicology studies of the best cocktail or dendrimer.

**Target IV:** Preparation of IND file (Investigetional New Drug) for Clinical Trials.
Chemistry
Myelin Epitopes (MBP) Cyclic Analogues
Towards Clinical Investigation of Elmyelin

gp Cyclo(2-9) (Ala⁸)MBP74-85

gp Cyclo(2-9) MBP74-85

Cyclo (1-17) (Ala⁹¹)MBP83-99

Cyclo (1-17) (Arg⁹¹)MBP83-99

Cyclo (1-17) (Phe⁹¹)MBP83-99

Cyclo (1-17) (Tyr⁹¹)MBP83-99
GREECE – AUSTRALIA
Mannan-MBP CONJUGATES
POTENTIAL PHARMACEUTICAL DRUGS FOR THE IMMUNOTHERAPY OF MS
# MYELIN II PATENTS - MULTIPLE SCLEROSIS
## CONJUGATES COMPRISING MANNAN AND MYELIN OLIGODENTROCYTE GLYCOPROTEIN (MOG) WITH KG LINKER
## CONJUGATES COMPRISING MANNAN AND MYELIN BASIC PROTEIN (MBP) WITH KG LINKER

<table>
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<tr>
<th>PCT: No. PCT/IB2009/000382, Filed 22nd January, 2009</th>
<th><strong>Short Title:</strong> &quot;Immunotherapy of Multiple Sclerosis Using Mannosylated Peptides as Therapeutic Vaccines&quot;</th>
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<td><strong>Short Description:</strong> MBP and MOG analogues conjugated with mannan via KGₙ bridge</td>
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<th>Matsoukas et al European: No. 09703874.9, Filed 22nd January, 2009</th>
<th><strong>Short Description:</strong> MBP analogues conjugated with mannan via KGₙ bridge</th>
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<th>Matsoukas et al European (divisional): No. 14156495.5, Filed 25th February, 2014</th>
<th><strong>Short Description:</strong> MOG analogues conjugated with mannan via KGₙ bridge</th>
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<th>Matsoukas et al US: No. 12/864,019, Filed 22nd January, 2009</th>
<th><strong>Short Title:</strong> “Conjugates comprising mannan and myelin oligodendrocyte glycoprotein (MOG)”</th>
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<td><strong>Short Description:</strong> MOG analogues conjugated with mannan via KGₙ bridge</td>
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<th>Matsoukas et al US (divisional): No. 14/877,679, Filed 7th October, 2015</th>
<th><strong>Short Title:</strong> “Conjugates comprising mannan and myelin basic protein (MOG)”</th>
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<th>Matsoukas et al Australia: No. 2009207345, Filed 22nd January, 2009</th>
<th><strong>Short Description:</strong> MOG analogues conjugated with mannan using KGₙ bridge</th>
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<th>Matsoukas et al Australia (divisional): No. 2014200921, Filed 21st February, 2014</th>
<th><strong>Short Description:</strong> MBP analogues conjugated with mannan using KGₙ bridge</th>
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# MYELIN I PATENTS - MULTIPLE SCLEROSIS
## CONJUGATES COMPRISING MANNAN AND MYELIN BASIC PROTEIN (MBP) WITH KLH LINKER

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<tr>
<th>PCT: No. PCT/IB2008/003493, Filed 20th November, 2008</th>
<th><strong>Short Title:</strong> &quot;Peptide Analogues &amp; Conjugates Thereof&quot;</th>
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<td><strong>Short Description:</strong> MBP and MOG analogues conjugated with mannan via KLH bridge</td>
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<th>Matsoukas et al European: No. 08851987.1, Filed 20th November, 2008</th>
<th><strong>Short Description:</strong> MBP analogues conjugated with mannan using KLH bridge</th>
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<td><strong>Status:</strong> Pending, under examination</td>
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Dimitris Monos, Professor of Immunology, University of Pennsylvania, USA
Eldrug, Patras Science Park, 2012
The People Behind
Bright minds do not have a country

Dr Elizabeth Matsouka
Biologist, Doctor of Chemistry
Academy Award
How Jim Watson & Francis Crick Discovered the Double Helix

Cambridge University, 1953

Karolinska Institute, Sweden, Nobel Award, 1962
James D. Watson in the University of Patras (2011) invited by the Post-Graduate Program “Medicinal Chemistry”
James D. Watson in University of Patras
“Discovering the Double Helix of DNA”
(April 14, 2011)
Andrew V. Schally  
(Nobel in Medicine and Physiology)  
in PSP, Eldrug’s Laboratory
Ada Yonath
(Nobel in Chemistry)
in PSP, Eldrug’s Laboratory
Harald zur Hausen,
Nobel in Medicine and Physiology
in PSP, Eldrug’s Laboratory
Ruth Padel, Professor of Poetry
Darwin's grand grand grand daughter
in PSP, Eldrug’s Laboratory
Top Scientists and Nobel Laureates in Conferences of the Post-graduate Program “Medicinal Chemistry: Drug Discovery, Design and Development”
16 Nobel Laureates Support Research in Greece
Ολυμπιακοί Αγώνες, Αθήνα 2004
Τελετή Λήξης
STATEMENTS OF DISTINGUISHED SCIENTISTS IN THE FIELD ABOUT FINDINGS IN THE MULTIPLE SCLEROSIS PROJECT
(Com-ordinator Professor John Matsoukas)

“Dear Professor Matsoukas, Bioassay results for your Cyclic MBP1-11 peptides first time to be reported in transgenic models are stunning”.
Prof. D. Wraith, Dept. of Pathology, University of Bristol, U.K.

“Dear Professor Matsoukas, You have one of the most interesting MBP-derivatives of the world. The cyclic peptides are as potent as the linear peptides!! To my Knowledge, this is the first time that a cyclic peptide binds to the MHC. As the cyclic peptide is surely more stable than the linear one, the therapeutic approach for treatment of Multiple Sclerosis is quite realistic....”
Prof. H. Kalbacher, University of Tubingen, Germany,

“Dear Professor Matsoukas, I am thrilled by the findings in this network and congratulations to this team for the excellent work. I think we can try the cyclic-MBP peptide in normal individuals and then try it in patients with Multiple Sclerosis....”
Prof. V. Apostolopoulou, Austin Research Institute, Australia

“Congratulations for the excellent work and activity”
James Watson, Nobel Laureate, Cold Spring Harbor Laboratory, U.S.A
Research on Multiple Sclerosis!

Contribution to a Better World!

THANK YOU!