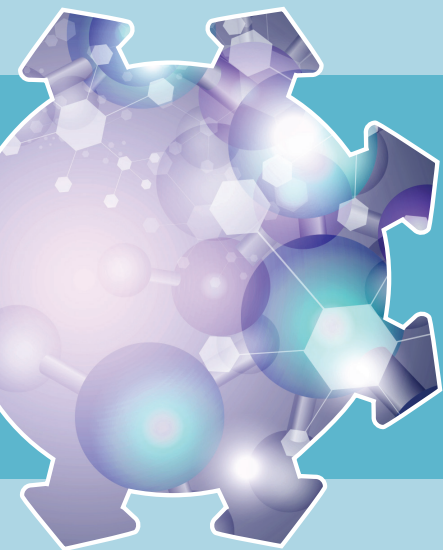


# 4th International Multithematic Scientific Bio-Medical Congress



## Bio-medical Scientific Cyprus

- Friday 4 - Saturday 5, November 2016
- Cultural Center,  
European University Cyprus

**8 Credits of Continued Medical Education (CME)**  
will be awarded

The Congress is under the auspices  
of the Ministry of Health and the  
Cyprus Medical Association (CYMA)



REPUBLIC OF CYPRUS  
MINISTRY OF HEALTH



CYPRUS  
MEDICAL ASSOCIATION

**Organized & Supervised by:** Professor Dr Ioannis Patrikios



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### Βιβλιογραφικές αναφορές

1. Βάση Δεδομένων IMS Health MIDAS: Μητρώος Πωλήσεων από Οκτώβριο 2008 ως Ιούνιο 2015

Πριν τη συνταγογράφηση συμβουλευθείτε την Περίληψη Χαρακτηριστικών του Προϊόντος.  
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Συμπληρώνοντας την «ΚΤΡΙΝΗ ΚΑΡΤΑ»



# Welcome Address

By the Rector Professor Kostas Gouliamos



Distinguished Guests and Honorable Participants,

It is with great pleasure that I welcome you to the European University Cyprus, and with honor that I address the 4th Multidisciplinary Scientific Bio-Medical Congress, entitled "Biomedical Scientific Cyprus".

European University Cyprus is an agile academic institution undergoing rapid growth while maintaining highest quality teaching and research; it has an exceptionally gifted student body, a world renowned faculty and innovative specialized disciplines; it is surrounded by first-rate research centers and laboratories and cutting-edge high tech infrastructures that open up a world of possibilities for multidisciplinary education and research.

European University Cyprus belongs to the largest international university organization, Laureate International Universities-- a worldwide network of Higher Education institutions founded in 1998 in the United States; it is currently made of more than 80 institutions in 28 countries, with more than 200 campuses and over 1,000,000 students worldwide.

Furthermore, Laureate International Universities is an international community of universities that encourages learning without boundaries, with a unique multicultural perspective.

European University Cyprus has secured a momentous distinction following the assessment by QS TOP UNIVERSITIES (QS Stars™), the independent and most authoritative university-rating tool globally which places European University Cyprus among the top universities in the world with the highest distinction of 5-Stars in Teaching, Facilities, Inclusiveness, Social Responsibility and Internationalization. European University Cyprus is the only University in Cyprus to be rated by QS TOP UNIVERSITIES.

European University Cyprus promotes opportunities for long – term strategic partnerships with an impact on academics, the economy and society at large. Such a strategic Partnership was created when Microsoft s selected European University Cyprus to be its partner institution for the establishment of the only Microsoft Innovation Center in Cyprus and one of 100 that operate globally. The operation of The Microsoft Innovation Center (MIC) at European University Cyprus will have an enormous impact on students, faculty, IT professionals, researchers, the economy and society at large.

Dear Colleagues,

The European University Cyprus is becoming an Institution with high quality targets aiming to new frontiers of science, innovation, research and excellence. We are investing with particular emphasis on Bio-Sciences like the opening of the Medical School at our University in September 2013.

High caliber events and symposia like this one, with distinguished scientists as speakers and participants from all over the world, are the vehicles driving to the accomplishment of our goals and they have our full support.

It is really an honor for us to have world known scientists and of your caliber participating and lecturing in our Institution.

Saying this, I salute and welcome every and each one of the congress participants and congratulate Professor Dr Ioannis Patrikios, Acting Chairman and Faculty member of the School of Medicine for his initiative and hard work to organize and give flesh and bones to his idea; and for giving us the opportunity to successfully be here today.

I would also like to acknowledge Bayer / Novagem Ltd, the sole sponsor of the congress for their genuine, valuable contribution.

I wish you all a successful and productive congress.

Professor Kostas Gouliamos  
*Rector, European University Cyprus*



## Welcome Address

By Professor Dr George Petrikkos



Distinguished Guests and Honourable Participants,

It is with great pleasure that I welcome you to European University Cyprus and with honour that I address the 4th Multidisciplinary Scientific Bio-Medical Congress, entitled "Biomedical Scientific Cyprus".

The European University Cyprus is becoming an Institution with high quality targets aiming to new frontiers of science, innovation, research and excellence. We are investing with particular emphasis on Bio-Sciences like the opening of the Medical School at our university in September 2013.

High calibre events and symposia like this one, with distinguished scientists as speakers including Nobelists and participants from all over the world are the vehicles driving to the accomplishment of our goals and they have our full support.

Saying this, I salute and welcome every and each one of the congress participants and congratulate the Chairman Professor Dr. Ioannis Patrikios, Faculty member of the School of Medicine for his initiative and hard work to organize this event; and for giving us the opportunity to successfully be here today.

I would also like to acknowledge Bayer/Novagem Ltd, the sole sponsor of the congress for their genuine, valuable contribution.

I wish you all a successful and productive congress.

Professor George Petrikkos  
*Acting Dean  
School of Medicine, European University Cyprus*

## Welcome Address

By Professor Dr Ioannis Patrikios



Dear Congress participants and guests

It is my great pleasure to welcome you to the 4th International Bio-Medical Scientific Cyprus Congress of the School of Medicine of European University Cyprus (EUC) that is taking place in Nicosia, Cyprus on the 4th of November 2016.

The School of Medicine of the EUC and I personally welcome all distinguished invited speakers and the scientific community of Cyprus that is attending this high quality Multidisciplinary Scientific Symposium.

As the founder and general organizer of the congress, I would like to thank the Ministry of Health and the Cyprus Medical Association for their support and recognition.

I would like to thank all of my fellow colleagues and friends that accepted the invitation to participate, travel, attend and share with us their unique and innovative scientific work of excellence as well as the executives of the European University of Cyprus (EUC) for their backing and trust to me and my abilities to organize this event at the highest possible level. I thank all of my colleagues participating as chairmen of the session committees; but also my colleagues here at the School of Medicine for their genuine support and willingness to help making this an unforgettable date of our calendar through the years.

It was my strong desire to establish this congress: "Biomedical Scientific Cyprus, (BSC)" to become an annual event with global recognition. I am more than sure that this is an achievable target and here we are for a fourth consecutive year.

Not only that; this year, as it is upgraded from one to two-days event with participation of scientific papers in a "Poster Session" it is expected to exceed all expectations and any previous participation. This alone indicates the quality, seriousness and scientific prestige of the conference that was launched exactly with the opening of the Medical School, three years ago and managed today to become an ordinance.

Finally, I would like to thank the sponsor of the congress, Bayer and NOVAGEM LTD and especially Mr. Mario Christodoulou, the General Director of the aforementioned companies in Cyprus, for his genuine support; investing on continued learning, knowledge, innovation and excellence. Bayer/NOVAGEM is the sponsors of this event since our first meeting. We hope to have them for many more.

The conference is being held in early November one of the best times to visit the island and enjoy its natural beauty as well as history. I thank each and every one of you for being here with us. I wish you all the best and a productive Congress

Professor Dr Ioannis Patrikios  
*Acting Chair, Faculty of Medicine, School of Medicine, European University Cyprus,  
General Congress Supervisor*



# Chairmen / Speakers

## Chairmen

- Dr Christodoulos Kaisis
- Dr Petros Agathaggelou
- Dr Ioannis Patrikios
- PhD Cand. George Loucaides
- Dr Tassos Georgiou
- Dr George Miltiadous
- Dr Andreas Zachariades
- Dr George Hadjigeorgiou
- Dr Christina Christodoulou
- Dr Nikos Gregoriades
- Dr Elpida Nikolousi
- Dr Michales Hadjigavriel
- Dr Anastasis Stephanou
- Dr Kyriakos Ioannou
- Dr Adamos Hadjipanagis
- Dr Marios Pantzaris
- Dr Savvas Savva
- Dr Dinos Mavromoustakis
- Dr Elisabeth Johnson
- Dr Demetres Ntourakis

## Keynote Speaker

- Prof. Dr Tomas Lindahl

## Plenary Speakers

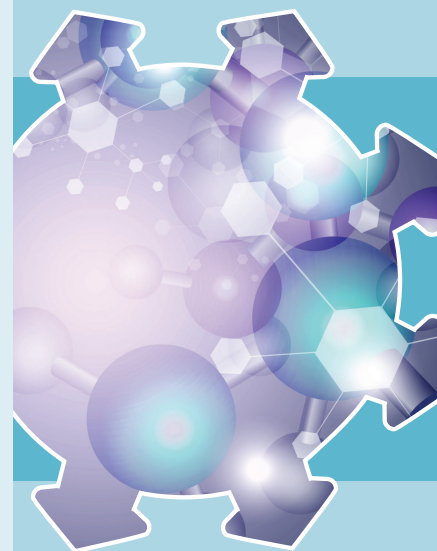
- Prof. Dr Philip Calder
- Prof. Dr Gerry Melino
- Ass. Prof. Dr Marios Pantzaris
- Prof. Dr Nikos Gregoriades
- Dr Andreas Lysandropoulos
- Prof. Dr Theodoros Kyriakides
- Prof. Dr Leonidas Phylactou
- Prof. Dr Constantinos Deltas
- Prof. Dr Theodoros Xanthos
- Dr Petros Agathaggelou
- Prof. Dr Kyriacos Kyriacou
- Prof. Dr Anastasis Stephanou
- Asst. Prof. Dr Antonis Kirmizis

**Special Thanks** to the Medical Students that have contributed to the organization of the conference; and Professor Dr Elisabeth Johnson for her leading role organizing the students.

- |                          |                       |
|--------------------------|-----------------------|
| - Alexandros Samouilides | - Mariana Balta       |
| - Alexander Aschermann   | - Mikaela Michaelidou |
| - Apostolos Sidiropoulos | - Panos Jeropoulos    |
| - Andreas Ellinides      | - Panos Kakouris      |
| - Angela Ishak           | - Paschalis Kasapidis |
| - Arya Harikrishna       | - Richard Saad        |
| - Guy Sydney             | - Smaragda Arkouli    |
| - George Pikis           | - Sophia Hadjiosif    |
| - Haris Christodoulou    | - Themis Demitriou    |
| - Kostantinos Petridis   | - Tereza Gedeon       |
| - Marios Mouseles        | - Tonia Pangali       |
| - Maria Vamvaka          |                       |

## Independent Poster Award Committee

- Prof. Dr Gerry Melino
- Prof. Dr George Hadjigeorgiou
- Dr Dinos Mavromoustakis



# Bio-medical Scientific Cyprus

## Program



# Program

## FRIDAY, NOVEMBER 4, 2016

- 1:30 – 3:30 REGISTRATION / COFFEE**
- 3:30 – 4:00** **Symphonic Band of European University Cyprus performing a diverse repertoire including Cypriot, Greek and Czech folk songs, themes from movies and Latin melodies**  
Yiannis Miralis, Associate Professor European University Cyprus, Conductor
- 4.00 – 4:30 OPENING CEREMONY**  
Introduction to the European University Cyprus School of Medicine
- Welcome Addresses
- Prof. Dr Ioannis Patrikios, Congress Chair and Acting Chair of the School of Medicine, European University Cyprus
  - Prof. Dr George Petrikos, Acting Dean of the School of Medicine, European University Cyprus
  - Prof. Andreas Makris, Vice Rector of Academic Affairs, European University Cyprus  
Representatives of Cypriot Government and Cyprus Medical Association
  - Dr George Pamborides, Minister of Health
  - Prof Costas Kadis, Minister of Education
  - Dr Petros Agathaggelou, President of Cyprus Medical Association
- 4:30 – 5:45 CONFERMENT CEREMONY**  
Doctor Honoris Causa  
Prof. Dr Tomas Lindahl, Nobel Laureate Chemistry, 2015 – for:  
“mechanistic studies of DNA repair”; Francis Crick Institute, UK
- Announcement of Professor Dr. Tomas Lindahl as a Honorary Professor of the School of Medicine, European University Cyprus;  
By the Vice Rector of Academic Affairs Professor Andreas Makris
- The Research of Excellence  
Prof. Dr Tomas Lindahl, Nobel Laureate, 2015; Francis Crick Institute, UK  
“The Intrinsic Fragility of DNA”. The Nobel Lecture  
Chairs: Christodoulos Kaisis, Petros Agathaggelou, Ioannis Patrikios
- 5:45 – 5:55 RECOGNITION OF INVITED SPEAKERS**  
Professor Andreas Efstathiou, Vice Rector of Research, European University Cyprus
- 5:55 – 7:00 COFFEE AND SNACK**

## SATURDAY NOVEMBER 5, 2016

- 8:00 – 9:25 REGISTRATION / COFFEE**
- 9:15 – 9:25** **Introduction to the European University Cyprus School of Medicine**  
**Welcome / Opening**  
**NEURODEGENERATIVE DISEASES-MULTIPLE SCLEROSIS-NEUROPROTECTION, NEUROREGENERATION**  
**VS**  
**NATURAL ANTI-INFLAMMATORY STRUCTURED MOLECULES (PUFA) AND ANTIOXIDANT VITAMINS/NUTRIENTS**  
**- NEW TREATMENT OPPORTUNITIES**
- 9:25 – 10:00** **Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?**  
Prof. Dr Philip Calder, School of Medicine, University of Southampton, UK  
Chairs: Petros Agathaggelou, George Loucaides, Tassos Georgiou
- 10:00 – 10:25** **Neuroaspis®plp10: The Adjuvant Treatment for Multiple Sclerosis**  
Ass. Prof. Dr Marios Pantzaris, Cyprus Institute of Neurology and Genetics- Cyprus School of Molecular Medicine, Cyprus  
Chairs: George Miltiadous, Andreas Zachariades
- GENETICS AS A TOOL FOR NEW THERAPIES OF CHRONIC NEURODEGENERATIVE DISEASES**
- 10:25 – 10:50** **MS treatment dilemmas: anti-T- or anti-B- cell regimens?**  
Prof. Dr Nikos Gregoriades, AHEPA University Hospital, Thessaloniki, Greece  
Chairs: George Hadjigeorgiou, George Miltiadous
- 10:50 – 11:05 COFFEE BREAK**
- 11:05 – 11:30** **HLA genotype as a marker of Multiple Sclerosis prognosis**  
Dr Andreas Lysandropoulos, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium  
Chairs: George Hadjigeorgiou, Christina Christodoulou
- 11:30 – 11:55** **A biologic marker of disease severity in Multiple Sclerosis; the search for the Holy Grail in a Hellenic cohort**  
Prof. Dr Theodoros Kyriakides, Cyprus Institute of Neurology and Genetics-Cyprus School of Molecular Medicine, Cyprus  
Chairs: Nikos Gregoriades, Elpida Nikolousi
- 11:55 – 12:20** **Oligonucleotide-based gene therapy for Muscular Dystrophy...**  
Prof. Dr Leonidas Phylactou, Cyprus Institute of Neurology and Genetics - Cyprus School of Molecular Medicine, Cyprus  
Chairs: Michales Hadjigavriel, Anastasis Stephanou



**12:20 – 12:45 Biobanking in Cyprus: Present and future prospects for research**

Prof. Dr Constantinos Deltas, *Cyprus University*

Chairs: Kyriakos Ioannou, Dimitris Ntourakis

**12:45 – 2:30 LUNCH BUFFET  
POSTER SESSION**

**CARDIOVASCULAR DISEASES - DIAGNOSIS AND TREATMENT**

**2:30 – 2:55 Research in Resuscitation: the beginning of an exciting new era**

Prof. Dr Theodoros Xanthos, *School of Medicine, European University Cyprus*

Chairs: Christodoulos Kasis, Kyriakos Ioannou

**2:55 – 3:20 Recent Advances and the future of Stroke Prevention in Non-Valvular Atrial Fibrillation – The role of NOACS – The Newer oral anticoagulant**

Dr Petros Agathagelou, *President of the Cyprus Medical Association*

Chairs: Christodoulos Kasis, Marios Pantzaris

**CANCER RESEARCH AND NEW POTENTIAL TREATMENT APPROACHES**

**3:20 – 3:50 The p53 family in cancer biology**

Prof. Dr Gerry Melino, *University of Rome Tor Vergata, Italy*

Chairs: Dinos Mavromoustakis, Anastasis Stephanou, Michales Hadjigavriel

**3:50 – 4:05 COFFEE BREAK**

**4:05 – 4:30 Familial breast cancer genetics: the experience in Cyprus**

Prof. Dr Kyriacos Kyriacou, *The Cyprus School of Molecular Medicine at*

*The Cyprus Institute of Neurology & Genetics*

Chairs: Dinos Mavromoustakis, Savvas Savva

**4:30 – 4:55 Novel targets for the anti-cancer properties of phytochemicals**

Prof. Dr Anastasis Stephanou, *School of Medicine, European University Cyprus*

Chairs: Elisabeth Johnson; Tassos Georgiou

**4:55 – 5:20 Epigenetics of aging and cancer: from mechanisms to therapeutic targets**

Asst. Prof. Dr Antonis Kirmizis, *University of Cyprus*

Chairs: Andreas Zachariades, Adamos Hadjipanagis

**5:20 – 5:35 COFFEE BREAK**

**ORAL PRESENTATION (SELECTED ABSTRACTS)**

**5:35 – 5:45**

**“Reduced apoptotic death of peripheral blood lymphocytes on patients with relapsing- remitting multiple sclerosis receiving glatiramer acetate, compared to interferon  $\beta$ -1 $\alpha$  - preliminary results”**

Marina-Kleopatra Boziki; *AHEPA University Hospital, Thessaloniki, Greece*

**5:45 – 5:55**

**“Transcriptome sequencing yields new insights into the pathogenesis of Chinese DLBCL ”**

Apostolos Zaravinos; *European University Cyprus*

**5:55 – 6:05**

**“AQP4 tag SNPs in patients with intracerebral hemorrhage in Greek and Polish population ”**

Maria Sokratous; *University of Thessaly School of Medicine, Larissa*

**6:05 – 6:15**

**“Neuroprotective effects of omega-3 fatty acids in a rat model of anterior ischemic optic neuropathy”**

Maria Kalogerou; *Ophthalmos Research and Educational Institute*

**6:15 – 6:25**

**“The Prevalence of the Epstein - Barr virus in Cypriot Multiple Sclerosis Patients: A Pilot Study ”**

Elie Deebea; *The Cyprus Institute of Neurology and Genetics*

**6:25 – 6:35**

**“Down regulation of LRSAM1 significantly affects neuronal cell growth and viability”**

Minaidou Anna; *The Cyprus Institute of Neurology and Genetics*

**6:35 – 6:45**

**“Looking beyond the electrical stimulation for hearing restoration in cochlear implantation: laboratory and clinical”**

Anagiotos Andreas.; *Nicosia General Hospital/ Larnaca General Hospital*

**6:45 – 6:55**

**“Biomarker Signatures in Fecal, Blood and Colon Biopsy samples for risk assessment of Colon Cancer”**

Elena Kamilari; *University of Cyprus*

Chairs: Elisabeth Johnson, Adamos Hadjipanagis

**6:55 – 7:00**

**Poster Awards**

Dr Ioannis Patrikios, *Congress Chair*

**CLOSING CEREMONY**

Founder and Congress Chair: Prof. Dr Ioannis Patrikios

All coffee breaks and lunch are sponsored by Bayer Novagem.





## Speakers CVs



### **Professor Dr Tomas Lindahl**

*Emeritus Principal Scientist of the Francis Crick Institute, Clare Hall Laboratories  
Nobel Prize in Chemistry in 2015*

Tomas Lindahl MD FRS carried out initial medical studies at the Karolinska Institute in Stockholm in the late 1950's, and has since then consistently been active in research. He worked as a postdoctoral fellow on nucleic acid biochemistry and enzymology at Princeton University and at Rockefeller University. He obtained an independent research position of the Swedish Natural Sciences Research Council, located at the Karolinska Institute in 1969.

He became Professor of Medical Chemistry at the University of Gothenburg in 1978. In 1981 he was appointed Head of the Mutagenesis laboratory at the ICRF Mill Hill Laboratories in London, subsequently the Clare Hall Laboratories. From 1984 to 2006 he was Director of the Clare Hall Laboratories of ICRF and Cancer Research UK, also serving as Deputy Director of Research. He remained Principal Scientist as Head of the Mutagenesis laboratory until 2009.

Amongst many honors, Tomas Lindahl is a member of EMBO, a fellow of the Royal Swedish Academy of Sciences, and the Royal Society, London. He was the Royal Society Croonian Lecturer in 1996 and received a Royal Medal in 2007, INSERM Prix Etranger in 2009, and the Copley Medal in 2010 of the Royal Society. He was awarded the Nobel Prize in Chemistry in 2015. He is now Emeritus Principal Scientist of the Francis Crick Institute, Clare Hall Laboratories, and involved in scientific activities as follows:

2010	Chair of Scientific Advisory Board, Institute of Molecular Oncology (IFOM)
2007	Chair of Scientific Advisory Board, Cancer Center Karolinska, Stockholm
2010	Member of Scientific Advisory Board, Cancer and Ageing Centre, University of Nice, France
2010 - 2012	Hon. Professor in Medical Oncology, University of Sheffield
2009 - 2012	Visiting professor of the Chinese Academy of Science and Scientific Advisor, Beijing Institute of Genomics



### **Professor Dr Philip Calder**

*Faculty of Medicine at the University of Southampton in the UK*

Philip Calder is Professor of Nutritional Immunology within the Human Development and Health Academic Unit of the Faculty of Medicine at the University of Southampton in the UK. He has broad interests in nutritional modulation of immunity, inflammation and cardio-metabolic disease risk. Much of his work has been devoted to exploring the metabolism and functionality of fatty acids with an emphasis on the roles of omega-3 fatty acids. Dr Calder has received several awards for his work including the Sir David Cuthbertson Medal (1995), the Nutricia International Award (2007), the ESPEN Cuthbertson Lecture (2008), the Louisiana State University Chancellor's Award in Neuroscience and Medicine (2011) the German Society for Fat Science's Normann Medal (2012), the American Oil Chemists' Society Ralph Holman Lifetime Achievement Award (2015), the BAPEN Pennington Lecture (2015), the British Nutrition Foundation Prize (2015) and the prestigious Danone International Prize for Nutrition (2016). He has served on many committees of professional societies and was for three years President of the International Society for the Study of Fatty Acids and Lipids (2009-2012). Dr Calder is currently Chair of the Scientific Committee of the European Society for Clinical Nutrition and Metabolism (ESPEN) and President-Elect of the Nutrition Society. He has over 500 scientific publications, his work has been cited over 20000 times, and he is listed as an ISI Highly Cited Researcher. Dr Calder was Editor-in-Chief of the British Journal of Nutrition from 2006 to 2013 and he is currently an Associate Editor of Clinical Science, Journal of Nutrition, Clinical Nutrition, Lipids, and Nutrition Research. He is a member of the several other Editorial Boards of journals in the nutrition, clinical science and lipidology fields.



## Speakers CVs



### **Professor Dr Gerry Melino**

*Department Director and Professor of Molecular Biology at the University of Rome "Tor Vergata" in Italy*

Professor Gerry Melino currently works as Department Director and Professor of Molecular Biology at the University of Rome "Tor Vergata" in Italy (Email: melino@uniroma2.it). He is also Programme Leader for the Medical Research Council (MRC) Toxicology Unit, in Leicester, UK His training originated in Italy and in particular Rome, where he obtained his M.D. (1978, University of Rome) followed by clinical specialisations in Paediatrics (1981, University of Rome) and Clinical Oncology (1985, University of Rome). He obtained his Ph.D in 1984 at the University of London in the Chemical Pathology Department, Charing Cross & Westminster Medical School. Upon graduation, he worked as Research Fellow, then Lecturer and later Senior Lecturer (Honorary Consultant) until 1987. Professor Melino returned to Italy in 1988 to the University of Rome as a Lecturer before becoming a full Professor in 1994. Professor Melino has acted as Consultant and Scientific Advisor for several companies and government institutions. He also has significant Editorial activity as Founder and Editor-in-Chief of the Nature-Publishing-Group journals Cell Death and Disease ([www.nature.com/cddis](http://www.nature.com/cddis)) and Cell Death and Differentiation (Impact Factor 9.050. [www.nature.com/cdd](http://www.nature.com/cdd)) as well as serving on the editorial boards of several other scientific journals. The Scientific Interests of Professor Melino are focused on Programmed Cell Death or apoptosis, in neural and epidermal models. Originally, he worked on the Molecular mechanisms of cell death in the skin, a process known as cornification or formation of the cornified envelope. The molecular events were investigated in vitro and in animal models as well as in human genetic pathologies. The role of transglutaminases (type 1, 2, 3, and 5) and their substrates (SPRs, loricrin, keratins) were investigated at biochemical and genetic levels. While still keeping an interest on these aspects, his current work is focused on The p53/p63/p73 family. DNA damage elicit repair mechanisms involving the tumour suppressor gene p53 and the two newer members of the same family: p63 and p73. The molecular events driven by DNA damage to elicit the function of p63/p73 and their transcriptional regulation, is investigated in vitro. The molecular mechanisms of apoptosis and their protein stability and degradation is also under investigation. Transgenic mice and knock-out work for p63 or p73 is in progress.



### **Professor Dr Nikolaos Gregoriadis**

*Aristotle University of Thessaloniki*

Dr Nikolaos Grigoriadis graduated from the Faculty of Medicine of the Aristotle University of Thessaloniki. He did his PhD thesis and residency in Neurology in the same institution. He has been specialized in clinical and experimental Neuroimmunology and CNS immunopathology in a number of research centers and institutions abroad.

He is now Professor of Neurology at the Aristotle University of Thessaloniki and Head of the MS Centre and the Laboratory of Experimental Neurology and Neuroimmunology of the B' Dept of Neurology, AHEPA University Hospital.

Professor Grigoriadis is member of various international scientific committees such as the European School of Neuroimmunology, ParadigMS, the subcommittee of ENS for Multiple Sclerosis, the ECTRIMS committee (until 2010), Co-founder and Secretary of the Hellenic Academy of Neuroimmunology. He is Ad Hoc reviewer in more than 32 international scientific journals, co-ordinator in more than 40 multicenter clinical trials for MS and principal investigator in collaborative research projects for cell therapies in CNS autoimmune demyelination.

His field of interests are: Neuroimmunology; Multiple sclerosis; experimental models of autoimmune diseases (EAE etc); neurodegeneration; immunomodulation; cell therapies. He has published more than 130 papers in peer reviewed journals, and his current citation index is more than 3500 with an H-index: 30.



## Speakers CVs



### **Professor Dr Theodoros Kyriakides**

*Professor at the Cyprus School of Molecular Medicine and Senior Consultant at the Cyprus Institute of Neurology and Genetics*

Dr Theodoros Kyriakides is a Professor at the Cyprus School of Molecular Medicine and Senior Consultant at the Cyprus Institute of Neurology and Genetics. He graduated Physiology (BSc Hons) and Medicine (MB ChB) from Bristol University in 1983.

He trained in General Medicine and Neurology in the UK and in Neuropathology in Perth Australia. Since 1992 he works as a Senior Consultant in the Cyprus Institute of Neurology and Genetics where he is also in charge of the Neuropathology Lab. His clinical interests are neuromuscular disorders and Multiple Sclerosis. He sees a variety of myopathies and neuropathies and he is particularly interested in TTRMet30 amyloidotic neuropathy.

He joined the University of Nicosia Medical School in 2011 and he is currently the Module Convenor for Life Control for CS and T Year as well as Lead for NeuroPlus in P Year for the St. George's University of London Medical Programme delivered in Cyprus at the University of Nicosia. In 2013 he was appointed Professor at the University of Nicosia.

His main research interests include the study of Complement C1Q and C5aR inhibition in a mouse model of TTRMet30 amyloidotic neuropathy, the study of modifier genes and epigenetics in disease severity in Multiple Sclerosis.



### **Professor Dr Kyriakos Kyriakou**

*Head of the Department of Electron Microscopy/Molecular Pathology; the Cyprus School of Molecular Medicine and the Cyprus Institute of Neurology and Genetics*

Dr K. Kyriakou got his PhD in 1982 from the Department of Oral Pathology, King's College Schools of Medicine and Dentistry on "Nerve induced secretory processes in rabbit submandibular glands". His Academic appointments include: Lecturer in Biochemistry, Medical School at King's College Hospital, London, 1982; Research Associate and Head of the Electron Microscope Department, at King Saud University, Riyadh Saudi Arabia, 1985; Senior Scientist, at the Cyprus Institute of Neurology and Genetics, Head of the department of Electron Microscopy/Molecular Pathology 1991. He served as Professor, Cyprus School of Molecular Medicine, 2012 and Dean, The Cyprus School of Molecular Medicine, 2014. His research interests include the genetic epidemiology and molecular pathology of cancer, with emphasis on breast cancer genetics. Instrumental in characterising the spectrum of mutations in the BRCA genes in Cypriot families and also obtained substantial funding, towards the establishment of the first Electron microscope department in Cyprus. His group has carried out the largest epidemiological study on breast cancer in Cyprus and has identified factors which lower the risk of breast cancer in Cypriot women such as the Mediterranean diet. Through this research unique mutations were also identified in Cypriot families, in BRCA1 and BRCA2 genes and these results led to the establishment of an important cancer genetics service for the whole Cyprus population. He is the co-ordinator of several research projects, has obtained more than 8,000,000 euros in competitive funding and has published more than 120 articles, in peer reviewed journals. He represents Cyprus in several Societies and advisory committees in Europe. He has been appointed by the Cyprus Research Promotion Foundation as the representative of Cyprus, in the EU-COST programme, in the BMBS (Biomedicine and Biomolecular Sciences) domain. He is an active member of the Electron Microscopy working group which is part of the European Society of Pathology and a Consultant to ESMO on Educational Courses on Genetics. He serves as a reviewer for several scientific journals and research funding bodies. Professor Kyriakou has organized several scientific conferences, at both National and International levels and has been invited to deliver many lectures both locally and at International Conferences. At Present Professor K. Kyriakou is the Dean of The Cyprus School of Molecular Medicine, Nicosia, Cyprus.



# Speakers CVs



**Professor Dr Leonidas Phylactou**  
*Chief Executive and Medical Director of the Cyprus Institute of Neurology and Genetics*

Leonidas Phylactou studied Medical Biochemistry at the University of Birmingham in UK and then did a PhD in Molecular Genetics and Gene Therapy in the same University, although most of the time was spent at the University of Connecticut Health Centre in USA. He then moved back to UK at the University of Oxford for as a post-doctoral scientist, where he set up a team working on gene therapy for Myotonic Dystrophy.

In 1998 he established a research group at the Cyprus Institute of Neurology and Genetics working on the gene function and gene therapy. In 2005 he was appointed Head of the Department of Molecular Function and Therapy in which apart from the research activities he is responsible for diagnostic services in Medical Genetics. He is also the Chief Executive and Medical Director of the Cyprus Institute of Neurology and Genetics.

Leonidas Phylactou sits on the Editorial Boards of the journals *Molecules* and *Pharmaceuticals* and has secured international funding from several organisations such as the Association Francaise contres Les Myopathies, the Human Frontiers Sciences Program and the Muscular Dystrophy Campaign of UK. He participates in several European Networks and published extensively in the areas of expertise.



**Associate Professor Dr Marios Pantzaris**  
*Associate Professor at the Cyprus School of Molecular Medicine and Senior Consultant at the Cyprus Institute of Neurology and Genetics*

He got his training in Neurology in 1995 in Thessaloniki, Greece. He has been trained in Carotid Duplex – Doppler ultrasonography in London, St Mary's Hospital in 1995 and in 1999 he was a visiting doctor in an acute stroke unit in Massachusetts General Hospital, Harvard University Boston, USA.

He is working at the Cyprus Institute of Neurology and Genetics as a Senior Neurologist in the Neurological Dept and he is the Head of the Neurology Clinic C and the Neurovascular Department.

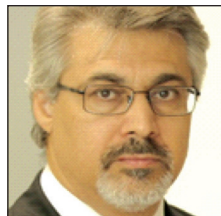
He has a huge experience in carotids – transcranial ultrasound and he has participated in many research projects and publications in this area.

He is also head of the Multiple Sclerosis (MS) clinic with more than 1000 patients where he is running research projects towards the etiology and therapy of MS. He is also interested in movement disorders and pain. With the collaboration of the Cyprus Functional Neurosurgery team they have started operating patients with Parkinson disease (Deep Brain Stimulation) as well as neuropathic pain (Spinal Cord Stimulation).

He has given many lectures about MS, carotids ultrasound stroke and Parkinson's disease in Cyprus and abroad. Dr Pantzaris is one of the three scientists that found and developed the food supplement "Neuroaspis plp10" for the Multiple Sclerosis patients and director of PALUPA Medical Ltd, a R&D company. He has more than 80 published papers in a high impact peer reviewed journals and currently is involved in a phase III multicenter clinical trial for multiple sclerosis and a Phase II single center clinical trial for Parkinson's disease testing the efficacy of "Neuroaspis plp10".



## Speakers CVs



### **Professor Dr Constantinos Deltas**

*Director of the Molecular Medicine Research Centre, University of Cyprus*

Prof. Deltas graduated in 1982 from the National and Kapodistrian University of Athens with a degree in Pharmaceutics. He then earned a PhD degree in Biochemistry, from Rutgers University, The State University of New Jersey, USA in 1988. Between the years 1988-1990 he worked as Instructor in Medicine, Jefferson Institute of Molecular Medicine, Thomas Jefferson University in Philadelphia, PA, USA. He worked as a Research Associate in the Division of Neurology at Duke University Medical Center, at Durham, North Carolina, USA. In 1991 he returned to his home country, Cyprus, at the newly established Cyprus Institute of Neurology and Genetics. He created and directed the Department of Molecular Genetics with emphasis on molecular diagnostics and genetics research, mostly engaged in inherited kidney disorders. In 2002 he was elected Professor of Genetics in the newly created Department of Biological Sciences of the University of Cyprus. He served twice as elected Chairman and had a key role in establishing the Department, implementing undergraduate and graduate programs of study and hiring new faculty. He is Director of the Molecular Medicine Research Center and teaches undergraduate and graduate courses on human molecular and medical genetics. He served two terms as member of the Cyprus National Bioethics Committee and served as a representative of Cyprus to the Standing Committee of the European Medical Research Council of the European Science Foundation and as a Coordinator of the Committee of the Cyprus Council of the Recognition of Higher Education Qualifications (KY.S.A.T.S.), on the subject of Biology-Biochemistry. He served as an elected member of the Council School of Pure and Applied Sciences and the Senate of the University of Cyprus. Presently he is a member of the newly appointed Cyprus Council for Medically Assisted Reproduction.

His research activities focus on Nephrogenetics while he is developing tools for better understanding of molecular pathomechanisms at cellular and animal level. Recently, with competitive funding of 2m Euro by the European Regional Development Fund and the Republic of Cyprus through the Cyprus Research Promotion Foundation, he founded the Molecular Medicine Research Center at the University of Cyprus ([www.ucy.ac.cy/mmrc](http://www.ucy.ac.cy/mmrc)), of which he is the Director. Also, with competitive funding, he started and established the first Biobank in the country, upon approval by the Cyprus National Bioethics Committee, for archiving samples and patients with genetic diseases. His work has been reported in more than 120 original and review peer-reviewed publications in international journals and in additional local journals.

In 2008, Prof. Deltas was elected as "Eminent Scientist 2008" by the International Research Promotion Foundation, which honoured his innovative research on "Nephrology and Human Genetics" and awarded him with its prestigious Millennium Golden International Award for Europe. Also, he was awarded with the "Cyprus Research Award-Distinguished Researcher 2014" upon nomination by the Cyprus Research Promotion Foundation, based on long standing research experience in Cyprus and demonstration of outstanding achievements with local and international impact honoring Cyprus, significant publication record in high impact journals, development of innovative molecular diagnostic methods, success on attracting competitive research funding, the creation of significant research infrastructures and the training/guidance of young researchers



### **Dr Andreas P. Lysandropoulos**

*University Hospital "Erasmé" in Brussels, BELGIUM*

Andreas P. Lysandropoulos, MD, is a Neurologist and MS specialist at the University Hospital "Erasmé" in Brussels, BELGIUM.

His major research interests are clinical spectrum of MS and CD8+ T cells, vitamin D, HLA and EBV in MS, MRI in MS and QoL.

He received his MD from Athens Medical School in Greece and completed his internship and residency in Neurology/Neuroimmunology at the University Hospital of Lausanne, Switzerland. At the same time, he developed his own research project at the Laboratory of Immunology of the University Hospital of Lausanne. Since June 2012, he is running the Neuroimmunology Unit at the University Hospital "Erasmé" in Brussels. He got his PhD in 2016 from the University Hospital of Brussels.

Dr. Lysandropoulos develops research projects in MS and participates as principal investigator to several clinical studies.

He is the writer of medical articles in peer-reviewed journals and he is an invited author to national medical journals. Dr Lysandropoulos is a member of national and international MS advisory boards.



## Speakers CVs



**Professor Dr Theodoros Xanthos**  
*Professor at Medicine for European University Cyprus*

Theodoros Xanthos was born in Athens. He has finished medicine in the National and Capodistrian University of Athens and he has specialized in Internal Medicine and Cardiology in Athens and London UK. He holds an MSc Degree on Clinical Toxicology (University of Cardiff) and an MRes on Education (University of Liverpool). He also holds a Doctorate degree from Athens. He is a Fellow of the Higher Education Academy, a Fellow of the Academy of Medical Educators, a Fellow of the European Resuscitation Council (ERC), a Fellow of the American College of Pharmacology and he is also a Fellow of the European Society of Cardiology. Moreover, he is the only European Certified Toxicologist in Cyprus. He is a Professor of Resuscitation for the University of Cagliari and a Professor of Pharmacology for Midwestern University of Chicago. He is an ERC Medical Educator for Europe. He has an impact factor of 700, an h-index of 25 and his total number of citations are more than 3000. He is currently a Professor of Medicine for European University Cyprus.

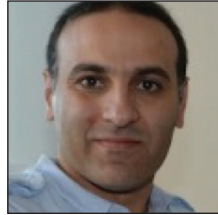


**Professor Dr Anastasis Stephanou**  
*Professor of Cell and Molecular Biology at the European University Cyprus, School of Medicine*

Professor Stephanou is currently Professor of Cell and Molecular Biology at the European University, School of Medicine (2014 – present). He completed his PhD at the Westminster and Charing Cross Medical School, University of London in 1992. He then did his Post-doctoral training (1992-1995) in the Department of Endocrinology, Cincinnati Children's Hospital, USA, working transcriptional gene regulation. In 1995, he moved as a postdoctoral fellow to the laboratory at the Windeyer Institute of Medical Sciences, UCL where he studied the regulation of heat shock proteins and their cytoprotective properties. During his postdoctoral work, he developed his interest in the Signal Transducers and Activators of Transcription (STATs) factors as key regulators of apoptosis. In 2002, Dr Stephanou became a Lecturer at UCL and in 2005 was promoted to a Reader/Associate Professor. His main research interests is in the field of signal transduction in pathways and mechanisms of cell death (apoptosis), cell cycle regulation and autophagy in disease models such as myocardial infarction injury and also in cancer. Other interests include collaborating with a colleague in the Mechanical Engineering Department at UCL, who has developed a novel technique called bio-electrospraying (BES) for deposition and other controlled jetting of primary neonatal cardiac myocytes, primary cardiac and endothelial cells, as well as creating a beating cardiac tissue graft and are hoping to use such protocols for transplantation and treatment of severe heart failure models. He has recently edited a book entitled "JAK-STAT Pathway in Diseases" and also in 2012 became Editor-In-Chief of the journal JAK-STAT. He has authored over 150 peer-reviewed articles.



## Speakers CVs



**Assist. Professor Dr Antonis Kirmizis**  
*University of Cyprus*

Dr. Antonis Kirmizis received his PhD in Molecular and Cellular Biology at the University of Wisconsin-Madison, USA. He then completed his post-doctoral work in the field of Epigenetics as an EMBO and Marie Curie fellow at the Gurdon Institute of Cambridge University, UK. In 2010, Antonis established his research laboratory within the Department of Biological Sciences at the University of Cyprus ([www.kirmizislab.com](http://www.kirmizislab.com)). The following year, he received a prestigious ERC-starting grant to identify novel regulators of epigenetic modifications and to define the molecular mechanisms employed by these regulators in order to control gene expression. His studies are driven by the fact that deregulation of epigenetic mechanisms leads to diseases like cancer and the overall goal of his research is to identify new targets and strategies for cancer therapy. Antonis is a member of various professional societies and associations including the EpiGeneSys Network of excellence.



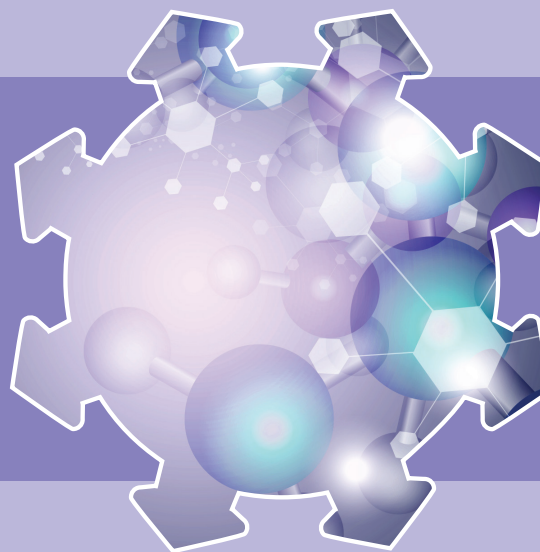
**Dr Petros Agathangelou**  
*Cardiologist, President of the Cyprus Medical Association*

Dr Petros Agathangelou was born in 1961 in Famagusta, Cyprus. He studied Medicine at the University of Chicago in the US, he gained Bachelor's degree in Human Physiology and subsequently he specialized in Cardiology. Since then, he operates as Consultant Cardiologist at Evangelistria Medical Centre, Nicosia Heart Institute, as well as in his private practice.

At present, he is the President of the Cyprus Medical Association. He has served as Immediate Past President of the Cyprus Society of Cardiology for the period 2012-2014. Moreover, he is an active member of various Scientific Committees in Cyprus, he is the president of the Committee of the Supreme Health Council for Athletes at the official Cyprus Sports Authority and, he is leading of the program for the prevention of sudden cardiac death in young athletes. He is a member of the nucleus of European Society of Cardiology – Council for the clinical cardiology practice and member of the European Committee for National Societies in Heart Failure and Cyprus official representative in UEMS for the specialty in Cardiology.

He has participated in several scientific studies that have been published in the Greek and international medical press, as well as in several medical conferences in Cyprus and abroad.





# Bio-medical Scientific Cyprus

## **Abstracts**

- Invited Abstracts
- Selected Abstracts
- Poster Abstracts



# Invited Abstracts

## The Intrinsic Fragility of DNA

**Tomas Lindahl**

*Francis Crick Institute, UK*

The DNA in our cells undergoes unavoidable damage, caused by the intracellular water and by reactive oxygen and metabolites. In consequence, our DNA is being repaired continuously. Several repair pathways are involved, the quantitatively most important of these is base excision-repair to counteract occasional loss of adenine and guanine basis from DNA.

## Omega-3 fatty acids and inflammation: from mechanisms to clinical practice

**Philip Calder**

*School of Medicine, University of Southampton, UK*

Inflammation is a condition which contributes to a range of human diseases. It involves a multitude of cell types, chemical mediators, and interactions. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 (n-3) fatty acids found in oily fish and fish oil supplements. These fatty acids are able to partly inhibit a number of aspects of inflammation including leucocyte chemotaxis, adhesion molecule expression and leucocyte-endothelial adhesive interactions, production of eicosanoids like prostaglandins and leukotrienes from the n-6 fatty acid arachidonic acid, and production of inflammatory cytokines. In addition, EPA gives rise to eicosanoids that often have lower biological potency than those produced from arachidonic acid and EPA and DHA give rise to anti-inflammatory and inflammation resolving mediators called resolvins, protectins and maresins. Mechanisms underlying the anti-inflammatory actions of marine n-3 fatty acids include altered cell membrane phospholipid fatty acid composition, disruption of lipid rafts, inhibition of activation of the pro-inflammatory transcription factor nuclear factor kappa B so reducing expression of inflammatory genes, activation of the anti-inflammatory transcription factor peroxisome proliferator activated receptor - and binding to the G protein coupled receptor GPR120. These mechanisms are interlinked, although the full extent of this is not yet elucidated. Animal experiments demonstrate benefit from marine n-3 fatty acids in a range of models of inflammatory conditions including arthritis, IBD, MS and endotoxemia. Human trials demonstrate benefit of oral n-3 fatty acids in some inflammatory diseases, with the strongest evidence in arthritis and some evidence in a number of other diseases. There is growing interest in whether these effects of marine n-3 fatty acids may be useful in the chronic low-grade inflammation that accompanies cardio-metabolic disease.



## The p53 family in cancer biology

Ivano Amelio

MRC Toxicology Unit, Leicester LE1 9HN, United Kingdom

Francesca Bernassola

University of Rome Tor Vergata, Rome, Italy

Giovanni Chillemi

SCAI-SuperComputing Applications & Innovation Department, CINECA, Rome, Italy

Tak Wah Mak

The Campbell Family Cancer Research Institute, Toronto, Ontario M5G 2M9, Canada

Gerry Melino

MRC Toxicology Unit, Leicester LE1 9HN, United Kingdom, University of Rome Tor Vergata, Rome, Italy

The p53 family members p73 and p63 are involved in female infertility maternal reproduction (Nature Rev MCB 2011;12,4:259) and as well as in cancer formation (TiBS 2014;39,4:191). We identified their activation during DNA damage, several transcriptional targets, the mechanisms of regulation of cell death, and the protein degradation pathway. To understand the p53 structure-function relationship, we performed a molecular dynamics study, showing an induced-fit interaction of the C-terminal domain with the DNA-binding domain. Direct intra- and intermonomeric long-range communications between the tetramerization and DNA-binding domains are noted, providing a biophysical rationale for the reported functional regulation of the p53 C-terminal region. We also detect 'dynamic' deformations switched on and off by particular p53 tetrameric conformations and measured by the roll and twist parameters in the same base pairs. These different conformations can indeed modulate the electrostatic potential isosurfaces of the whole p53-DNA complex (Oncogene, in press PMID: 26477317). While TAp73<sup>-/-</sup> mice show high tumor incidence with hippocampal dysgenesis, they show an elevated cancer incidence. Accordingly, TAp73 opposes HIF-1 activity, affecting tumour angiogenesis. TAp73 interacts with HIF-1 $\alpha$ , promoting HIF-1 $\alpha$  polyubiquitination and consequent proteasomal degradation. These findings demonstrate a novel mechanism for HIF-1 regulation and provide an additional explanation for the molecular basis of the growth, progression, and invasiveness of human cancers. (PNAS-USA 2015;112,1:226) (TiBS 2015;40,8:425). P63 is a determinant of skin development. Using a MMTV-ErbB2 murine model, we found that  $\Delta$ Np63 regulates mammary Cancer Stem Cells self-renewal and breast tumorigenesis via the direct transactivation of Sonic Hedgehog (Shh), GLI family zinc finger 2 (Gli2), and Patched1 (Ptch1) genes. (PNAS-USA 2015. 112,11: 3499-504. PMID: 25739959). At least in part, this seems to be exerted by regulation of the metabolism via Hexokinase II (PNAS-USA 2015. 112,37: 11577-82. PMID: 26324887).

## MS treatment dilemmas: anti-T- or anti-B- cell regimens?

Nikolaos Grigoriadis

B' Dept. of Neurology, AHEPA University Hospital, Aristotle University of Thessaloniki

Multiple Sclerosis (MS) is a chronic autoimmune demyelinating disorder of the central nervous system (CNS), mainly affecting young people. More than 13 disease modifying treatments are (DMTs) are currently available, aiming to control the disease activity, i.e., the inflammatory demyelinating attacks in the CNS parenchyma clinically expressed as relapses and the ongoing disability progression. The vast majority of DMTs target the activation of adaptive immunity, particularly the T-cells and concomitant T-cell – related immune reactions. However, there is increasing evidence that B-cells are also important players in the underlying immunopathology of the disease, as indicated by the presence of plasma cells, myelin-specific antibodies (Abs) and, to a lesser extent, B cells in both chronic MS plaques and acute MS lesions. In addition, the presence of immunoglobulins in MS CNS tissues, lymphoid-like tissues in MS CNS, B cells and plasma cells in MS cerebrospinal fluid (CSF), immunoglobulins in CSF and autoantibodies targeting myelin proteins in MS patients highlight the B-cell involvement in MS immunopathology. In addition, it has been shown that Abs extracted from MS CNS tissue are often autoreactive with myelin Abs to non-protein CNS antigens in MS (though not specific for the disease) whereas the presence of B cells characterizes the subtype II MS lesions which benefit from plasmapheresis. B cells can contribute to the pathogenesis of MS through cytokine production, antigen presentation and formation of autoantibodies. T- and B- cells do not function independently. B cells can activate autoreactive T cells; in return, T cells signal to B cells to enable maturation to plasma cells, which produce highly specific antibodies.

These findings indicate the importance of targeting B-cells in order to control the disease activity. Interestingly enough, there is some evidence that even currently available DMTs have some effect on B-cells thus resulting in a shift in circulating B-cell immunophenotypes, thus increasing the relative frequency of immature and naive B cells, decreasing the proportion of memory B-cells, increased B-cell production of IL-10 with concurrent suppression of proinflammatory cytokine secretion. B cells from DMT-treated patients are generally less able to support a proinflammatory T-cell response.

There is increasing recent evidence that DMTs targeting exclusively CD-20 on B-cells are able to control MS relapses and ongoing disability progression even in progressive forms of the disease. Among them, rituximab and ocrelizumab are typical examples of anti-CD20 MAbs in MS. Evidently, despite the enrichment of our armamentarium to control MS, there is much concern in which cases should the anti- B- cell treatment be used and what the biomarkers indicating the predominant activity in an individual case might be.



## **A biologic marker of disease severity in Multiple Sclerosis; the search for the Holy Grail in a Hellenic cohort**

**Theodoros Kyriakides**

*The Cyprus Institute of Neurology and Genetics-Cyprus School of Molecular Medicine, Cyprus*

Background: Multiple sclerosis (MS) is characterized by a remarkable heterogeneity in disability progression but there is a lack of biological markers that can be used to prognosticate on an individual patient basis. There is convincing evidence that genetic variants may contribute to accrual of disability. Genes involved in innate and adaptive immunity have been implicated but molecules involved in leukocyte trafficking to the CNS also appear appealing. Methods: In total, 389 MS cases and 336 controls were recruited from three MS centres in Cyprus and Greece. We genotyped 147 tagging SNPs in 9 genes (P-selectin, integrins ITGA4, ITGB1 and ITGB7, adhesion molecules ICAM1, VCAM1 and MADCAM-1, Fibronectin 1 and Osteopontin) involved in lymphocyte adhesion processes and trafficking to the central nervous system. Severity was measured by the multiple sclerosis severity score (MSSS). Results: A significant association was detected between rs6721763 of ITGA4 gene and susceptibility to MS as well as with disease severity as measured with MSSS ( $p=1.46E-5$ ). Conclusion: The current provides evidence that variants encoding adhesion molecules, responsible for lymphocyte trafficking and activation impact on MS clinical severity. These findings may have implications for prognosis, treatment options and in the selection of potential therapeutic targets.

## **Bio banking in Cyprus: Present and Future Prospects for Research**

**Constantinos Deltas**

*Molecular Medicine Research Center, University of Cyprus*

Biobanks are organized collections of medical records and biospecimens, aimed to support biomedical research, serving as repositories and distribution centers. The use of Biobanks as research infrastructures has been in progress for many decades but during more recent years there has been intense effort to further develop Biobanking in Europe and create and sustain more Biobanks for promoting translational research and diagnostics projects. It has become clear that only the systematic archiving of records under high standard specifications and with quality management systems can take full advantage of the medical information and associated biological material for unraveling the mysteries of the rare monogenic disorders as well as the more frequent complex conditions. These include, among others, the conditions that consume most of us during aging, such as Alzheimer and related dementias, cardiovascular conditions, diabetes and diabetic nephropathy, rheumatic disorders, etc. Research that uses high quality trustworthy material and information from certified Biobanks aspires to improve existing and develop innovative approaches for diagnostics and novel drug discovery. A serious Pan-European effort for enhancing and at the same time raising the quality of Biobanking, was launched in 2008 within the framework of an FP7 program led by the Medical University Graz, which developed the preparatory phase of BBMRI (Biobanking & BioMolecular resources Research Infrastructure). Another important aim has been the harmonization of Biobanking procedures across Europe and across the globe. Subsequently, BBMRI was upgraded to a European Research Infrastructure Consortium, BBMRI-ERIC, representing the largest family of Biobanks in Europe. BBMRI-ERIC has 19 members, 14 full members and 5 Observers. Upon our initiative, Cyprus has applied and become Observer to BBMRI-ERIC since April 2016, having participated already in several meetings of the Assembly of Members and the Management Committee.

Cyprus started Biobank only 5-years ago, with competitive funding by the European Regional Development Fund and the Republic of Cyprus, through the Research Promotion Foundation. This was a 2m euro project to our group at UCY, with duration of 55 months, aimed at establishing the first Biobank in Cyprus and at supporting a major ongoing research project on inherited nephropathies. This led to the establishment of the Molecular Medicine Research Center (MMRC), which recently moved to its contemporary dedicated premises at the Shokolas Educational Centre of Clinical Medicine, next to the Nicosia General Hospital. The activity of the UCY-Biobank, approved by the Cyprus National Bioethics Committee for Biobanking on all genetic disorders, monogenic and complex, served already several important research projects and several faculty in various medical fields, with high impact findings and peer-reviewed publications. It is anticipated that the infrastructure available and the momentum created shall foster further developments aimed at the delineation of the mysteries of inherited disorders, the study of multifactorial conditions, better diagnostics and foster collaborations with networks in Cyprus and abroad, to the benefit of all citizens.



## Familial breast cancer genetics: the experience in Cyprus

A. Hadjisavvas, M. Loizidou, P. Pirpa, K. Kyriacou

Department of EM/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Cyprus

Y. Markou, E. Kakouri

Bank of Cyprus Oncology Centre, Cyprus

M. Daniel, S. Malas

Department of Oncology, Limassol General Hospital

E. Spanou, T. Delikurt, G. Tanteles, V. Anastasiadou

Clinical Genetics Department, The Cyprus Institute of Neurology and Genetics and Makarios III Hospital, Cyprus

### Breast cancer: incidence, mortality and epidemiology

Breast cancer is the most common cancer in women and in 2008 there were over 1.4 million new cases, with nearly 0.5 million deaths. The incidence is increasing even in countries with established population screening programs and in Europe breast cancer affects approximately 1 in 8 women. The epidemiology of breast cancer has been studied more extensively than any other human disease. A spectrum of risk factors is associated with the development of breast cancer, including duration of estrogen exposure, late first pregnancy and family history. In contrast higher parity and longer duration of lactation lower the risk. However in most women with breast cancer, a specific risk factor cannot be identified.

### Breast cancer: familial genetics

The most important risk factor is a family history and it has been recognized for many years that about 10% of breast cancer patients present with a positive family history. The Hereditary Breast Ovarian Cancer syndrome (HBOC, MIM113705) is the most common form of inherited breast cancer and this is associated with the BRCA1 and BRCA2 genes. Germline mutations in the BRCA1 and BRCA2 genes greatly increase the risk of developing not only breast, but ovarian, and other cancers. A bewildering number of mutations has been characterized, and in Cypriot families a unique spectrum of mutations has been identified including a founder mutation in BRCA2. The discovery of the BRCA genes has provided an important tool for identifying individuals at high risk. It is now possible to identify causative mutations in the BRCA genes and genetic testing, for susceptibility to HBOC, forms an integral part of contemporary oncological practice. Such services should be offered following genetic counseling and must respect the ethical and psychological issues pertaining to patient's welfare.

### Breast cancer: pathology in BRCA carriers

Breast cancers that arise in women with BRCA1/2 mutations frequently manifest characteristic pathological and histological features, such as high histologic grade, hormone receptor negativity and aneuploidy. Despite this, women with BRCA mutations and breast or ovarian cancer, experience better survival than women without mutations, possibly due to enhanced susceptibility to chemotherapy. The genetic hallmark of BRCA deficient cells is inadequate DNA repair which leads to mutational events and eventually cancer. This property is being exploited in pharmacogenomics to design highly targeted and more effective therapies for breast tumors arising in BRCA carriers.

## HLA genotype as a marker of Multiple Sclerosis prognosis

A. Lysandropoulos, E.H. Kaoutar

Neurology, CUB-Erasme, Brussels

A. Ribbens, A. Maertens, W. Van Hecke

Icometrix, Leuven, Belgium

**Introduction:** Scientific evidence suggests that the Human Leucocyte Antigen system (HLA) is linked to multiple sclerosis (MS) [1,2]. The objective of this study is to further examine whether HLA is a marker of MS prognosis.

**Methods:** A total of 117 MS patients underwent HLA class I and II typing. Disease progression was assessed between two time points (years 2013 & 2015) based on clinical parameters, including Expanded Disability Scale Score (EDSS), Multiple Sclerosis Severity Score (MSSS), Timed 25-foot walk (T25W), Symbol Digit Modality Test (SDMT) and on magnetic resonance imaging (MRI). The percentage of brain volume change was assessed on the brain MRI scans using MSmetrix [3]. In order to investigate the correlation between HLA and clinical and MRI variables, the appropriate statistical approaches are performed. The effect of covariates gender, first versus second line treatment and days since the first MS symptom was also evaluated. Results: Analysis is still ongoing. Patients harboring HLA A2 have lower MSSS and lower aPBVC (multivariate analysis: p-value 0.01). However, only the effect for MSSS is significant (individual p-value 0.01) for this group. The effect of both MSSS and aPBVC are significant for the subset harboring the combination of both HLA A2 and DRB15. For this subset, not only the multivariate analysis provides a p-value of 0.007, but also the individual tests indicate a p-value of 0.04 for MSSS and p-value of 0.02 for aPBVC.

**Conclusion:** Our preliminary analysis suggests that HLA A2, in particular in combination with DRB15, is a marker of better prognosis in MS with respect to MSSS and brain volume changes. Data with regards to the other Class I and II HLA alleles will be presented.

### References:

- (1) Fogdell-Hahn et al., Tissue Antigen 2000
- (2) Zivadinov et al., J Neuroimmunol 2009
- (3) Jain et al., Neurolmage Clinical 2015

**Disclosure:** All authors have nothing to disclose

**Source of funding:** Department of Neurology, Erasme Hospital, Brussels, Belgium



# Invited Abstracts

## Novel Targets for the Anti-cancer properties of Phytochemicals

**Patrikios I., Yiallouris A., Stephanou A.**

*School of Medicine, European University Cyprus*

**Fedosova NV**

*Dept. of Biomedicine, Aarhus University, Denmark*

**De Ford C.**

*Dept Pharmaceutical Biology, University Freiberg, Germany*

**Graier W.**

*Medical University of Graz*

Tripterygium wilfordii Hook F, amygdalin and graviola phyto-compounds have been reported to have anti-cancer activity. However, the precise target of action for these plant-based anti-cancer agents has not been well characterized. Importantly, no studies have yet been reported on whether these anti-cancer plant-based agents have death-promoting effects on normal non-transformed cells. Therefore, in order to determine the direct effects of the three phyto-compounds we investigated their death-promoting ability in several cancer cell lines as well as in normal non-transformed cells. All three phyto-compounds showed morphological changes of apoptotic cell death in all cancer cell lines. In contrast, treatment with Tripterygium wilfordii, amygdalin and graviola had no effect in normal non-transformed cells. In silico studies on the most abundant molecules found in Tripterygium wilfordii and graviola but not amygdalin indicated for the first time a possible association with the Na<sup>+</sup>/K<sup>+</sup> ATPase and SERCA ATPase channel activity. Importantly experiments were performed that were able to validate our in silico findings. These results strongly indicated that the above phyto-chemicals have death promoting activity in cancer cells but not in normal cells. The present data indicate that these natural phyto-compounds may have distinct targets with reduced drug toxicity for the treatment and prevention of cancers

## Oligonucleotide-based gene therapy for Muscular Dystrophy

**Leonidas A. Phylactou**

*The Cyprus Institute of Neurology & Genetics and School of Molecular Medicine*

Muscular Dystrophy is a group of inherited diseases with common characteristics the progressive weakness and loss of muscle. Among the most known muscular dystrophies is Duchenne Muscular Dystrophy and Myotonic Dystrophy.

Antisense oligonucleotides (AON) are short pieces of nucleic acids which can be designed to target cellular mRNA and inhibit or modulate endogenous gene expression.

In our laboratory we design and use AON in order to correct the genetic defects and consequently the disease phenotype of muscular dystrophies.

During the presentation, data will be shown which support that AON are good candidates for gene therapy of muscular dystrophies.

## Epigenetics of aging and cancer: from mechanisms to therapeutic targets

**Antonis Kirmizis**

*University of Cyprus*

Epigenetic mechanisms including DNA methylation and histone modifications are important regulators of chromatin structure and gene expression. Malfunction of these epigenetic mechanisms is a prominent hallmark in both the aging process and cancer. The fact that epigenetic aberrations can be potentially reversed, understanding these epigenetic mechanisms can offer new opportunities for therapeutic interventions or extension of healthy lifespan. During this presentation I will describe data, which identify the histone N-terminal acetyltransferase Naa40 as a novel epigenetic regulator of cellular aging. Specifically, I will show that loss of Naa40 activity towards histone H4 extends cellular lifespan by inducing cellular stress-response pathways in a manner that mimics the effect of calorie-restriction. In addition, I will show that in colon cancer cells Naa40 functions as an anti-apoptotic factor and will propose that this epigenetic enzyme should be considered as a therapeutic target.



# Invited Abstracts

## Neuroaspis® plp10: Adjuvant Treatment for Multiple Sclerosis

**Marios Pantzaris, George Loucaides**

*The Cyprus Institute of Neurology and Genetics-Cyprus School of Molecular Medicine, Cyprus*

**Ioannis Patrikios**

*School of Medicine, European University Cyprus*

Multiple Sclerosis (MS) is a chronic autoimmune and degenerative disease that affects central and peripheral nervous system causing severe damage to neuronal axons and myelin sheath leading to permanent disability to the patients. It is a complex, multi-factorial disease that involves many pathogenic pathways and interaction of all these pathways (the system) in a way that within the whole system they are all mutually affected. Current medical philosophy is using single pathogenic pathways to approach treatment (reductionism), either ignoring or overlooking the whole system and its interactions! Holistic approach, on the other hand and treatment decisions following this approach is in line with what Hippocrates had said: "It's far more important to know what person the disease has than what disease the person has". This approach in the gold standard for personalized medicine! Neuroaspis® plp10 is a liquid mixture of specific Polyunsaturated Fatty Acids (PUFAs) at specific amount and ratios together with specific antioxidant vitamins that fulfills the criteria of having "biologic effects" to potentially succeed a holistic treatment model approach in MS, by synchronized and simultaneous manipulation of all involved pathogenic mechanisms and complex dynamic interconnected pathways. Neuroaspis® plp10 has been clinically tested as an adjuvant treatment in MS patients in Cyprus in a double blind phase II clinical trial and has proven its effectiveness by significantly reducing both the clinical relapses and the accumulation of disability. Another ongoing larger double blind phase III multicenter clinical trial is attempting to repeat the positive results of Neuroaspis plp10 in MS patients making the position of this liquid nutraceutical formulation centered to the treatment of MS and other complex immunological and/or degenerative neurological and not only diseases.

**Disclosure:** Authors declare conflict of interest as they are all shareholder of PALUPA Medical, a R&D company owner of the aforementioned intervention.

## Research in Resuscitation: the beginning of an exciting new era

**Theodoros Xanthos**

*School of Medicine, European University Cyprus*

Cardiac arrest remains the ultimate medical emergency with more than one million victims every year in the United States of America and Europe. Its incidence has been reported in various countries in Europe and each of the countries has variable survival to hospital discharge depending on many different factors, such as implementation of cardiopulmonary resuscitation (CPR) guidelines, training of healthcare professionals, availability of automated external defibrillators and sufficient legislation to permit usage of these defibrillators by trained lay people, just to name a few. The annual incidence of out-of-hospital cardiac arrest is 38 per 100,000 population and the incidence of in-hospital cardiac arrest ranges from 1 to 5 per 1,000 admissions. The International Liaison Committee on Resuscitation (ILCOR) is a joined initiative from various continents, including the European Resuscitation Council (ERC) and the American Heart Association (AHA), aiming at harmonization of the guidelines on CPR. These guidelines are reviewed every 5 years and they are updated to mirror both advancement in science and the current consensus of experts. Despite the fact that every possible measure is taken in the formulation of the consensus statements, the guidelines only include data derived from human studies; and while this is easily understood for any other discipline in medicine, human randomized trials are scarce in resuscitation, because of the difficulty from the part of the researchers to obtain consent from people who are "recruited" in these studies. As cardiac arrest victims are unconscious, this means that informed consent needs to be weaved and several ethical committees are reluctant to allow inclusion of patients in studies. This lack of human data often results in guidelines that show little or minimal change from the previous versions. In CPR, the most daring change was noted in 2010 when the compression ventilation ratio changed from 15 compressions to 2 ventilations to 30 compressions to 2 ventilations in a non-secured airway. On the other hand, researchers who do mainly animal research have found several promising receptors, pharmacological agents among others that await evaluation in humans.



## Invited Abstracts

### **Recent Advances and the future of Stroke Prevention in Non-Valvular Atrial Fibrillation – The role of NOACS – The Newer oral anticoagulant**

**Petros Agathangelou**

*Cardiologist, President of the Cyprus Medical Association*

AF is an Independent Risk Factor for Stroke. AF patients have a near 5-fold increased risk of stroke and 1 in every 6 strokes occurs in a patient with AF. Ischemic stroke associated with AF is typically more severe than stroke due to other causes and stroke risk persists even in asymptomatic AF.

A study projected that AF prevalence will increase by more than 2.5 fold to affect more than 5.6 million people by the year 2050.

The newer oral anticoagulants are used for AF and antithrombotic treatment and for the prevention of Stroke in Patients with AF. The lack of data regarding arterial blood pressure control, the lack of specific antidotes and their actions and the economical aspects are some points of future research.

## Selected Abstracts

### **SA01 Looking Beyond the electrical stimulation for hearing restoration in cochlear implantation: laboratory and clinical data**

**Anagiotos A.**

*ENT Department, Nicosia General Hospital & Larnaca General Hospital, Cyprus*

**Gostian AO, Schwarz D., Lüers JC., Pazen D., Lang-Roth R., Klünter HD., Hamdan N., Nünning M., Mitropoulou P., Hüttenbrink KB., Beutner D.**

*University of Cologne, Medical Faculty, Department of Otorhinolaryngology, Head and Neck Surgery, Germany*

Cochlear implantation (CI) is the standard procedure for restoration of hearing in cases of severe to profound hearing loss or deafness. Given the excellent hearing performance of CI recipients, research is now oriented toward preserving the remaining functions of the cochlea, namely residual hearing and vestibular function. Using the CI patients' database and performing experimental temporal bone measurements at the ENT Department of the University Hospital of Cologne, Germany, we investigated the influence of implantation on middle and inner ear residual functions.

Firstly, we evaluated the influence of intracochlear electrode arrays on middle ear vibration transmission and on inner ear fluid dynamics in six non fixated human temporal bones using the laser vibrometer. We found that intracochlear electrode arrays do not change the mechanical properties of middle and inner ear to a point of clinical relevance independent of electrode type and insertion path.

In addition, we conducted a retrospective chart review of all patients who underwent primary CI with a conventional full-length electrode in a six years period (n=422). The results showed preservation of residual hearing in almost half of the recipients. Interestingly, hearing preservation and its extent were significantly better in children and adolescents compared to those in adults. Results were even better when performing electrode array insertion with our underwater technique in order to reduce the mechanical trauma of the cochlea. As far as the vestibular function is concerned, it is known from previous studies that CI may cause alterations of the vestibular organ. Consequently, we continued the database analysis including the implantations performed in a seven years period (n=560) and examined possible common changes in patients' audiogram and caloric stimulation of the vestibular organ. The rate of postoperative vestibular function drop didn't show significant differences between the groups of preservation and loss of residual hearing.

In conclusion, despite opening of the intracochlear structures and insertion of the CI electrode array, the inner and middle ear show a high rate of preservation of their functions. Moreover, an alteration of one of the inner ear functions doesn't necessarily mean disturbance of the other. Preservation of the residual inner ear functions could be facilitated through gentle techniques that respect the physiology of the cochlea and minimize trauma during cochlear opening and implantation, such as our underwater technique.

#### **Financial Disclosures / Conflicts of Interest:**

Part of this study was supported by Cochlear Research and Development Ltd.



## SA02 Neuroprotective effects of omega-3 fatty acids in a rat model of anterior ischemic optic neuropathy

Tassos Georgiou, Panagiotis Kolovos, Maria Kalogerou, Katerina Prokopiou,

Anastasia Neokleous, *Ophthalmos Research and Educational Institute, Nicosia, Cyprus*

Yao-Tseng Wen, *Institute of Eye Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*

Chung-Hsing Chang, *Department of Dermatology, School of Medicine, College of Medicine, China Medical University, Taichung, Taiwan, Department of Dermatology, China Medical University Hospital, Taichung, Taiwan*

Chin-Te Huang, *Department of Dermatology, China Medical University Hospital, Taichung, Taiwan*

Rong-Kung Tsai, *Institute of Eye Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan*

**Background:** The purpose of this study was to investigate the therapeutic effect of omega 3 polyunsaturated fatty acids ( $\omega$ -3 PUFAs) administration in a rat model of anterior ischemic optic neuropathy (rAION). **Methods:** Gas chromatographic analysis was used to assess the level of blood arachidonic acid/eicosapentaenoic acid (AA/EPA) following treatment of  $\omega$ -3 PUFAs for 14 days in six wild type rats, to determine the optimal dosage of  $\omega$ -3 PUFAs (when AA/EPA  $\leq$  1.5). The rAION-induced rats were daily administered by gavage fish oil (1 g/day EPA and docosahexaenoic acid (DHA)) or phosphate buffered saline (PBS) for 10 consecutive days (day 3 pre-rAION to day 7 post-rAION induction). Animals underwent flash visual-evoked potentials (FVEP) to assess visual function and were euthanized 4 weeks post-infarct. Density of retinal ganglion cells (RGCs) was counted using Fluoro-Gold retrograde labeling. TUNEL apoptosis assay of RGCs in the retinas, macrophage activation and M2 polarization in the optic nerve (ON) were measured by immunohistochemical staining of ED1 and CD206. The mRNA levels of M2 macrophage markers and the pro-inflammatory related genes were measured by quantitative qRT-PCR in the ON.

**Results:** Blood fatty acid analysis showed that the AA/EPA ratio was greatly reduced to  $\leq$  1.5 after 10 days of fish oil treatment. The RGC densities and P1-N2 amplitude were significantly higher in the  $\omega$ -3 PUFA-treated group, compared to the PBS-treated group ( $p < 0.05$ ). The number of the apoptotic cells in the RGC layer of the  $\omega$ -3 PUFA-treated rats was significantly decreased ( $p < 0.05$ ) compared to that of the PBS-treated rats. Treatment with  $\omega$ -3 PUFAs reduced 3.17-fold of macrophage recruitment at the ON site in the rAION model. The M2 macrophage markers, which decrease inflammation, were induced in the  $\omega$ -3 PUFA-treated group in contrast to the PBS-treated group. In addition, the mRNA levels of tumor necrosis factor alpha, interleukin-1 beta and inducible nitric oxide synthase were significantly reduced in the  $\omega$ -3 PUFA-treated group.

**Conclusions:** Administration of  $\omega$ -3 PUFAs at an optimal dosage have neuroprotective effects in rAION, possibly through dual actions of anti-apoptosis of RGCs and anti-inflammation via decreasing inflammatory cells infiltration and regulation of macrophage polarization to decrease cytokine-induced injury on the ON.

## SA03 Down regulation of LRSAM1 significantly affects neuronal cell growth and viability

A. Minaidou, P. Nicolaou, K. Christodoulou

*The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,*

*The Cyprus School of Molecular Medicine, Nicosia, Cyprus*

**Introduction:** Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy of the peripheral nervous system affecting 1 in 2500 individuals. Recently we identified a novel LRSAM1 gene mutation (c.2047-1G>A) in a large Sardinian family co-segregating with autosomal dominant axonal CMT2 disease. LRSAM1 is an E3 ubiquitin ligase that has a significant role in endocytic and adhesion pathways in neuronal cells and mediates monoubiquitination of TSG101 at multiple sites. To date, four other LRSAM1 mutations have been associated with CMT neuropathy with dominant and recessive inheritance. This study is investigating the role of LRSAM1 in CMT pathogenesis.

**Materials and methods:** Down regulation of LRSAM1 was performed using the Silencer® Select siRNAs technology. SH-SY5Y cells were double transfected with a specific siRNA against LRSAM1. The cell growth and proliferation were observed every 24 hours. Furthermore, proteins were extracted from transfected cells for Western blot analysis.

**Results:** Down regulation of LRSAM1 affected the morphology of the cells. Moreover, counting of the cells showed that down regulated cells displayed a reduced growth rate as compared to the controls. Western blot analysis revealed that double transfection of LRSAM1 siRNA caused 70% decrease on LRSAM1 expression.

**Conclusions:** The LRSAM1 gene has recently been implicated in the CMT pathways and very little is currently known about its role. We hereby prove that down regulation of LRSAM1 significantly affects cell growth and viability of SH-SY5Y cells, an in vitro model of neuronal function.

**Acknowledgement:** This study is funded by the Cyprus TELETHON (Grant #: 73115).



## SA04 AQP4 tag SNPs in patients with intracerebral hemorrhage in Greek and Polish population

Maria Sokratous, Vasileios Siokas, Efthimios Dardiotis, Georgia Xiromerisiou, Amalia Michalopoulou, Zisis Tsouris, Persia Kountra, Vana Tsimourtou, Hadjigeorgiou M. Georgios

Department of Neurology, Laboratory of Neurogenetics, University of Thessaly, University Hospital of Larissa, Larissa, Greece

Jeremiasz Jagiella

Department of Neurology, Medical College Jagiellonian University, Krakow, Poland

**BACKGROUND:** A small number of genetic variants have been associated with the risk of intracerebral hemorrhage (ICH). Aquaporin-4 (AQP4) has been reported to be implicated to pathophysiological processes during the development of ICH.

**OBJECTIVE:** The present candidate gene association study was aimed to investigate possible contribution of AQP4 gene region polymorphisms to the risk of ICH.

**METHODS:** 250 Greek and 193 Polish patients with primary ICH and 250 and 322 Polish controls were enrolled during this study. 7 AQP4 tag single nucleotide polymorphisms (SNPs) were tested for associations with ICH risk, lobar ICH risk and non-lobar ICH. Cox regression models were used to test the effect of AQP4 tag SNPs on the ICH age of onset.

**RESULTS:** In Greek cohort the rs3875089 TC genotype were tended to be associated with higher risk of primary ICH and of lobar ICH compared to the TT, [Odds Ratio, OR (95% confidence interval, C.I.): 1.82 (1.13-2.93),  $p=0.031$ ] and [OR (95%C.I.): 1.89 (1.04-3.46),  $p=0.022$ ]. Furthermore, individuals with TC genotype of the same SNP had significantly higher probability for lobar ICH onset compared to the wildtype genotypes [Hazard Ratio (95%C.I.): 1.75 (1.07-2.84),  $p=0.025$ ]. In Polish population, rs3763043 TT genotype were tended to be associated with lower risk of primary ICH and of non-lobar ICH compared to the CC, [OR (95%C.I.): 0.31 (0.13-0.72),  $p=0.014$ ] and [OR (95%C.I.): 0.22 (0.07-0.70),  $p=0.012$ ] respectively and the rs335931 GG [OR (95%C.I.): 3.55 (1.11-11.36),  $p=0.032$ ] with lower risk of non-lobar ICH compared to the AA. Furthermore, individuals with TT genotype of the rs3763043 tag SNP had significantly lower probability for onset of ICH and non-lobar ICH onset compared to the wildtype genotypes [HR (95%C.I.): 0.42 (0.021-0.83),  $p=0.013$ ] and [OR (95%C.I.): 0.26 (0.10-0.73),  $p=0.01$ ] respectively. These results were not replicated in the other cohort and didn't survive Bonferroni correction.

**CONCLUSIONS:** In conclusion, the present study provides an indication that AQP4 gene variants might affect susceptibility for primary ICH and probably influence the age at onset of ICH in an ethnicity-specific variability.

**Disclosure:** No conflict of interest

**Funding:** The study was supported in part by a research grant of the Research Committee of the University of Thessaly, Greece (Code: 2845)

## SA05 Reduced apoptotic death of peripheral blood lymphocytes on patients with relapsing- remitting multiple sclerosis receiving glatiramer acetate, compared to interferon $\beta$ -1 $\alpha$ - preliminary results

Marina Boziki, Roza Lagoudaki, Theano Tatsi, Dimitrios Karacostas, Nikolaos Grigoriadis

2nd Neurological Department, AHEPA University General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

**Background:** Glatiramer acetate (GA) and interferon (IFN)  $\beta$ -1 $\alpha$  are disease modifying treatments (DMTs) widely used for the management of relapsing – remitting multiple sclerosis (RRMS), known to affect lymphocytes' phenotypical and functional attributes. Aim: The present study addresses molecules mediating pro-and anti-apoptotic functions on peripheral blood lymphocytes in RRMS patients under treatment with GA or IFN  $\beta$ -1 $\alpha$ . Methods: Apoptotic flow cytometry markers were used on peripheral blood lymphocytes from patients receiving GA and IFN  $\beta$ -1 $\alpha$ . Values were analyzed by independent – samples T-test and results were indicated as mean  $\pm$  standard error of mean. Results: 15 patients receiving IFN  $\beta$ -1 $\alpha$  and 8 patients under GA were included. Patients' groups were age (IFN  $\beta$ -1 $\alpha$ :  $39.36 \pm 3.33$ ; GA:  $44.25 \pm 4.97$ ;  $p=0.45$ ) and gender matched (IFN  $\beta$ -1 $\alpha$ :  $m=4, f=11$ ; GA:  $m=3, f=5$ ;  $p=0.84$ ). Mean disease duration (years) did not differ among groups (IFN  $\beta$ -1 $\alpha$ :  $9.9 \pm 1.79$ ; GA  $11.83 \pm 1.9$ ;  $p=0.5$ ). Also, mean treatment duration (months) (IFN  $\beta$ -1 $\alpha$ :  $80.46 \pm 14.5$ ; GA  $54.42 \pm 8.09$ ;  $p=0.23$ ), and mean EDSS score did not differ between groups (IFN  $\beta$ -1 $\alpha$ :  $3.57 \pm 0.51$ ; GA  $2.62 \pm 0.65$ ;  $p=0.3$ ). Mean frequency (% of CD4+ lymphocytes) of Annex V+ lymphocytes for patients receiving IFN  $\beta$ -1 $\alpha$  and GA was  $45.32 \pm 13.7$  and  $29.67 \pm 12.1$  ( $p=0.4$ ), respectively. Furthermore, mean frequency (% of CD4+ lymphocytes) of Bcl2+ lymphocytes for patients receiving IFN  $\beta$ -1 $\alpha$ , and GA was  $20.66 \pm 4.83$  and  $12.62 \pm 3.84$  ( $p=0.25$ ), respectively, whereas the mean frequency (% of CD4+ lymphocytes) of caspase3+ lymphocytes for patients receiving IFN  $\beta$ -1 $\alpha$  and GA was  $8.78 \pm 2.68$  and  $10.98 \pm 8.13$  ( $p=0.6$ ), respectively. Conclusion: We hereby provide evidence of a trend for reduced frequency of peripheral blood lymphocytes undergoing apoptotic cell death, defined as CD4+Annexin V+ lymphocytes, in patients receiving GA, compared to patients under IFN  $\beta$ -1 $\alpha$  treatment.



## SA06 The Prevalence of the Epstein - Barr virus in Cypriot Multiple Sclerosis Patients: A Pilot Study

Elie Deeba, Georges Krashias, Dana Koptides, Astero Constantinou, Christina Christodoulou  
Department of Neurology, Laboratory of Neurogenetics, University of Thessaly, University Hospital of Larissa, Larissa, Greece

Efthychia Gaglia, Marios Pantzaris

Neurology Clinic C, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

**BACKGROUND:** Multiple Sclerosis (MS) is a chronic, demyelinating, inflammatory autoimmune disease of the central nervous system (CNS). Several genetic and environmental factors have been suggested to contribute in MS. A microbial candidate proposed to be involved in the onset of MS and/or induction of subsequent exacerbations is the Epstein-Barr virus (EBV). The possible involvement of EBV in MS is highlighted by a number of national epidemiological studies showing a higher percentage of EBV seropositivity and EBV DNA in MS patients. This study aims to evaluate for the first time the prevalence of EBV in Cypriot MS patients.

**METHODS:** The serum of 53 MS patients and 50 healthy controls was used to determine the positivity index for EBV nuclear antigen-1 (ENBA-1) IgG, early antigen-D (EA-D) IgG, and EA-D IgM, using ELISA. Additionally, blood samples from 37 of these MS patients and 25 of the healthy controls were evaluated for the presence of EBV DNA using real time PCR.

**RESULTS:** All MS patients were seropositive for EBNA-1 IgG as compared to 96% of the healthy group, furthermore, the positivity index of the antibody was significantly higher in MS patients. There was no significant difference in the presence/absence of EA-D IgG among the 2 groups nor in the corresponding positivity index. Lastly, EA-D IgM was shown to be more present in MS patients than in healthy controls with a significantly higher positivity index in the former. Regarding the EBV DNA, it was more frequently detected in MS patients compared to healthy controls.

**CONCLUSION:** Results of EBNA-1 IgG higher seropositivity in MS patients seem to conquer with previous findings of studies in other countries, thereby further asserting the theory of EBV involvement in MS. The significance of EA-D IgM presence in MS patients remains unclear, however, more data should be collected with a bigger sample pool as well as more detailed clinical histories of the patients involved (relapse dates, medications, MS progression, etc.). Further analysis will also attempt to quantify and compare the EBV DNA in MS patients and healthy controls.

## SA07 Biomarker Signatures in Fecal, Blood and Colon Biopsy samples for risk assessment of Colon Cancer

Elena Kamilari (presenting author), Yiorgos Apidianakis, George Potamitis  
Department of Biological Sciences, University of Cyprus

**Finding:** Marie Curie, Career Integration Award and UCY intramural funding.  
The authors declare no conflict of interest.

Approximately 5%, or 1 in 20, of the western world humans will be diagnosed with cancer of the colon or rectum in their lifetime, 90% of which in people 50 and older. Accordingly, colonoscopy over the age of 50 and every 5 years thereafter is recommended indiscriminately to all but those with inflammatory bowel disease or with a family history of colorectal cancer (CRC). Following surgical excision adenomatous polyps often show recurrence, indicating that even normal-appearing colorectal tissues may retain molecular or cellular alterations that impose a risk for developing a neoplasm. Furthermore, genetic, epigenetic and environmental – including microbial – factors are extensively related to elevated risk of CRC. Our goal is to establish molecular colonoscopy - a cutting edge molecular risk assessment tool - in the clinical practice against colon cancer in Cyprus and internationally by identifying biomarker signatures related to the normal-appearing mucosa (NAM) of the colon and rectum, which may predict the risk for the development of colorectal neoplasia. Considering the necessity for a personalized approach in colon cancer predisposition assessment, we hypothesize that combinations of: (1) mitosis and regenerative inflammatory signaling biomarkers of macroscopically normal colon, (2) bacterial signatures in the colonic NAM, and (3) blood and fecal biomarkers linked to systemic inflammation, bacterial signatures and mitosis may predict the risk of colonic neoplasias in humans. Our hypothesis is supported by our unpublished results in mice and *Drosophila* showing that a high mitosis, intestinal infection and regenerative inflammation is linked to increased tumorigenesis. Individual biomarkers have been selected and will be analyzed combinatorically. Out of at least 500 individuals screened primarily via colonoscopy, 20 will be selected for bearing adenocarcinomas, 40 for bearing colonic adenomas and 60 for being macroscopically normal. The pertinent human protocol (number EEBK/EP/2015/38) is approved the Cyprus National Bioethics Committee. Mucosa biopsy samples are being assessed quantitatively via RT-qPCR, ELISA and immunohistochemistry for the expression of genes indicating mitosis, regenerative inflammation, overall bacterial load and genus/species specific bacterial load along the colon. In addition, we evaluate for the first time the fecal colon cell mitosis marker M2PK, fecal 16S-based bacterial profiles and blood metabolites & cytokines through ELISA, as adjunct biomarkers of risk. An innovative step in our approach is the combination of the various biomarkers in a sophisticated way linking them to the existence vs. absence of colonic adenomas or malignant tumors. This is particularly important because people are generally reluctant to follow up on their colonoscopy schedule, and more of them are likely to comply, if we are able to reveal their predisposition to disease.



## Selected Abstracts

### SA08 Transcriptome sequencing yields new insights into the pathogenesis of Chinese DLBCL

**Apostolos Zaravinos**

*Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institutet, SE-14186 Stockholm, Sweden, Department of Life Sciences, School of Sciences, European University Cyprus*

**Longyun Chen, Zhang Zi**

*Beijing Genomics Institute, Shenzhen, Shenzhen 518083*

**Weicheng Ren, Rozina Caridha, Qiang Pan-Hammarström**

*Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institutet, SE-14186 Stockholm, Sweden*

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin B cell lymphoma, accounting for 30-40% of diagnoses. Although its prognosis has improved significantly over the past decade due to the addition of the rituximab antibody to chemotherapy regimens, approximately 30% of the patients still do not respond to treatment or relapse rapidly. To investigate the genetic events that drive DLBCL lymphomagenesis we mapped and quantified the transcriptomes of 49 human B-cell lymphomas and 5 normal tonsils of Chinese origin. Our data reveal destabilization of the B cell genome in many levels, in terms of somatic mutations, differential gene expression (DEGs), gene fusions, prediction of novel transcripts, and alternative splicing. Most of the substitutions (44.05%) were T:A to C:G, which might have probably occurred during RNA-editing events during B cell transformation. We also detected a high percentage of C:G to T:A transitions (27.54%), characteristic of an impaired recognition/repair of G:T mismatches arising as a result of DNA polymerase errors. Furthermore, we identified numerous gene fusions as a result of translocation, interstitial deletion, or chromosomal inversion. The most recurrent somatic fusion transcripts included AKT2-IGHG1, UBA2-WTIP, JAK3-INS1, LYZ-RN7SL1/2, CD274-IGHG1 and CIITA-RN7SL2, and were verified using RT-PCR and Sanger sequencing. Further transcriptional profiling of DEGs identified a molecular classifier separating GC from non-GC B cell lymphomas. The expression of the top up- and down-regulated genes was validated using qRT-PCR. Protein-coding RNAs were distinguished from noncoding RNAs using the coding potential calculator and the function of novel transcripts was further investigated to assess their protein-coding potential. Alternative splicing events were also investigated per sample and differences in exon splicing were detected between DLBCL and control tonsils, as well as between GCB and non-GCB lymphomas. This study demonstrates that transcriptome sequencing can yield new insights into the pathogenesis of Chinese DLBCL.

## Poster Abstracts

### PA01 Towards understanding the role of Naa40 in gene regulation and cancer

**Christina Demetriadou, Demetria Pavlou, Antonis Kirmizis**

*Department of Biological Sciences, University of Cyprus*

N-terminal acetyltransferases (NATs) are evolutionarily conserved enzymes which catalyze the acetylation of the  $\alpha$ -amino group of the first amino acid residue of a protein. So far, six different NAT protein members have been identified in eukaryotes, noted as NatA-NatF. Naa40 (Nat4) is a unique NAT that specifically acetylates histones H4 and H2A only but its regulatory function still remains elusive. Very recently evidence from our lab showed that Nat-ac of H4 by Naa40 in yeast, interplays with the adjacent H4R3me2 histone mark to activate the transcription of rDNA.

Furthermore, we have recently shown that depletion of Naa40 in HCT-116 and HT-29 colon cancer cells triggers apoptosis, whilst non-cancerous mouse fibroblasts remain viable. Here we examine the effects of Nat-ac by Naa40 on chromatin and gene regulation in human cells. Upon Naa40 knockdown the global levels of different activating and repressive histone modifications in the epigenome of colon cancer cells are altered. Specifically, we observe a robust decrease in the total levels of H4R3me2, which coincides with reduced expression of PRMT5, the enzyme that catalyzes H4R3me2. In line with the above results, we find that depletion of Naa40 results in altered expression of known PRMT5 direct target genes that are either oncogenes or tumour suppressors. Overall, the results demonstrate that Naa40 may control the expression of cancer related genes by regulating the expression of PRMT5 enzyme. Elucidating the molecular mechanisms underlying Naa40 gene regulation will be important to assess its potential as a therapeutic target.



## PA02 Neuroaspis® PLP10 Intervention Efficacy and Correlation to the Molecular Alteration of the Red blood Cells' Membranes

Ioannis S. Patrikios

Neurology Clinic C, The Cyprus Institute of Neurology and Genetics (CING), PALUPA Medical Ltd., School of Medicine, European University Cyprus, Correspondence should be addressed to e-mail: I.Patrikios@euc.ac.cy

George N. Loukaides

Neurology Clinic C, The Cyprus Institute of Neurology and Genetics (CING), PALUPA Medical Ltd.

Marios C. Pantzaris

Neurology Clinic C, The Cyprus Institute of Neurology and Genetics (CING), PALUPA Medical Ltd. Correspondence should be addressed to e-mail: pantzari@cing.ac.cy

**Introduction:** Multiple sclerosis (MS) treatments are products of reductionism, partially effective with no (re)myelinating/neuroprotective abilities associated with significant side-effects. We aimed to assess whether our novel interventions, formulated based on systems medicine (SM), comprising specific polyunsaturated fatty acids (PUFA) and vitamins reduce disease activity in patients with relapsing remitting (RR) MS who were either treated with disease modifying treatment (DMT) or untreated.

**Methods:** We contacted a 30-month randomized, double-blind, placebo-controlled, proof-of-concept clinical study at the CING. Of a total of 80 patients, 20 were randomly assigned to receive intervention A (docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) (3:1 w/w) omega-3, linoleic acid (LA)/gamma(g)-linolenic acid (GLA) (2:1 w/w) omega-6 fatty acids, omega-3/omega-6 (1:1 w/w), other specific PUFA, monounsaturated fatty acids (MUFA), minor quantity of specific saturated fatty acids (SFA), vitamin A and vitamin E), 20 to receive gamma-tocopherol, intervention C, 20 to receive the combination of A and C, intervention B (PLP10) and 20 to receive placebo, as an oral solution, once daily. The primary end point was the annualized relapse rate (ARR) and the key secondary end point was the time to disability progression. The red blood cells (RBC) from each patient blood collection sample at every prescheduled assessment were used as the cells of reference; for evaluation of any correlation between possible efficacy and PUFA profile within the RBC membrane. ISRCTN87818535.

**Results:** PLP10 reduced the ARR by 70% ( $p=0.003$ ), in relation to the baseline ARR and the placebo increased by 46% ( $p=0.354$ ). For the primary end point, PLP10 reduced the ARR by 58% (95% CI 0.10 to 0.79,  $p=0.016$ ) and for the secondary end point, significantly reduced the risk of sustained progression of disability by 86% over the 2-year period (Hr, 0.11; 95% CI 0.01-0.97,  $p=0.047$ ) vs. placebo. More patients in the PLP10 group (72%) vs. placebo group (20%) were free from new or enlarging T2-weighted lesions on brain MRI scans over the 2-year study. No adverse events were reported. Interventions A and C showed no significant efficacy. The RBC lipid profile was supportive to the reported PLP10 efficacy by the statistically significant increased quantitative content of the aforementioned PUFA within the RBC membrane as well as by the increased significant release of arachidonic acid (inflammation initiator molecule) from the RBC membrane when gamma-tocopherol was present (intervention B); supporting the synergistic theory of all PLP10 ingredients for activity.

**Discussion:** PLP10 treatment significantly reduced the ARR, and the risk of sustained disability progression without any adverse or significant side effects. This is the first clinical study of SM approach medical nutrient formula that holds strong promise as an effective treatment for RRMS.

## PA03 Amygdalin Extract Promotes Selective Cell Death

Patrikios Ioannis, Yiallouris Andreas, Stephanou Anastasis

School of Medicine, European University Cyprus, Nicosia Cyprus

Cristian de ford

Institute of Pharmaceutical sciences, University of Freiburg, Germany

Fedosova Natalia

Department of Biomedicine - Physiology and Biophysics at Aarhus University

Wolfgang Graier

Department of Pharmacology School of Health Sciences University of Thessaly

Konstantinos Dimas, Evangelia Sereti

Department of EM/Molecular Pathology, Nicosia, Institute of Neurology and Genetics

Kleitos Socratous

**Presented by:** Angela Ishak, Arya Harykrishna

Natural plant extracts have been used for centuries for treating many types of diseases including inflammatory related diseases as well as cancer. One of these extracts is amygdalin which is a natural compound mostly extracted from the seeds of bitter almonds and apricots. Due to its cyanate containing structure, amygdalin has been reported to have anti-tumor properties by promoting apoptosis. In the present study, we treated various cancer cell lines with different doses of amygdalin. Our experiments on cancer cell lines such as Pancreatic, Prostate, Breast and laryngocarcinoma, revealed that treatment with amygdalin results cell death in a dose dependent manner with approximately 75% death in cancer cell lines at a dose of 1 mg/ml. Moreover, experiments in normal cells like peripheral blood mononuclear cells (PBMC's) and MCF10A MCF12F (breast cells) shows that amygdalin has no toxic effect. From our results we show that amygdalin may indeed have anti-cancer properties and therefore be a promising therapeutic agent use for cancer treatment.



## Poster Abstracts

### **PA04 Graviola Extract: A non-toxic Compound that Promotes cell death in Cancer Cells**

**Patrikios Ioannis, Yiallouris Andreas, Stephanou Anastasis**

*School of Medicine, European University Cyprus, Nicosia Cyprus*

**Cristian de ford**

*Institute of Pharmaceutical sciences, University of Freiburg, Germany*

**Fedosova Natalia**

*Department of Biomedicine - Physiology and Biophysics at Aarhus University*

**Wolfgang Graier**

*Department of Pharmacology School of Health Sciences University of Thessaly*

**Konstantinos Dimas, Evangelia Sereti**

*Department of EM/Molecular Pathology, Nicosia, Institute of Neurology and Genetics*

Phyto-compounds are beneficial in healthcare sector that have contributed in many aspects in Medicine. Studies have demonstrated the progressive effects of phyto-compounds on immune related diseases, quality of life of cancer patients. Graviola is a tree where its extracts (leaves, seeds) are known to be beneficial against cancer. Graviola has been reported to have anti-tumor properties by promoting cell death and apoptosis. Here, we are demonstrating the effect of Graviola in different cancer cell lines such as HeLa, Pancreatic, Prostate, Breast and laryngocarcinoma. Our results reveal an approximately 80% cell death in dose of 1mg/ml. The key finding of the present study is that Graviola promotes cell death (apoptosis) via downregulation of Na<sup>+</sup>/K<sup>+</sup>-ATPase and SERCA pumps. Moreover, the effects on normal cells was also examined both in vitro and in vivo showing limited/no toxic effects on normal cells. Overall our results suggest that this compound could be a strong agent for cancer cure, treatment and prevention.

### **PA05 Tripterygium Wilfordii Promotes Selective Cell Death via a Novel Na/K ATP-ase Pathway**

**Patrikios Ioannis, Yiallouris Andreas, Stephanou Anastasis**

*School of Medicine, European University Cyprus, Nicosia Cyprus*

**Cristian de ford**

*Institute of Pharmaceutical sciences, University of Freiburg, Germany*

**Fedosova Natalia**

*Department of Biomedicine - Physiology and Biophysics at Aarhus University*

**Wolfgang Graier**

*Department of Pharmacology School of Health Sciences University of Thessaly*

**Konstantinos Dimas, Evangelia Sereti**

*Department of EM/Molecular Pathology, Nicosia, Institute of Neurology and Genetics*

**Presented by:** Anthony Lisacek - Kiosoglous Benjamin, Andrew Georgiou

There has been an enormous interest in the literature that phyto-compounds have therapeutic and beneficial effects in various diseases including inflammatory associated arthritis, diabetes, hypertension, parasitic infections, and cancer. A natural extract isolated from the leaves and the root of the Chinese herb *Tripterygium wilfordii* Hook "F" shown to have anti-cancer effects by promoting cancer cell death (apoptosis, necrosis, necroptosis and autophagy). The precise target of action for this plant-base anti-cancer has not been well characterized. Importantly, studies performed with *Tripterygium wilfordii* have not indicated whether these anti-cancer plant-base agents have any toxic effects on normal cells. In the present study we show that *Tripterygium* extract exposed to different cancer cell lines including HeLa, Pancreatic, Prostate, Breast and laryngocarcinoma caused cell death in a dose-dependent manner, with 85% death in cancer cells at a dose of 1mg/ml. Importantly, *Tripterygium* extract did not have cell death effects on normal cells (PBMC's, MCF12F and MCF10A). An in silico approach on the most abundant molecules found in *Tripterygium wilfordii* indicated a possible association with the Na<sup>+</sup>/K<sup>+</sup> ATPase and this was confirmed with specific in vitro studies. Thus, these results strongly indicated that *Tripterygium wilfordii* has death promoting activity in cancer cells but not normal cells. Moreover, we have also identified a novel mechanistic pathway on how the active.



## PA06 Long term efficacy of second – line disease modifying treatments on parents with relapsing – remitting multiple sclerosis: a single center retrospective study

Boziki M., Kallivoulos S., Bakirtzis C., Nikolaidis I., Polychroniadou E., Karacostas D., Grigoriadis N.

**Background:** BRACE treatment has long been used as first-line treatment for patients with relapsing – remitting multiple sclerosis (RRMS). However, not all patients respond equally and therefore, switch to second-line treatments is advisable under internationally applied guidelines of escalation. Factors that affect the efficacy of second – line treatments are age, degree of disability upon switch and disease duration.

**Aim:** A retrospective analysis of the efficacy of second – line treatments (NTZ) and fingolimod (FTY) in patients with RRMS poorly responding to BRACE treatment. Methods: Patients poorly responding to BRACE treatment were switched either to NTZ (N=66) or fingolimod (N=25). Expansion Disability Status Scale (EDSS) was assessed on a 6-month basis and annual MRI was evaluated for new T2 and Gd(+) lesions, for an up to 8-year follow up. Annualized Relapse Rate (ARR) was calculated, before and after switch. Age and disease duration, as well as EDSS score upon switch ( $\leq 3$  or  $> 3$ ) were used as confounding factors. Data were registered on iMED software. Analysis was conducted by the use of Repeated Measures General Linear Model (RM-GLM). Data were presented as mean  $\pm$  SD.

**Results:** Patients' mean age was  $36,8 \pm 9,51$  and  $34,8 \pm 7,81$  with a female to male ratio 2,9:1 and 2,44:1, for patients receiving NTZ and FTY, respectively. Mean EDSS upon switch was  $3,99 \pm 1,53$  and  $2,75 \pm 1,21$  for NTZ and FTY, respectively. Mean disease duration upon switch was  $9,63 \pm 5,74$  and  $10,37 \pm 5,84$  for NTZ and FTY, respectively. Compared to BRACE treatment, mean ARR decreased from  $1,2 \pm 0,86$  and  $0,8 \pm 0,67$ , to  $0,5 \pm 0,93$  and  $0,1 \pm 0,2$ , for NTZ ( $p < 0,001$ ) and FTY patients ( $p < 0,001$ ), respectively. A greater efficacy ( $p = 0,04$ ) was observed, in terms of ARR reduction, for NTZ treated patients with EDSS  $\leq 3$  upon switch from BRACE (from  $1,61 \pm 1,04$  to  $0,27 \pm 0,27$ ), compared to patients with EDSS  $> 3$  (from  $1,08 \pm 0,75$  to  $0,66 \pm 0,93$ ). A similar tendency was observed for FTY treated patients (ARR for patients with EDSS  $\leq 3$  decreased from  $0,88 \pm 0,74$  to  $0,09 \pm 0,21$ , whereas for EDSS  $> 3$  decreased from  $0,57 \pm 0,27$  to  $0,19 \pm 0,18$ ). However, this tendency did not reach statistical significance ( $p = 0,076$ ), underlying the need for larger sample size. Age and disease duration did not contribute significantly ( $p > 0,05$ ).

**Conclusions:** We hereby report that, based on evidence stemming from the present study, switch for RRMS patients that respond poorly to BRACE treatment to second – line treatment at low rates of disability may exert beneficiary effect on the disease course by contributing to greater ARR reduction.

## PA07 Development of a novel targeted assay for non-invasive prenatal testing of fetal trisomies exhibits near-diagnostic accuracy.

Christina Demetriadou, Demetria Pavlou, Antonis Kirmizis

Department of Biological Sciences, University of Cyprus

George Koumbaris, Elena Kypri, Elisavet A. Papageorgiou, Philippos C. Patsalis

NIPD Genetics, Ltd, Nicosia, Cyprus, AAK Ultrasound and Fetal Medicine Center, Nicosia, Cyprus

Kyriakos Tsangaras, Achilleas Achilleos, Petros Mina, Voula Velissariou, Christopoulou Georgia, Marios Ioannides

NIPD Genetics, Ltd, Nicosia, Cyprus

Maria Neofytou

AAK Ultrasound and Fetal Medicine Center, Nicosia, Cyprus

Ioannis Kallikas

AAK Ultrasound and Fetal Medicine Center, Nicosia, Cyprus

Alicia González-Liñán

Andros Day Surgery Clinic – Reproductive Medicine Unit. Palermo, Italy

Egle Benusiene

Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Anna Latos-Bielenska

Center for Medical Genetics Genesis, Poznan, Poland

Pietryga Marek

Gynecology and Obstetrics, Poznan University of Medical Sciences, Poznan, Poland

Alfredo Santana

Clinical Genetics, Childhood Hospital Materno – Infantil. Las Palmas GC. Canary Islands. Spain

Nikoletta Nagy9, Márta Széll

Department of Medical Genetics, University of Szeged, Szeged, Hungary

Piotr Laudanski

Department of Perinatology and Obstetrics Medical University of Bialystok, Bialystok, Poland

There is a great need for the development of highly accurate, cost effective technologies, which can facilitate the widespread adoption of Non Invasive Prenatal Testing (NIPT). We hereby present a novel cost effective assay of unparalleled accuracy which overcomes the limitations of current technologies.

### Materials and Methods

This method enables the targeted analysis of selected genomic regions at very high sequencing depth and allows highly accurate fetal fraction determination to ensure extremely accurate aneuploidy detection. The analytical performance of the assay was evaluated in a blind study, which comprised 631 samples derived from pregnancies of at least 10 weeks of gestation that had also undergone invasive testing.



## Results

The blind study exhibited 100% sensitivity and specificity and correctly classified 52/52 (95% CI: 93.2-100%) cases of trisomy 21, 16/16 (95% CI: 79.4-100%) cases of trisomy 18, 5/5 (95% CI: 47.8-100%) cases of trisomy 13, and 538/538 (95% CI: 99.3-100%) normal cases. The test also correctly identified fetal sex in all cases (95% CI: 99.4-100%). One sample failed pre-specified assay quality control criteria, and 19 samples were non-reportable due to low fetal fraction.

## Conclusion

The clinical impact of free fetal DNA (ffDNA) testing has been significant as indicated by its quick adoption in prenatal care. Our novel technology overcomes limitations of current technologies and exhibits near diagnostic performance. We believe that this method enables accurate and cost-effective non-invasive fetal aneuploidy detection of trisomy 21, 18 and 13, which is critical for widespread adoption of NIPT.

## PA08 Neuroprotective effects of EPA and DHA fatty acids in the DBA/2J

Kalogerou Maria, Kolovos Panagiotis, Prokopiou Katerina, Malas Stavros, Papagregoriou Gregoris, Deltas Constantinos, Georgiou Tassos

**Purpose:** Glaucoma, the second leading cause of blindness, is characterised by progressive death of retinal ganglion cells (RGCs) and loss of RGC axons in the optic nerve. Chronic low-grade inflammation has been shown to play a major role in the degeneration of RGCs in glaucoma. The purpose of this study was to determine whether omega-3 Polyunsaturated Fatty Acid ( $\omega$ -3 PUFA) supplementation used alone or in combination with Timolol eye-drops, protect against inflammation and RGC loss in DBA/2J mouse model of hereditary glaucoma.

**Method:** DBA/2J mice were assigned to the following treatment groups (n=20/group) with balanced sex:  $\omega$ -3 PUFAs plus Timolol,  $\omega$ -3 PUFAs, Timolol and untreated. All treatments were started at the age of 8.5 months and continued until sacrifice at 11.5 months of age. Mice received daily gavage administration of fish oil (eicosapentaenoic acid (EPA)/ docosahexaenoic acid (DHA) = 2:1) and/or topical instillation of Timolol (0.5%) once a day until the end of the treatment. Gas chromatographic analysis was used for lipid analysis. The dosage of EPA and DHA was adjusted so that the arachidonic acid (AA)/EPA blood ratio was within therapeutic range of 1 to 1.5. Animals used in this study underwent intraocular pressure measurements once a week on both eyes using TonoLab and Slit lamp biomicroscopy to monitor the disease progression. Density of RGCs was counted by using Brn3a antibody in retinal flat-mounts. Brn3a-positive RGCs were then manually quantified with the Fiji image analysis software. Western blot analysis was performed to determine the protein expression levels of Interleukin 18 (IL-18) and Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) inflammatory molecules in the retinas. Statistical analyses for these experiments were performed using ANOVA with the Tukey Multiple Comparison test.

**Results:** The RGC densities were found significantly higher in the  $\omega$ -3 PUFAs plus Timolol (1303.77 cells/mm<sup>2</sup>),  $\omega$ -3 PUFAs (768.40 cells/mm<sup>2</sup>) and Timolol-treated groups (995.70 cells/mm<sup>2</sup>) than in the untreated group (323.39 cells/mm<sup>2</sup>). Furthermore, expression of IL-18 protein was significantly reduced in the retinas of  $\omega$ -3 PUFA treatment group as compared with the untreated group (n=4). The expression of TNF- $\alpha$  protein was significantly reduced in the retinas of  $\omega$ -3 PUFA and Timolol treatment groups as compared with the untreated group (n=4).

**Conclusion:** Our findings suggest that  $\omega$ -3 PUFA supplementation has a neuroprotective effect in the DBA/2J mouse model of hereditary glaucoma, as demonstrated by the RGC density analysis. The combination treatment with  $\omega$ -3 PUFAs and Timolol has a better neuroprotective effect than Timolol or  $\omega$ -3 PUFAs as monotherapy. The importance of assessing AA/EPA blood ratio (<1.5) is emphasized in order for the dosage of  $\omega$ -3 PUFA to be adjusted with the aim to provide the maximum therapeutic effect. These data provide insight into the role of inflammation in the pathogenesis of glaucoma and indicate that  $\omega$ -3 PUFA administration could be beneficial in controlling inflammation in the retina. However, the lack of blockage of inflammation following  $\omega$ -3 PUFAs plus Timolol treatment, suggests that IL-18 and TNF- $\alpha$  downregulation may not be the only participant in  $\omega$ -3 PUFA-mediated neuroprotection. Further studies are required in order to further clarify the detailed mechanisms of this effect. These novel data might indicate a remarkable turning point in the current treatment approach of patients with glaucoma.

## PA09 Recent advances on SSc: genes, pathways and molecular interactions

Chairta P., Nicolaou P., Christodoulou K.

*The Cyprus Institute of Neurology and Genetics, Neurogenetics Department, Nicosia, Cyprus, Cyprus School of Molecular Medicine, Nicosia, Cyprus*

Systemic sclerosis (SSc) is a multisystemic rheumatic disease characterised by fibrosis, vasculopathy and autoimmunity. The exact aetiology of SSc is still unclear, but several studies show that various genetic factors and pathways may be involved. The aim of this study is to assess HLA alleles as well as non-HLA variants that have thus far been associated with SSc and further extract pathways and molecular interactions that may be involved in SSc pathogenesis. A comprehensive search of three electronic databases using broad search terms was completed. Articles retrieved were assessed based on a set of criteria. Functional enrichment analysis of SSc associated genes was performed using several biological databases. A total of 121 publications passed the filters and were reviewed. These publications showed that alleles and variants in specific HLA and non-HLA genes, respectively, are statistically significantly associated with SSc. Pathways analysis revealed that interleukin, inflammation mediated by chemokine and cytokine and T cell activation pathways are among the common extracted pathways associated with SSc. Network interactions analysis, of bibliography extracted genes, showed that specific HLA and non-HLA genes are highlighted as main "hubs" of interaction networks. PPI Hub proteins tool showed that genes which were not extracted from bibliography are also identified as main interacting "hubs". In conclusion, this study gathers the genetic factors that have statistically significantly been associated with SSc and identifies the possible pathways and interactions of implicated molecules. More research is needed at the molecular and cellular level to elucidate the main and causal variants/alleles/genes implicated in SSc pathogenesis.



## PA10 Seroprevalences of Sandfly Fever Viruses and West Nile Virus infections in Cyprus

Gaetan Billioud, Christina Tryfonos, Jan Richter and Christina Christodoulou

*Cyprus Institute of Neurology and Genetics, Department of Molecular Virology, Nicosia, Cyprus*

**Background & Aim:** Sandfly fever is an incapacitating disease due to sandfly-transmitted Phlebovirus infection that can lead to aseptic meningitis or meningoencephalitis if not managed appropriately. West Nile virus, a mosquito-borne Flavivirus, can induce neuro-invasive disease manifested by meningitis, encephalitis or acute flaccid paralysis. Main vectors for West Nile infection are mosquitos from the Culex species. Both vectors, sandflies and Culex mosquitoes, are endemic in Cyprus and very active during summer which put at risk not only regions' inhabitants but also for travellers' health. Epidemiological data are therefore needed for risk assessment and surveillance.

**Methods:** 333 sera collected between vectors' active periods of 2013 and 2014 were tested for markers of Sandfly Fever Virus (SFV) and West Nile Virus (WNV) infections by indirect immunofluorescence assay and ELISA, respectively.

**Results:** The overall IgG seroprevalence for SFVs was 29%, significantly increasing with age. Antibody prevalence rates in men (33%) and women (26%) were not statistically significant ( $p > 0.05$ ). SFVs IgM, early markers of infection, were also detected in 8 of 123 (6%) selected patients presenting symptoms of infection. Prevalence rate for WNV IgG was found to be 5%, without statistically significant differences between genders. WNV IgM could also be detected in selected patients presenting symptoms of infection.

**Conclusion:** These findings confirm the circulation of those infectious agents in Cyprus that can pose significant public health problems.

**Keywords:** Sandfly fever virus, West Nile Virus, Arbovirus, Prevalence, Cyprus.

## PA11 The role of mitochondria in neurodegenerative diseases and cancer

Georgia Sourso

*Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia Greece*

Mitochondria are important intracellular organelles contributing as integrators of cellular function. They produce adenosine triphosphate for energy production through oxidative phosphorylation and they are involved in cell signaling, cellular metabolism, production of reactive oxygen species, cell survival and cell death. It has been known from the literature that the abnormal function of mitochondria is associated with neurodegenerative diseases. More specifically, disruptions in the regulation of mitochondrial dynamics and protein misfolding can lead to neuronal death, resulting to neurodegenerative diseases. Represented by the mitochondria shaping proteins MFN1, MFN2, which are the regulators of fusion at the level of outer mitochondrial membrane, and OPA1 which

is involved in the fusion of the inner mitochondrial membranes and also plays a role in controlling cell-death, mitochondria could be fewer or suffer from fragmentation or elongation depending on either over-expression or knock-down of the abovementioned proteins. Another aspect of mitochondrial dynamics is the decreased motility of mitochondria. Furthermore, they could result in poor cell growth which enhances susceptibility to apoptosis. As far as cancer is concerned, mitochondria are crucially positioned for establishing resistance to cell death and sustaining proliferative signalling. Their role is essential for the metabolic shift to glycolysis- common in tumor cells. It is noteworthy that mitochondrial pathways mutations of mitochondrial enzymes are a general feature of cancer cells. They are associated with elongated mitochondria, while cell death is usually accompanied by mitochondrial fragmentation. That evidence shows the involvement of mitochondrial dynamics in cancer development which is similar with their role in neurodegenerative diseases. In conclusion, the observations presented suggest a prominent role for mitochondria in a plethora of pathways from cell proliferation to resistance to apoptotic stimuli and from cell mutation to decreased movement of mitochondria. However, these observations also suggest the potential for many new findings to come regarding the existing knowledge of basic mechanisms underlying pathologies and their relationship with mitochondrial morphology alterations in order to draw up novel strategies, to highlight how mitochondria are responsible for neurodegeneration work and if there is a similarity activity with cancer so as to improve the prognosis of an increasing number of patients.

## PA12 Gene-Mediterranean diet interactions studied by targeted metabolomics in serum of Greek-Cypriot females

Maria G. Kakkoura, Kleitos Sokratous, Maria A. Loizidou, Andreas Hadjisavvas, Kyriacos Kyriacou

*Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

Christiana A. Demetriou

*The Cyprus School of Molecular Medicine, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, Neurology Clinic D, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

**Background:** Reduction in breast cancer (BC) risk was previously observed to be significantly associated with a high adherence to the Mediterranean diet (MD), among the Greek-Cypriot women of the MASTOS study. Interestingly, further nutrigenetic studies in the same study population, have also shown that specific genotypes of common polymorphisms, which are involved in metabolic pathways, can enhance the protection of the high MD adherence against BC risk. A holistic approach of combining nutrigenetics with nutrimental metabolomics could help us shed light into the effects of gene-diet interactions on the levels of metabolites. Alterations on the levels of metabolites can be related to the degree of dietary adherence or enzymatic activity, which itself is affected by polymorphisms.



## Poster Abstracts

**Objective:** To assess the effect of the degree of the MD adherence as well as the effect of polymorphisms-MD interactions on the serum levels of specific metabolites (targeted metabolomics.)

**Methods:** We conducted a cross-sectional study that included 564 BC control (healthy with respect to BC) Greek-Cypriot women of the MASTOS case-control study, who had either the lowest or the highest adherence to the MD. The adherence to the MD (high loadings of vegetables, fruit, legumes and fish) was previously assessed by principal component analysis. Women of the study were also genotyped before for 9 polymorphisms in genes encoding enzymes that are involved in one-carbon metabolism, oxidative stress and xenobiotic metabolism. We developed an ultra-performance liquid chromatography-tandem mass spectrometry method, to measure in a single run, the serum concentration of 14 metabolites, which are markers of dietary intake or key players involved with the aforementioned enzymes.

**Results:** Increasing adherence to the MD significantly increased levels of 5-methyltetrahydrofolate (5-MTHF). There was a significant interaction between the GSTM1 deletion polymorphism and adherence to the MD, affecting the levels of either flavin mononucleotide (FMN) or 5-MTHF metabolites. Particularly, women with the homozygous null GSTM1 genotype and an increasing adherence to the MD had significantly increased levels of FMN or 5-MTHF. The MTHFR (rs1801133) polymorphism also significantly interacted with MD adherence on the levels of 5-MTHF. In particular, women with the homozygous wild type MTHFR genotype and an increasing adherence to the MD were observed to have significantly increased levels of 5-MTHF.

**Conclusions:** The healthy women, who carried either a homozygous null GSTM1 genotype or a homozygous wild type MTHFR genotype and had a high MD adherence, were found to have increased levels of antioxidant-related FMN and 5-MTHF, respectively. These novel findings of the interactions between polymorphisms and MD on the serum levels of key metabolites, suggest that there is a synergy between the high MD adherence and the genetic variation of the Greek-Cypriot women, in exerting the antioxidative effect of the MD.

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### PA13 Detection of the paternally inherited fetal alleles using fast temperature-tolerant COLD PCR for the prenatal diagnosis of $\beta$ -thalassaemia

Byrou S., Papasavva Th., Kleanthous M.

*The Cyprus Institute of Neurology and Genetics, Molecular Genetics Thalassaemia Department, Nicosia, Cyprus*

Makrigiorgos M.

*Dana Farber Cancer Institute, Harvard Medical School, Department of Radiation Oncology, Divisions of Genomic Stability and DNA repair, Physics and Radiation Therapy, Boston, USA*

E-mail: stefaniab@cing.ac.cy

$\beta$ -thalassaemia is the most common autosomal recessive single-gene disorder in Cyprus. Prenatal tests for at-risk pregnancies use invasive methods, so that development of a non-invasive prenatal diagnostic (NIPD) method is of paramount importance to prevent unnecessary risks inherent to invasive methods. The development of a NIPD assay for  $\beta$ -thalassaemia is based on the analysis of maternal plasma DNA for the detection of the paternally inherited fetal alleles. Herein, we present the successful detection of paternally inherited alleles in maternal plasma using a variation of Fast Temperature-Tolerant COLD PCR.

Fast Temperature-Tolerant COLD PCR is a rapid and inexpensive technique that uses a gradual increase of the denaturation temperature during thermo-cycling. This allows the minor allele to be enriched over the overwhelming background of a major allele. The sensitivity and specificity of the method, as well as its efficiency and applicability in the discrimination and detection of the minor paternally inherited alleles in the maternal background were evaluated and investigated. Two single-nucleotide polymorphisms (SNPs) located on the  $\beta$ -globin gene cluster with a high degree of heterozygosity in the Cypriot population were selected to be evaluated. Using spiked genomic DNA that mimics the fetomaternal relationship, the specificity and efficiency of this variation of Fast TT-COLD-PCR was examined setting the detection limit of minor alleles to 5%. In total seventeen maternal plasma samples from pregnancies at risk for  $\beta$ -thalassaemia were analysed in at least triplicate reactions for the selected SNPs using this variation of Fast TT-COLD-PCR. The paternal inherited allele was correctly detected in 92.96% of reactions tested (66/71) for SNP1 and in 97.34% (73/75) for SNP2. The results of this assay on the maternal plasma samples were confirmed by analysis of the corresponding CVS sample. These results demonstrate the efficiency and sensitivity of this variation of Fast TT-COLD-PCR in detecting the minor paternally inherited alleles in maternal plasma. Our results are extremely promising for using NIPD for  $\beta$ -thalassaemia as a service in Cyprus and the Eastern Mediterranean region.

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## PA14 Genome editing by non-homologous end joining as high-efficiency gene therapy for sickle-cell disease and $\beta$ -thalassaemia

Loucari C.

*Cyprus School of Molecular Medicine and Molecular Genetics of Thalassaemia Department, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

Van Dijk TB., Philipsen S.

*Erasmus University Medical Centre, Rotterdam, The Netherlands*

Lederer, CW., Kleanthous M.

*Cyprus School of Molecular Medicine and Molecular Genetics of Thalassaemia Department, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus. These authors have contributed equally to this work*

Email: lederer@cing.ac.cy

**Background:** Sickle-cell disease (SCD) and  $\beta$ -thalassaemia are amongst the most common and life-threatening haemoglobinopathies, and through decades of study have become paradigms for the management and therapy of monogenic diseases. Of note, pathology for both disorders is significantly ameliorated by elevated levels of the fetal  $\beta$ -like globin,  $\gamma$ -globin, which forms fetal haemoglobin (HbF:  $\alpha_2\gamma_2$ ), whose expression is curtailed in most adults by the BCL11A transcription factor.

**Aims:** The recently emerged RNA-guided endonuclease (RGEN) CRISPR/Cas9 genome editing system is utilized to achieve knockout of the  $\gamma$ -globin repressor BCL11A and thus re-activation of  $\gamma$ -globin expression as a universal therapeutic approach for SCD and  $\beta$ -thalassaemia. For disruption, BCL11A may be targeted by genome editing and efficient repair by non-homologous end joining (NHEJ), which would disrupt the edited sequence and thus in most cases affect BCL11A function. BCL11A also being required in non-erythroid cells prompted us to target the extra-long (XL) isoform of BCL11A, which is particularly abundant in erythroid cells. Besides a concurrent investigation of the structure-function relationship for the BCL11A transcription factor, the key aim of this study is to achieve therapeutic expression levels of  $\gamma$ -globin via its derepression in human erythroid thalassaemia cells.

**Methodology/Results:** Three pairs of short guide RNAs (sgRNAs) were designed to target genomic areas encoding BCL11A-XL. Additionally, as positive control for the induction of  $\gamma$ -globin derepression by BCL11A disruption, two sgRNAs were designed aiming to disrupt the BCL11A translation initiation site. To our knowledge this is the first time this approach has been taken to create functional knockouts by RGENs. All sgRNAs were initially evaluated in the HEK293T cell line, using a transient transfection approach with sgRNA- and Cas9-expressing plasmids. Thus, the most effective sgRNAs, in terms of genomic disruption, were selected and introduced into lentiviral vectors for further experiments in a HUDEP-2 cell line stably expressing Cas9. In addition to the previous experiments,

the non-integrating lentiviral vector system was utilized in order to establish genome-edited HUDEP-2 cells with reduced risk of off-target events compared to RGEN expression from integrating proviruses. In this way, the effect of BCL11A-XL knockout was investigated in a human erythroid cell line representing adult erythropoiesis.

**Conclusion:** At this point, highly efficient sgRNAs have been identified for different target sites. The results in HUDEP-2 cells indicate that BCL11A-XL-specific knockouts and corresponding  $\gamma$ -globin derepression may be therapeutic, pending analysis of efficiencies and tolerability in erythroid and non-erythroid progeny of patient-derived CD34+ cells. Further experiments in CD34+ cells, isolated from controls and thalassaemic patients, are required in order to demonstrate that the sgRNA-mediated  $\gamma$ -globin derepression is not accompanied by any disruption of B- and T-cell development and formation, an observation that was proven upon complete loss.

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## PA15 Characterisation of gene-therapy vectors for $\beta$ -thalassaemia

Stephanou C.

*Molecular Genetics Thalassaemia, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

Zanti M., Papasavva P., Loucari C.

*Molecular Genetics Thalassaemia, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, Cyprus School of Molecular Medicine, Nicosia, Cyprus*

Lederer CW., Kleanthous

*Molecular Genetics Thalassaemia, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, Cyprus School of Molecular Medicine, Nicosia, Cyprus. These authors have contributed equally to this work*

Patsali P.

*Molecular Genetics Thalassaemia, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, King's College London, London, United Kingdom*

Antoniou M.

*King's College London, London, United Kingdom*

Breda L., Rivella S.

*Haematology, Children's Hospital of Philadelphia, Philadelphia, PA, USA*

E-mail: coraleas@cing.ac.cy

Human  $\beta$ -globin disorders are severe hereditary anaemias of global significance. Of these,  $\beta$ -thalassaemia represents a quantitative  $\beta$ -globin defect and is of particular prevalence in Cyprus. Genetic



and experimental evidence suggests that either overexpression of a functional copy of  $\beta$ -globin or activation of the endogenous  $\beta$ -globin-like  $\gamma$ -globin gene would be therapeutic for patients with  $\beta$ -thalassaemia. Gene therapy (GT) by permanent transfer of  $\beta$ -globin into haematopoietic stem cells has already emerged in several clinical trials as a successful treatment option for mostly mild forms of  $\beta$ -thalassaemia. Towards an evaluation of existing and emerging GT vector systems and the treatment of severe forms of the disease, the present work used two of the most advanced GT platforms, the vectors GLOBE and T9W, for correction of  $\beta$ -thalassaemia in primary erythroid cells from transfusion-dependent patients. In addition to the prototype vectors GLOBE and T9W, this study included potentially  $\gamma$ -globin-inducing GLOBE derivatives that targeted the negative regulator of  $\gamma$ -globin, BCL11A, by RNA interference (RNAi). Aim of these RNApol-II-driven RNAi vectors was to achieve concurrent  $\beta$ -globin expression and  $\gamma$ -globin induction for potentially improved therapeutic efficiency compared to GLOBE. GLOBE, T9W, the T9W-derived AnkT9W vector, which harbours an ankyrin barrier insulator, a series of modified GLOBE-based RNAi vectors and RNApol-III-driven RNAi control vectors were used in same-sample experiments to compare performance and potentially identify derivatives with improved efficacy compared to prototype vectors. Using the same technical conditions throughout, we assessed vector titres, transduction efficiencies and key markers of disease severity, such as haemoglobin levels and phenotypic erythroid cell correction. Our preliminary data suggest that the independently developed prototype vectors showed equivalent performance, while overall function of GLOBE-based RNAi vectors was compromised, indicating that design improvements would be required to restore and possibly improve upon the performance of the GLOBE vector by RNAi-based BCL11A co-suppression. Overall, this type of head-to-head comparison of therapeutic vectors developed by two nominally competing laboratories is unprecedented and a paradigm of collaborative effort and transparency in the development of GT tools.

**Disclaimer:** The authors have no conflicts of interest to declare.

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**Additional Information:** The study received written informed consent from all subjects, and experimental protocols were approved by the Cyprus National Bioethics Committee (Applications EEBK/EP/2012/02 and EEBK/EP/2013/23).

## PA16 Induction of $\gamma$ -globin by antioxidants and natural modulators of metabolic pathways for the treatment of $\beta$ -thalassaemia

Stephanou C., Pandelis S., Phylactides M., Kleanthous M.

*1The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

E-mail:coraleas@cing.ac.cy

Beta-thalassemia is a disorder characterized by reduced synthesis of functional  $\beta$ -globin chains, leading to an imbalanced globin chain ratio, ineffective erythropoiesis and anaemia. Regular blood transfusions and iron chelation therapy constitute the main form of disease management. An alternative therapeutic approach is the production of fetal haemoglobin (HbF) through pharmacological re-activation of the endogenous  $\beta$ -like fetal  $\gamma$ -globin genes, which can substitute for the absent or reduced adult  $\beta$ -globin.

Oxidative stress is an important issue in  $\beta$ -thalassaemia. This is mainly attributed to iron overload due to increased iron absorption in the gastrointestinal tract, blood transfusions and the increased intracellular denaturation of unpaired  $\alpha$ -globin chains resulting in the release of iron into the body. Labile non-transferrin bound iron can be involved in chemical reactions that generate reactive oxygen species (ROS), which affect various cellular components, damaging vital organs including the heart, liver and endocrine system. In addition, endogenous antioxidant mechanisms are depleted due to the increased need to neutralize the oxidative stress. Oral administration of antioxidants in patients was shown to decrease ROS production in red blood cells.

Identification of compounds which combine antioxidant and HbF inducing activities would be ideal therapeutic agents for this disease, although it is unlikely that the two activities are directly related. Here we present screening data for  $\alpha$ -Lipoic acid, dipicolinic acid, penicillamine,  $\beta$ -carotene, L-carnitine, Acetyl-L-carnitine, L-Dopa, 3,3',5-Triiodo-L-Thyronine (the first 6 compounds have antioxidant activity), in order to evaluate their potential as HbF inducers in GM979 cells and/or primary erythroid progenitor cells. 3,3',5-Triiodo-L-Thyronine, which is not an antioxidant, was the only compound with substantial activity on the  $\gamma$ -globin gene promoter in GM979 cells. Further tests in primary erythroid progenitors showed that 3,3', 5-Triiodo-L-Thyronine also caused a substantial increase in HbF, F-cell and  $\gamma$ -globin mRNA levels.

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## PA17 Exosomal Muscle-Specific miRNAs in Serum of Myotonic Dystrophy Patients Relate to Muscle Disease Progress

**Andrie Koutsoulidou, Marinos Photiades, Kristia Georgiou, Leonidas A. Phylactou**  
*Department of Molecular Genetics, Function & Therapy, Cyprus Institute of Neurology & Genetics, P.O. Box 2346, 1683 Nicosia, Cyprus.*

### Tassos C. Kyriakides

*Yale Center for Analytical Sciences, Yale School of Public Health, 300 George Street, Suite 555, New Haven, CT 06520, United States of America*

### George K. Papadimas, Evangelia Kararizou

*Department of Neurology, Eginitio hospital, Medical School of Athens, 74 Vasilissis Sofias, 11528, Athens, Greece*

### Yiolanda Christou, Eleni Zamba Papanicolaou

*Neurology Clinic D, Cyprus Institute of Neurology & Genetics, P.O. Box 2346, 1683 Nicosia, Cyprus*

### Anna Łusakowska

*Department of Neurology, Medical University of Warsaw, Warsaw, Poland*

Myotonic dystrophy type 1 (DM1) is the most common form of adult-onset muscular dystrophy that primarily affects skeletal muscle and is characterised by progressive skeletal muscle wasting. Muscle wasting is currently being monitored through physical examination and electromyography. The discovery of reliable blood-based biomarkers is essential for the diagnosis and monitoring of the disease. Four muscle-specific miRNAs, miR-1, miR-133a, miR-133b and miR-206, were reported to be correlated with the progression of muscle wasting observed in DM1 patients. Specifically, the levels of the four muscle-specific miRNAs were found to be elevated in the serum of DM1 patients compared to healthy participants and are also elevated in the serum of progressive muscle wasting DM1 patients compared to disease stable DM1 patients. Although the fact that miRNAs stably exist in human serum is well established, the underlying reasons for this high stability remain largely unknown. The aim of this work was to investigate the ontology of the four muscle-specific miRNAs in the blood circulation of DM1 patients. We showed that the four muscle-specific miRNAs are encapsulated within exosomes isolated from DM1 patients. More interestingly, the levels of the four exosomal muscle-specific miRNAs were found to be associated with the progression of muscle wasting in DM1 patients. There has been a growing interest regarding the clinical applications of exosomes and their role in prognosis and therapy of various diseases. Based on our results, we propose that exosomal muscle-specific miRNAs may be useful molecular biomarkers for monitoring the progress of muscle wasting in DM1 patients.

## PA18 Molecular Typing and Epidemiology of Enteroviruses in Cyprus 2008-2010

**C. Tryfonos, J. Richter, C. Christodoulou**

*Department of Molecular Virology, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

**E-mail: tryfonos@cing.ac.cy**

Worldwide, human enteroviruses (HEVs) are responsible for a wide spectrum of clinical diseases. Even though usually associated with non-specific febrile illness, they can also cause more severe infections of the central nervous system manifested as meningitis, encephalitis or flaccid paralysis and therefore pose a serious public health problem especially during outbreaks. For this reason, the identification of HEV serotypes in clinical specimens is important for epidemiological surveillance and public health management.

Between 2008 and 2010, clinical specimens from a total of 107 patients with viral meningitis and/or symptoms of enteroviral infection were diagnosed enterovirus positive using a diagnostic Real-Time RT-PCR assay targeting the 5'non-coding region (5'NC). The number of positive samples appears to be significant given the small size of the Cypriot population (approx. 820,000 total, 0-14 years: 17%, 15-64 years: 70%, <65 years: 13%). Typing of viruses was carried out by sequencing a 300bp part of the VP1 capsid protein region, which has been shown to correlate well with the classical serotype classification. The serotype of each isolate was determined by BLAST search of the VP1 amplicon sequence against GenBank. In addition, a phylogenetic analysis of the 5'NC and VP1 region was carried out.

Twelve different serotypes were identified. The most predominant enterovirus species identified were Echovirus 30 (56.1%), followed by Echovirus 6 (10.3%) and Coxsackievirus A6 (7.5%). Coxsackievirus B5 and Echovirus 9 were detected only rarely. Phylogenetic analysis showed that the clustering of the strains in the 5'NC was in high concordance with the VP1 tree clustering. Serotype distribution corresponded essentially with observations reported from other European countries during the same period. Sequence comparisons revealed high homogeneity in the part of the VP1 region investigated between strains isolated in Cyprus and strains isolated elsewhere.



## PA19 Therapeutic effects of omega-3 supplementation in a dry macular degeneration model

Ekatherine Prokopiou, Tassos Georgiou, Panagiotis Kolovos, Maria Kalogerou, Anastasia Neokleous,

*Ophthalmos Research and Educational Institute, Nicosia, Cyprus*

Gregoris Papagregoriou, Constantinos Deltas,

*Laboratory of Molecular and Medical Genetics, Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*

Stavros Malas

*Developmental and Functional Genetics Group, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

**Corresponding authors:**

Ekatherine Prokopiou, Tassos Georgiou

*Ophthalmos Research and Educational Institute, Nicosia, Cyprus*

**Purpose:** One of the major causes of blindness in the elderly is age-related macular degeneration (AMD). Currently there are no guidelines for the treatment of dry AMD; therefore the aim of this study was to evaluate the therapeutic effects of omega-3 ( $\omega$ -3) and  $\omega$ -6 fatty acids (separately or in combination) in the CCL2<sup>-/-</sup> model of dry AMD. Monitoring the level of fatty acids in blood, including the eicosapentaenoic acid (EPA) and arachidonic acid (AA), served to adjust the treatment dosage (AA/EPA = 1-1.5). An increased level of EPA favours an anti-inflammatory effect, in contrast with high AA level which has been associated with pro-inflammatory actions.

**Methods:** Age-matched (9-months) animals were allocated to different groups: A) CCL2<sup>-/-</sup> untreated, B) CCL2<sup>-/-</sup> treated with  $\omega$ -3+ $\omega$ -6, C) CCL2<sup>-/-</sup> treated with  $\omega$ -3 and D) C57BL/6 untreated. Treatment was daily administered by gavage for 3 months. Blood samples were collected at different time points and eyes were processed at the end of the study for further analysis. Fatty acids analysis of blood and retina samples was investigated using a gas chromatographic technique and whole eyes were histologically examined using immunofluorescence staining, followed by confocal microscopy. An additional group of younger mice (3 months) was used for comparison purposes in the histological analysis. Real-time PCR (RT-PCR) and Western blot were performed using isolated retinas from each group in order to examine levels of some inflammatory mediators. Statistical comparison between groups was performed using the most appropriate test.

**Results:** Increased level of EPA and decreased level of AA were observed in both blood and retina samples in the  $\omega$ -3+ $\omega$ -6 and  $\omega$ -3 treated groups. Both treatment groups,  $\omega$ -3+ $\omega$ -6 and  $\omega$ -3 displayed significantly larger outer nuclear layer (ONL) thickness compared to the untreated CCL2<sup>-/-</sup> animals, with a more pronounced effect in the  $\omega$ -3 group ( $45.0 \pm 3.9 \mu\text{m}$  ( $p < 0.05$ ) and  $62.8 \pm 4.9$

$\mu\text{m}$  ( $p < 0.001$ ), respectively, compared to  $32.3 \pm 1.5 \mu\text{m}$ ). The mean ONL thickness in the  $\omega$ -3 group was even greater than that in the younger mice ( $62.8 \pm 4.9 \mu\text{m}$  compared to  $48.9 \pm 1.9 \mu\text{m}$ , respectively,  $p < 0.05$ ). RT-PCR results revealed a minor decreased expression level of Toll-like receptor 3 (TLR3) and a more significant reduction in nuclear factor- $\kappa$  B (NF- $\kappa$ B), in both treatment groups. From Western blot analysis, IL-18 protein levels demonstrated a significant reduction in the  $\omega$ -3 treated group only.

**Conclusion:** Supplementation with high doses of  $\omega$ -3+ $\omega$ -6 or  $\omega$ -3 alone in such a dose so that the blood AA/EPA = 1-1.5 suggests a protective mechanism in the CCL2<sup>-/-</sup> animal model of dry AMD, with a more beneficial effect when  $\omega$ -3 are used alone. It has been considered that the resolution of inflammation plays a critical role in the disease's progression; however, our findings indicated that inflammation is not the only determinant factor. An additional mechanism, perhaps a regenerative process might be involved following administration of  $\omega$ -3 fatty acids, which could justify the increased number in retinal photoreceptors. Further work is encouraging in order to establish a better understanding of this effect.

## PA20 Breast cancer risk prediction using a Polygenic Risk Score (PRS)

Kyriaki Michailidou

*Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, The Cyprus School of Molecular Medicine, Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge*

Maria Loizidou

*Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics*

Kyriacos Kyriacou, Andreas Hadjisavvas

*Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, The Cyprus School of Molecular Medicine*

Yiola Marcou, Eleni Kakouri, Maria Daniel, Panayiotis Papadopoulos

*Bank of Cyprus Oncology Center, Nicosia, Cyprus*

Simon Malas

*Department of Oncology, Limassol General Hospital, Limassol, Cyprus*

Breast cancer is the most common cancer in women in the world. In Cyprus over 500 new cases are being diagnosed annually. Rare mutations in high risk genes like BRCA1 and BRCA2 have been identified, accounting for a fraction of the excess familial relative risk of breast cancer. Previous work in the department led to the identification of novel mutations in both the BRCA1 and 2 genes, including a founder mutation in BRCA2. However these mutations in the BRCA1 and 2 genes explain only a small fraction of the total number of cases.



Genome-wide association studies (GWAS) have identified several common and low risk variants (Single Nucleotide Polymorphisms or SNPs) associated with breast cancer predisposition. These variants account for a small amount of the risk associated with the disease and thus cannot be used individually. Recently several approaches have been used in an effort to collectively use these SNPs to predict breast cancer risk (Polygenic Risk Score calculations). In order to evaluate the predictive performance of the common genetic variants in our population, we used a total of 14 SNPs that have been identified previously in European ancestry GWAS and genotyped in 1109 cases and 1177 controls from the MASTOS study in Cyprus.

The PRS constructed was significantly associated with increased risk of developing breast cancer with OR (95% CI) 1.73 (1.29-2.30),  $p$ -value= $2.15 \times 10^{-4}$ . This has important implications in breast cancer risk prediction in the Cypriot population and its potential use in population screening for breast cancer, since it can be used to provide a more accurate individual risk score.

## PA21 The effects of IgG autoantibodies from MS patients compared to healthy controls in astrocytes.

**Natalia Filippidou, Christina Christodoulou, Marios Pantzaris, Anastasia Lambrianides**  
*Cyprus School of Molecular Medicine, The Cyprus Institute of Neurology and Genetics.*

**Background:** Multiple Sclerosis is a chronic, multifactorial, inflammatory disease of the CNS which is characterised by neurological disability that appears usually in relapses (relapsing – remitting MS) and eventually evolves to gradual loss of neurologic function (secondary progressive MS). There is a strong autoimmune component in MS with numerous studies reporting autoreactive T-cells and B-cells and elevated levels of autoantibodies targeting CNS antigens such as myelin associated molecules, phospholipids and others. The aim of this study is to evaluate the potential role of IgG autoantibodies from MS patients in vitro in an astrocytic cell line.

**Methods:** HiTrap IgG Purification Columns (GE Healthcare) were used for purification of total IgG from a group of 10 Multiple Sclerosis (MS) patients found to be positive for IgG antibodies against cardiolipin and 10 Healthy Controls (HC) which were age and gender matched. The purified samples were then pooled together according to group and tested for endotoxin. 100µg/ml IgG were used to stimulate an astrocytic cell line, U87, at different time points including 10 minutes, 1 hour, 3 hours, 6 hours and 12 hours. Subsequently, the cells were lysed, and the cell lysates were quantified and analysed by immunoblot using antibodies to p38 MAPK and NFκB.

**Results:** Stimulations of U87 astrocytes with 100µg/ml IgG from MS patients and HC at different time points, showed that there was considerable increase of phospho p38 MAPK and phospho p65 NF-κB at 3 hours of stimulation with MS-derived IgG compared to HC-derived IgG.

**Conclusion:** This is a novel finding which can prove crucial for understanding the mechanism of the effects of these autoantibodies exerted on astrocytes and how they may be implicated in MS pathogenesis. Future studies are required to further evaluate the functional activity of IgG autoantibodies from MS patients and the particular implication of astrocytes as effector cells.

Disclosure: The authors have nothing to disclose.

## PA22 Nogo receptor complex kinetics in experimental autoimmune encephalomyelitis

**Paschalis Theotokis, Olga Touloumi, Roza Lagoudaki, Evangelia Nousiopoulou, Evangelia Kesidou, Nikolaos Grigoriadis**

*B' Department of Neurology, Laboratory of Experimental Neurology and Neuroimmunology, AHEPA University Hospital, Aristotle University of Thessaloniki, Stilponos Kiriakides str. 1, 54636, Thessaloniki, Central Macedonia, Greece.*

Numerous studies indicate the dynamic and high potential role of neurite outgrowth inhibitor Nogo and its receptor complex (NgR complex) to inhibit, guide and modulate the injured and demyelinating tissue in various central nervous system (CNS) disease models. The purpose of this study was to describe the spatiotemporal expression of NgR complex molecules LINGO-1, p75 and TROY within the inflammatory sites of the experimental model of Multiple Sclerosis (MS) in mice. 30 C57BL/6 mice were subcutaneously injected with the myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide and developed chronic experimental autoimmune encephalomyelitis (EAE). The study included acute (days 18-22) and chronic (day 50) time points that were compared to controls respectively. All animals were examined daily using a 6-grade scale. Localization and neuropathological study of NgR complex was performed with double immunofluorescence (dIF) on 6µm coronal paraffin sections while molecular analysis was performed with real-time PCR in spinal cord extracts.

MOG-inoculated animals developed a typical chronic-MOG EAE pattern with mean maximal score (MMS) =  $3.76 \pm 0.28$ . The levels of the NgR complex were found to fluctuate, depending on the stage studied; LINGO-1 was increased in perivascular inflammatory foci ( $467.8 \pm 48.18$  cells/mm<sup>2</sup>) of acute phase while an additional increase was detected in axonal structures (Integrated density,  $249.156 \pm 26.177$ ) of chronic phase. Expression of p75 was increased only in residual inflammatory foci of chronic phase (IntDen,  $121.521 \pm 15.709$ ) while TROY was restricted within inflammatory cells at the lesion sites of acute phase ( $106.7 \pm 9.57$  cells/mm<sup>2</sup>).

Such dynamic expression of the Nogo receptor complex, it may support an alternative role that could include the confinement of the inflammatory reaction or the rampant sprouting of axons in chronic EAE lesions.



## PA23 Immunization of mice with syngeneic neurospheres may lead to antibody elicitation to self antigens

Ioannis Paspaltsis

Prion Diseases Research Group, School of Pharmacy, Aristotle University of Thessaloniki, Greece,  
Laboratory of Experimental Neurology and Neuroimmunology, Aristotle University of Thessaloniki, Greece

Evangelia Kesidou, Eva Nousiopoulou, Olga Touloumi, Roza Lagoudaki, Theodoros Sklaviadis

Prion Diseases Research Group, School of Pharmacy, Aristotle University of Thessaloniki, Greece

Nikolaos Grigoriadis

Laboratory of Experimental Neurology and Neuroimmunology, Aristotle University of Thessaloniki, Greece

Neural precursor cells (NPCs) attracted much attention by providing new therapeutic approaches for the treatment of neurodegenerative diseases such as multiple sclerosis. Transplantation of NPCs has been proposed to improve the functionality of damaged regions of the central nervous system through both, immunomodulating and restoring mechanisms of action. Although NPCs are considered as non-immunogenic, previous observations in our laboratory showed activation of the immune system in mice upon immunization with NPCs protein lysates. Isolation of monoclonal antibodies from these mice would ultimately address this uncertainty, contributing also to the identification of possible antigens. C57bl/6 mice were immunized against syngeneic neurospheres. RNA was isolated from the spleens and reverse transcribed. The Fab portions from different IgG subclasses were amplified by PCR and inserted sequentially into an appropriate phagemid vector. The arisen Fab phage display library was subjected to three biopanning rounds against immobilized protein lysates derived from neurospheres. The inserted DNA-Fab fragment encoding sequence of plasmids isolated after the last round of biopanning was read by Sanger sequencing. Fab fragments of unique clones were expressed in *E. coli* cells and purified by affinity chromatography. Purified recombinant monoclonal Fab fragments were/are under evaluation for their ability to recognize NPCs antigens by western blot, immunohistochemistry and immunoprecipitation (IP). The antisera from immunized mice have been proved to be strongly immunoreactive against NPCs protein lysates as compared to its non-immunized counterpart. To isolate monoclonal Fab antibody fragments, a Fab phage display library was created with a titer of about  $2 \times 10^6$  clones. More than 30 phagemids isolated after the third round of biopanning were analyzed in regards to the insert and sequenced. Some IgG Fab fragments showed to be immunoreactive when used to probe NPCs or total brain lysates in western blots. Also positive signals were obtained in distinct brain regions by immunohistochemistry. Up to date no positive signal was obtained by IP, which could shed light to the nature of the specific antigens. IP conditions are currently under optimization. Antisera from mice immunized with NPCs lysates have been showed to be immunoreactive against protein extracts from the same cells in western blots. This is indicative for the existence of antigenic proteins.

Application of the Fab phage display technique provided monoclonal Fabs, which in turn would be helpful to identify the recognized antigens by means of IP and mass spectrometry. Antigen identification would provide insights about the antigenicity of transplanted NPCs and their therapeutic mode of action.

The authors certify that there is no conflict of interest to declare.

## PA24 Towards understanding the role of Naa40 in gene regulation and cancer

Christina Demetriadou, Demetria Pavlou, Antonis Kirmizis

Department of Biological Sciences, University of Cyprus

N-terminal acetyltransferases (NATs) are evolutionarily conserved enzymes which catalyze the acetylation of the  $\alpha$ -amino group of the first amino acid residue of a protein. So far, six different NAT protein members have been identified in eukaryotes, noted as NatA-NatF. Naa40 (Nat4) is a unique NAT that specifically acetylates histones H4 and H2A only but its regulatory function still remains elusive. Very recently evidence from our lab showed that Nat-ac of H4 by Naa40 in yeast, interplays with the adjacent H4R3me2 histone mark to activate the transcription of rDNA.

Furthermore, we have recently shown that depletion of Naa40 in HCT-116 and HT-29 colon cancer cells triggers apoptosis, whilst non-cancerous mouse fibroblasts remain viable. Here we examine the effects of Nat-ac by Naa40 on chromatin and gene regulation in human cells. Upon Naa40 knockdown the global levels of different activating and repressive histone modifications in the epigenome of colon cancer cells are altered. Specifically, we observe a robust decrease in the total levels of H4R3me2, which coincides with reduced expression of PRMT5, the enzyme that catalyzes H4R3me2. In line with the above results, we find that depletion of Naa40 results in altered expression of known PRMT5 direct target genes that are either oncogenes or tumour suppressors.

Overall, the results demonstrate that Naa40 may control the expression of cancer related genes by regulating the expression of PRMT5 enzyme. Elucidating the molecular mechanisms underlying Naa40 gene regulation will be important to assess its potential as a therapeutic target.



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