



Unlocking NRF2 Research for Future Clinical Therapies *20 – 21 February 2025, Sapienza University of Rome, Italy*

The European COST Action CA20121, titled “Bench to Bedside Transition for Pharmacological Regulation of NRF2 in Non-Communicable Diseases (BenBedPhar),” is organizing its third industry showcase meeting aimed at strengthening connections between experts in NRF2 research and biopharmaceutical companies. This event will provide a platform for companies to promote their NRF2-based drug development pipelines while fostering collaboration with basic researchers and clinicians. The meeting will be held in a hybrid format, offering both online and in-person participation. Stakeholders from various sectors of the entrepreneurial ecosystem are invited to join and engage in this exciting opportunity.

Organizers:

Fabio Di Domenico. Sapienza University of Rome, Rome, Italy

Marzia Perluigi. Sapienza University of Rome, Rome, Italy

Brigitta Buttari. Istituto Superiore di Sanità, Rome, Italy

Antonio Cuadrado. Autonomous University of Madrid, Madrid, Spain

Venue:

Aula Multimediale I, Department of Human Neuroscience, General Physiology and Anthropology (CU026), Sapienza University Viale Regina Elena 332, Rome, Italy.



Entrance to the University from Viale Regina Elena

Entrance to the Venue in front of the Cafeteria



Thursday 20th of February

09:00-09:15. Fabio Di Domenico. Sapienza University of Rome, IT.

Welcome.

09:15-09:30 Antonio Cuadrado. COST Action CA20121 chair. Autonomous University of Madrid, ES.

Introduction Bench to Bedside Transition for Pharmacological Regulation of NRF2 in non-communicable diseases”

Models and tools to study NRF2 pharmacology

SESSION I- Chair: Gina Manda. Victor Babes National Institute of Pathology, RO.

09:30-10:00 Ian Cople. University of Liverpool, UK

Biomarkers for monitoring NRF2 activity and drug response in chronic diseases

10:00-10.30 Roberto Motterlini (online). University Paris Est Créteil, FR.

The NRF2/heme oxygenase/CO pathway as a target for drug discovery

10:30-11.00 Mojgan Masoodi. University of Bern, CH

Investigating potential role of NRF2 activation on lipid metabolism in regression of MASH

11:00-11:30 Coffee Break

SESSION II- Chair: Christina Morgenstern. Medical University of Vienna, AT.

11:30-12:00 Albena Dinkova-Kostova. University of Dundee

Pharmacological regulation of the KEAP1/NRF2 axis

12:00-12:30 Geoffrey Wells. University College London, UK.

Biophysical evaluation of KEAP1 and beta-TrCP binders

12:30-13:00 Anders Bach (online). University of Copenhagen, DK.

Targeting Keap1 with fragment-based drug discovery

13:00-14:30 Lunch Break

SESSION III- Chair: Brigitta Buttari. Istituto Superiore di Sanità. IT.

14:30-15:00 Elke Heiss (online). University of Vienna, AT.

NRF2 at the interface between cellular energy metabolism and stress response

15:00-15.30 Anna Grochot-Przędzek. Jagiellonian University, PL.

NRF2 in cardiovascular disease

15:30-16.00 Sandra Tenreiro. Universidade NOVA de Lisboa, PT.

Targeting NRF2: An Effective In Vitro Model for Drug Discovery in Age-Related Macular Degeneration

16:00-16:30 Coffee Break

ROUND TABLE- Chair, Ian Cople. University of Liverpool, UK.

16:30-17:30 Open to all participants. Endpoints in preclinical and clinical studies involving to NRF2-related therapy

Friday 21st of February

Progress towards the clinic

SESSION IV-Chair: Fabio Di Domenico. *Sapienza University of Rome, IT.*

09:00-09:30 Ioannis Trougakos, GenCell-TSBiotech, GR.

Screening natural products for the identification of NRF2 activity modulators

09:30-10:00 Raquel Fernández-Ginés, Servatrix Biomed S.L., ES.

Targeting NRF2 in therapy against liver fibrosis

10:00-10:30 Christina Morgenstern. Medical University of Vienna, AT.

Unlocking the power of NRF2 Biomarkers, at crossroad of disease and therapy

10:30-11:00 Coffee Break

SESSION V- Chair: Santiago Cuevas. *BioMedical Research Institute of Murcia, ES.*

11:00-11:30 Anna-Liisa Levonen. University of Eastern Finland, FI.

Biomarkers of NRF2 activation in cancer

11:30-12:00 Harry Van Goor. University Medical Center Groningen, NL.

Sodium thiosulfate as a modulator of NRF2

12:00-12:30 Iveta Bernatova. Slovak Academy of Sciences, SK.

Vascular function in rat models of prehypertension with and without hypertriglyceridemia: can dimethyl fumarate therapy be beneficial?

12:30-14:00 Lunch Break

14:00-15:30 Networking activities

15:30-16:00 Coffee Break

SESSION VI-Chair: Rumen Kostov. *Milonex, UK*

16:00-16:30 Marzia Pierluigi. Sapienza University of Rome, IT.

Aberrant BACH1/Nrf2 axis in Down Syndrome brain contributes to early onset Alzheimer-like neuropathology

16:30-17:00 Gerasimos Sykiotis (online). University of Lausanne, CH.

Serendipity as a strategic tool to expand omaveloxolone's medical landscape

17:00-17:30 Nil Roy (online). Vividion, USA.

Inhibitors of NRF2 in cancer therapy

17:30-18:00

CLOSING COMMENTS

Fabio Di Domenico

Marzia Perluigi

Brigitta Buttari

Antonio Cuadrado

Unlocking NRF2 Research for Future Clinical Therapies

Introduction Bench to Bedside Transition for Pharmacological Regulation of NRF2 in non-communicable diseases

Chair: Antonio Cuadrado

Autonomous University of Madrid, Madrid, Spain

COST Action CA20121, known as BenBedPhar, focuses on the "Bench to Bedside transition for pharmacological regulation of NRF2 in non-communicable diseases." Non-communicable diseases (NCDs), including cancer, diabetes, cardiovascular, neurodegenerative, respiratory, and immune disorders, account for 77% of all deaths in Europe. The transcription factor NRF2 is a master regulator of cytoprotective responses and a promising target for drug development. BenBedPhar fosters collaboration to translate NRF2 research into innovative therapeutics, bridging the gap between scientific advancements and economic opportunities.

A key goal of BenBedPhar is the economic exploitation of NRF2-based pharmacology. By connecting academic researchers with biotech SMEs, pharmaceutical companies, and investors, the network fosters the commercialization of NRF2-targeting compounds. Industry showcases serve as crucial platforms for identifying market needs, forming partnerships, and accelerating drug development pipelines. The showcases in Madrid and Athens enabled direct dialogue between scientists and industry leaders, highlighting potential NRF2-based therapeutics for commercial development.

Our industry showcase in Rome will build upon previous discussions, providing a vital follow-up to ensure that promising NRF2-related discoveries move closer to market application. Strengthening these collaborations is essential for developing new drugs, securing funding, and enhancing Europe's position in NRF2-driven biotechnology. By fostering innovation and commercialization, BenBedPhar contributes to the economic growth of the pharmaceutical sector while addressing urgent medical needs

S1.01 Biomarkers for monitoring NRF2 activity and drug response in chronic diseases

Ian Copple

University of Liverpool, UK

Email: ian.copple@liverpool.ac.uk

The transcription factor NRF2 protects mammalian cells against chemical and oxidative stress. NRF2 is a therapeutic target of interest in a variety of diseases for many pharmaceutical companies. Whilst methods for determining if NRF2 has been activated in tissue samples are well established, these samples are difficult to access clinically. Instead, there is a need to develop a robust method for monitoring NRF2 activity non-invasively (e.g. using clinically accessible blood samples), but little work has been done in this area. This has limited our ability to demonstrate that drugs have the intended effect on NRF2 in humans, and to correlate this with therapeutic responses in clinical trials. In this presentation, I will describe non-clinical and clinical studies being conducted by my group to address this knowledge gap. The goal of this work is to determine the optimal strategy for monitoring the activity and therapeutic response of NRF2 non-invasively in humans, to support the development and use of NRF2 activators as novel therapies.



Ian Copple is Professor of Pharmacology & Toxicology and currently holds a 5-year Senior Nonclinical Fellowship from the UK Medical Research Council. Ian leads the StressResponse Lab, a group of 8 pre/post-doctoral researchers interested in pharmacological and toxicological aspects of stress response pathways, and particularly the NRF2 oxidative stress response. He is also academic lead of the Human Liver Research Facility in Liverpool (www.liverpool.ac.uk/hlrf).

He works extensively with the pharmaceutical industry and has participated in several EU Innovative Medicines Initiative academic-industry research programs. Ian has received several awards for his research from national and international societies, including the 2023 Early Career Toxicology Award from the American Society of Pharmacology & Experimental Therapeutics (ASPET) and the 2018 Early Career Investigator Prize from the British Toxicology Society. He is also a Fellow of the British Pharmacological Society and the current Chair of the Scientific Subcommittee of the British Toxicology Society.

S1.02 The NRF2/heme oxygenase/CO pathway as a target for drug discovery

Roberto Motterlini and Roberta Foresti

Faculty of Health, University Paris-Est Créteil, INSERM, Créteil, France

Email: roberto.motterlini@insrm.fr

Heme oxygenase-1 (HO-1) is a key antioxidant and cytoprotective protein which expression is regulated by NRF2 activation. HO-1 catalyzes the degradation of heme into carbon monoxide (CO), a gasotransmitter with well-established anti-inflammatory properties and the ability to modulate energy metabolism. To exploit the therapeutic potential of HO-1, our team has developed compounds that activate NRF2 and deliver CO both in vitro and in vivo. In this presentation, we will report on the efficacy of CORM-401, an orally active CO-releasing molecule, in preventing body weight gain in a mouse model of high-fat diet-induced obesity. Our results demonstrate that CORM-401 significantly improves glucose metabolism, enhances insulin sensitivity, and reprograms the gut microbiota to a healthier phenotype in obese mice. In the second part of this lecture, we will present data on HYCOs—hybrid compounds that link CO-releasing molecules with well-established NRF2/HO-1 inducers for treating inflammatory-related disorders. We will demonstrate that selected HYCOs efficiently release CO, upregulate Nrf2/HO-1, and significantly reduce anti-inflammatory markers in monocytes stimulated with lipopolysaccharide (LPS). Furthermore, administration of HYCOs in mice led to a variety of beneficial effects in models of skin disease, including accelerated skin wound healing and a reduction in psoriasis-induced inflammation. These results underscore the promising action of our compounds for treating metabolic dysfunction and inflammatory conditions.



Roberto Motterlini is Director of Research (DR1) at INSERM U955 within the Faculty of Health, University of Paris Est, France. He has a long-standing interest in the regulation, activity and biological significance of heme oxygenase-1 (HO-1), a ubiquitous defensive protein that degrades heme to carbon monoxide (CO) and biliverdin. His early research focused on the role of Nrf2 as a transcription factor in controlling HO-1 gene expression and uncovered the vasodilatory, anti-ischemic and anti-inflammatory properties of CO. Dr. Motterlini's studies led to the development of CO-releasing molecules (CO-RMs), small active compounds that deliver controlled amounts of CO in vivo and exert important

pharmacological actions to counteract vascular, inflammatory and metabolic disorders. Dr. Motterlini's group has also developed and characterized a new class of hybrid compounds, termed HYCOs, which can activate Nrf2 and simultaneously release CO. He has filed patents for the use of CO-RMs and HYCOs in the treatment of inflammatory and metabolic diseases, and he is currently investigating the gut microbiota as a potential target for CO's mechanisms of action.

S1.03 Investigating potential role of NRF2 activation on lipid metabolism in regression of MASH using relevant mouse model

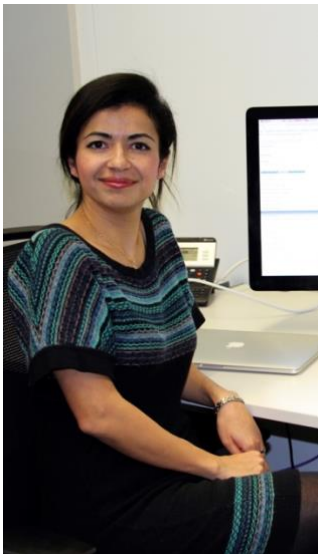
Prof. Dr. Mojgan Masoodi

University Hospital Bern, Switzerland

Email: Mojgan.masoodi@insel.ch

Metabolic dysfunction-associated fatty liver disease (MASLD) affects approximately 25–30% of the global population, posing a significant and growing public health concern. It can progress to metabolic dysfunction-associated steatohepatitis (MASH). Despite extensive global research, no effective therapy is currently available, partly due to the complex and multifactorial mechanisms underlying disease development and progression. MASH is characterized by steatosis, leading to toxic lipid accumulation, mitochondrial dysfunction, oxidative stress, and chronic inflammation. NRF2 plays a key role in suppressing the inflammatory phenotype of macrophages and proinflammatory enzymes such as COX-2 and NOS-2. Patients with chronic liver disease exhibit increased oxidative stress, chronic low-grade inflammation and lipid peroxidation.

To establish an appropriate experimental model, we have recently compared MASH mouse model (WD+CCL4) to human MASH and showed resemblance in terms of pathology and metabolic alterations. We observed a strong correlation between gene sets regulating NRF2 activation and those involved in fatty acid metabolism and oxidation in both human MASH and our mouse model. Additionally, mass spectrometry imaging identified zone-specific lipid metabolism and oxidative stress during MASH progression. Currently, we are investigating the role of NRF2 activation in regulating lipid metabolism in regression of MASH.



Mojgan Masoodi is currently an Associate Professor at Faculty of Medicine, University Hospital Bern in Switzerland. She is also an adjunct professor at Department of Nutritional Sciences, Faculty of Medicine, University of Toronto. She received her doctorate in pharmacology and targeted drug delivery in 2002 (Pharm D), and then completed her PhD in lipid metabolism and cell signalling in United Kingdom. In 2009, she joined the Medical Research Council (MRC) in Cambridge as an Investigator Scientist. She led a group in Nestle Institute of Health Sciences at EPFL campus with the focus on lipid metabolism and medical nutrition from 2012-2019 before joining University hospital Bern. Her group is investigating lipid metabolism and the function of lipids in the regulation of physiological processes associated to metabolic disorders, developing approaches for patient stratification and intervention that could improve lipid metabolism. Her lab uses combination of observational clinical studies that monitor metabolism during disease progress with in vivo/in vitro models that elucidate related mechanisms.

S2.01 Pharmacological Regulation of the KEAP1/NRF2 Axis

Albena T. Dinkova-Kostova

Division of Cancer Research, University of Dundee School of Medicine, Dundee, United Kingdom

Email: a.dinkovakostova@dundee.ac.uk

Transcription factor NRF2 and its principal negative regulator KEAP1 represent an attractive therapeutic target for chronic conditions with underlying oxidative stress and inflammation. The consequences of pharmacological NRF2 activation are long-lasting, allowing efficacy with intermittent drug administration. Small-molecule electrophilic and non-electrophilic NRF2 activators are in drug development, and two electrophiles, dimethyl fumarate and omaveloxolone, are clinically used. Electrophiles modify reactive cysteines in KEAP1, preventing NRF2 ubiquitination and free KEAP1 regeneration, resulting in accumulation of *de novo* synthesized NRF2. Non-electrophilic compounds include protein-protein interaction (PPI) inhibitors, which bind specifically to the NRF2-binding site of KEAP1, stabilizing NRF2 by preventing the KEAP1:NRF2 interaction. The mode of action of these two classes of NRF2 activators in the cellular environment can be distinguished by use of Förster resonance energy transfer-based multiphoton fluorescence lifetime imaging (FRET-FLIM) and the cellular thermal shift assay (CETSA). PPI inhibitors, but not electrophiles, increase the thermostability of KEAP1 and cause dissociation of the KEAP1:NRF2 protein complex. Furthermore, the amount of KEAP1-bound NRF2 increases upon treatment with electrophiles, whereas treatment with PPI inhibitors disrupt KEAP1 binding to NRF2, as well as other KEAP1-binding partners. In contrast to electrophiles, PPI inhibitors increase the levels of KEAP1, likely due to disrupted KEAP1 interaction with p62 and subsequent impaired KEAP1 autophagic degradation. The functional significance of the disruption of the other KEAP1 interactors is currently unknown.



Albena Dinkova-Kostova is a Professor of Chemical Biology at the University of Dundee School of Medicine (UK). She graduated in Biochemistry and Microbiology from Sofia University (Bulgaria) and obtained her PhD degree in Biochemistry and Biophysics from Washington State University (USA). She subsequently trained in Pharmacology at Johns Hopkins University School of Medicine (USA), where she continues to hold an Adjunct Professor position. She joined the University of Dundee in 2007 as a Research Councils UK Academic Fellow and a research group leader. Her group collaborates with basic scientists and clinicians, and with the pharmaceutical industry. In her research, at the interface of Chemical Biology and Medicine, she is committed to understanding how cells and organisms respond to oxidative, inflammatory, and metabolic stress, and is working

towards development of strategies for protection against chronic disease. She was named among the top influential academics in Clarivate's Highly Cited Researchers 2019, 2020, 2021, 2022, 2023 and 2024 lists.

S2.02 Biophysical evaluation of KEAP1 and β -TrCP binders

Geoff Wells

UCL School of Pharmacy, London UK

Email: g.wells@ucl.ac.uk

The therapeutic potential of Nrf2-inducing molecules spans several disease states including chronic neurodegenerative diseases, inflammatory conditions and possible roles in cancer chemoprevention. Binders for KEAP1 and β -TrCP have the potential to inhibit Nrf2 ubiquitination and thereby increase its transcriptional activity and influence the activity of other proteins that are regulated by these E3 ligases. Compounds that bind to KEAP1 in a reversible manner have been widely described, spanning a range of binding affinities and physicochemical properties. In contrast compounds that interact with β -TrCP have not been widely studied and their therapeutic potential is relatively unexplored. The two proteins have similarities and differences in their structural features, binding motifs and activity in relation to NRF2. This presentation will discuss some of our recent studies characterizing the binding behaviour of KEAP1 and β -TrCP-interactive compounds against the wild-type proteins and, in the case of KEAP1, selected mutant versions of the protein.



Dr **Geoff Wells** is an Associate Professor in the Department of Pharmaceutical and Biological Chemistry at UCL School of Pharmacy. He graduated in Pharmacy at the University of Nottingham. He pursued a PhD in medicinal chemistry at the University of Nottingham under the supervision of Professor Malcolm Stevens FRS. After a period as a postdoctoral researcher working on anticancer drug discovery projects in Nottingham, he spent four years in the Gene Targeted Drug Design Research Group at the School of Pharmacy, University of London. This was followed by two years working for Pharminox Ltd, an anticancer drug discovery company, as Drug Discovery Project Leader. In October 2007, Dr Wells took up the position of Lecturer in Medicinal Chemistry at The School of Pharmacy. His research work has focused on the design and synthesis of

compound classes that affect redox homeostasis, interact with DNA in a sequence selective manner and that have selective cytotoxicity profiles. His current interests include the rational design of agents that interact with molecular targets of relevance to the infectious diseases and the chemoprevention of cancer.

S2.03 Targeting Keap1 by fragment-based drug discovery to reduce oxidative stress and inflammation

Anders Bach

Department of Drug Design and Pharmacology, University of Copenhagen

E-mail: anders.bach@sund.ku.dk

Activating the cytoprotective response of nuclear factor erythroid 2-related factor 2 (Nrf2) can reduce oxidative stress and inflammation. A promising strategy is to inhibit the protein-protein interaction between Kelch-like ECH-associated protein 1 (Keap1) and Nrf2 using noncovalent compounds that target the Keap1 Kelch domain. These compounds offer the potential for greater specificity, compared to covalent Keap1-reacting Nrf2 activators. However, developing druglike noncovalent Keap1-Nrf2 inhibitors is challenging due to the size and polarity of the Kelch binding pocket. In this talk, I will present our latest efforts in generating and optimizing Keap1-Nrf2 inhibitors derived from our fragment-based drug discovery platform. I will discuss the challenges of achieving high cellular potency, druglike properties, and in vivo activity in one compound, and provide updates on advancing our most promising compounds as potential treatments for kidney and liver diseases linked to oxidative stress and inflammation.



Anders Bach earned his PhD in medicinal chemistry from the University of Copenhagen (UCPH) in 2009, where he developed compounds targeting PDZ domains involved in protein-protein interactions (PPIs) in the CNS. His key discovery was the dimeric peptidomimetic PSD-95 inhibitors, which led to the spinout company Avilex Pharma and a subsequent clinical trial. After a postdoc at UCPH, he joined the Italian Institute of Technology in Genoa in 2012, focusing on covalent small-molecule inhibitors targeting enzymes involved in lipid metabolism and signaling.

In 2016, Dr. Bach established his research group at UCPH's Department of Drug Design and Pharmacology, where he applied fragment-based drug discovery (FBDD) to develop small-molecule inhibitors of PPIs related to oxidative stress and inflammation. This work has resulted in significant advances against targets like Keap1 and NOX2. In addition to ongoing studies and optimization of Keap1 inhibitors, his FBDD platform is now being used to explore new protein targets relevant to CNS diseases, inflammation, and cancer. Dr. Bach was appointed professor in medicinal chemistry in 2023, and he serves as head of studies of the MSc program in medicinal chemistry and leads courses in drug discovery and development.

S3.01 Nrf2 at the interface between cellular energy metabolism and stress response

Elke Heiss

University of Vienna, Department of Pharmaceutical Sciences

Email: elke.heiss@univie.ac.at

Recently, it has become increasingly evident that metabolism is not just a passive bystander but actively drives or at least modifies various cellular reactions. Our team is intrigued by the question to what extent and how the NRF2- mediated stress response is driven or influenced by the cellular energy metabolism.

This presentation will highlight findings from two recent projects, (i) exploring the influence of the metabolic sensor kinase AMP-activated kinase (AMPK) in regulating the transcription factor Nrf2, and (ii) examining the potential metabolic basis underlying the anti-inflammatory activity of the isothiocyanate and Nrf2 activator sulforaphane. Our work has shown that AMPK activation leads to phosphorylation of three serine residues within Nrf2 with distinct influence on transactivation of NRF2 target genes or stability of Nrf2 in a Keap1-negative background. Moreover, the Nrf2 activator sulforaphane reduces cytokine expression in M1 (LPS) macrophages, which is reliant on preserved mitochondrial activity (intact TCA cycle and OXPHOS activity, no fission) and reduced nuclear acetyl-CoA availability for promoter acetylation. Overall, the cellular energy metabolism deserves appreciation as one key determinant for Nrf2- mediated stress responses.



Elke Heiss is Associate Professor at the Department of Pharmaceutical Sciences at the University of Vienna, Austria. Before establishing her research focus in Vienna, she trained as a biochemist in Germany and the UK, completed her PhD at the German Cancer Research Center, and undertook postdoctoral fellowships in the USA and Sweden. Her research centers on unraveling signal transduction processes and investigating the in vitro pharmacology of small (natural) chemical compounds, with a particular emphasis on redox biology, cellular stress resistance, and their interplay with cellular energy

S3.02 NRF2 in cardiovascular disease

Anna Grochot-Przeczek

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Email: anna.grochot-przeczek@gmail.com

Cardiovascular diseases (CVDs), a group of disorders affecting the heart and blood vessels, are the leading cause of death worldwide. Many of these diseases are associated with impaired defense mechanisms against oxidative stress. Therefore, NRF2, as a master orchestrator of the cellular stress response, is a compelling subject of cardiovascular research. The talk will provide an overview of the mechanisms underlying NRF2's role in the cardiovascular system, with a focus on angiogenesis, atherosclerosis, and abdominal aortic aneurysm. Moreover, several important aspects of the NRF2 models and data interpretation will be discussed.



Anna Grochot-Przeczek is an associate professor in the Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics, and Biotechnology, Jagiellonian University in Krakow, Poland. She studies the molecular mechanisms that regulate the function of endothelial cells and blood vessels with a focus on the NRF2/KEAP1 pathway, ageing, and protein S-nitrosation. Currently, she investigates the importance of NRF2/KEAP1 imbalance and loss of proteostasis in blood vessel function.

S3.03 Targeting NRF2: an effective *in vitro* model for drug discovery in Age-Related Macular Degeneration

Ana S Falcão, Margarida Pedro, Luisa de Lemos, Shuvajit Rakshit, Pedro Antas, Miguel C. Seabra and **Sandra Tenreiro**

iNOVA4Health, NOVA Medical School|Faculdade de Ciências Médicas, NMS|FCM, Universidade Nova de Lisboa, Lisboa, Portugal,

Email: stenreiro@nms.unl.pt

One of the main pathologic features of age-related macular degeneration (AMD) is the degeneration of the retinal pigment epithelium (RPE), due to several factors including lipofuscin accumulation. Evidence suggests that the undigested autofluorescent material is a source of oxidative stress, impairing RPE health. The nuclear factor NRF2 is a known anti-oxidative stress sensor with a role in anti-inflammatory and autophagy regulation. Thus, we are exploring NRF2 as a therapeutic target for AMD.

We have developed an *in vitro* model system that recapitulates some AMD features by feeding human RPE monolayers with porcine photoreceptor outer segments resulting in the accumulation of autofluorescent granules (AFG) similar to lipofuscin *in vivo*. We are using this model to identify NRF2 activators able to reduce AFGs load, assessed by Flow cytometry. As a control, we are using a NRF2 KO generated based on CRISPR/Cas9. We observed that known NRF2 activators such as dimethyl fumarate and Omaveloxolone resolve lipofuscin-like AFGs accumulation in our *in vitro* model of AMD. We are also using this model to evaluate the potential of not yet described NRF2 activators.

Our model is suitable to be used as a platform for drug discovery aiming to identify new NRF2 activators with potential therapeutic applications in AMD and other chronic diseases involving this transcription factor.

Funding: Project funded by “La Caixa Foundation” (NASCENT HR22-00569), R&D unit [iNOVA4Health] (UIDB/04462/2020 and UIDP/04462/2020) and LS4FUTURE Associated Laboratory (LA/P/0087/2020) funded by FCT.



Sandra Tenreiro is the Group Leader of the Degeneration and Ageing Lab and her research is focused in clarifying the molecular mechanisms of retinal degeneration in the context of ageing diseases, using 2D cellular models and 3D retinal organoids. She holds a PhD degree in Biotechnology since 2001. She is also collaborating with ophthalmologists and actively involved in clinical studies. She was Principal Investigator (PI) and Co-PI in several research projects and has participated extensively in advanced training. Globally, her scientific career is reflected in 61 peer-reviewed publications with an H-INDEX of 32 (according to Scopus).

S4.01 Screening natural products for the identification of NRF2 activity modulators

Ioannis P. Trougakos^{1,2}

¹ Group of “Ageing and Age-related diseases” (<http://scholar.uoa.gr/itrougakos/home>), Faculty of Biology, National and Kapodistrian University of Athens, 15784, Athens, Greece

² GenCell-^{TS}Biotech (<https://gencell.gr/>), Athens, Greece

Email: itrougakos@biol.uoa.gr

GenCell-^{TS}Biotech specializes in the field of systems biology with a primary focus on combating ageing and age-related diseases. Ageing is a complex biological process caused by the time-dependent loss of cellular homeodynamics and consequently of physiological organismal functions. This process is affected by both genetic and environmental (e.g., diet) factors, as well as by their constant interaction. We translate our research at (among others) on-demand screening assays (both cell-free and cell-based, as well as at *in vivo* experimental models) aiming to (among others) reveal bioactive molecules with anti-ageing and/or anti-age-related diseases (e.g., cancer, neurodegeneration, etc.) activity. The antioxidant response system comprising (among others) the ubiquitously expressed NFE2-related transcription factor 2 (NRF2) and its redox-sensitive cytoplasmic inhibitor Kelch-like ECH-associated protein 1 (KEAP1) defends tissues against oxidative stress, thereby protecting against pathologies that relate to DNA, protein, and/or lipid damage. Therefore, it represents a promising target for the identification of novel biomolecules with a possible anti-ageing and/or anti-age-related diseases activity. Our rational and experimental platforms for identifying modulators of NRF2 activity, along with some relevant recent findings will be presented.



Ioannis Trougakos obtained his Ph.D. in Cellular-Developmental Biology from the National and Kapodistrian University of Athens (NKUA), Greece. He has worked as Research Scientist at EMBL, Germany, CBM “Severo Ochoa”, Spain and at NHRF, Athens, Greece; he was also research visitor at EMBL and at the Netherlands Cancer Institute. Prof. Trougakos was elected Research Lecturer at NHRF and currently serves as Professor and Director of the “Cell Biology” lab at the Faculty of Biology, NKUA. He is also appointed Adjunct Professor of “Systems Biology of Ageing and Cancer” at the European University of Cyprus. He is the Head of the “Ageing and Age-Related

Diseases” group (<http://scholar.uoa.gr/itrougakos>) at NKUA and founder/CEO of the NKUA spin-off company *GenCell-^{TS}Biotech* (<https://gencell.gr/>). Prof. Trougakos has published articles in high-ranking journals, chapters in international books; he is also co-inventor in several patents. His group is funded by private (GR, EU, USA) and public (GR, EU) entities; also, the group participates in contractual activities with the Industry.

S4.02 Targeting NRF2 in therapy against liver fibrosis

Raquel Fernández-Ginés^{1,2,3}, Ana I. Rojo^{2,3}, José Antonio Encinar⁴ and Antonio Cuadrado^{1,2,3}

¹ Servatrix Biomed S.L.

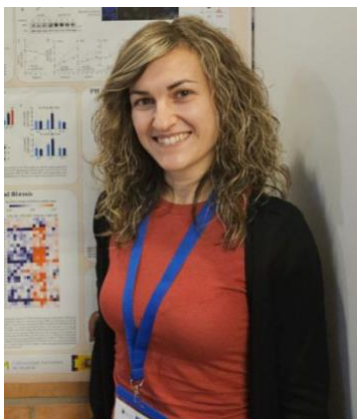
² Instituto de Investigación Sanitaria La Paz (IdiPaz). Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (Ciberneted). Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid (UAM), Madrid, Spain.

³ Instituto de Investigaciones Biomédicas “Alberto Sols” CSIC-UAM. Madrid, Spain

⁴ Institute of Research, Development and Innovation in Biotechnology of Elche (IDiBE) and Molecular and Cell Biology Institute (IBMC), Miguel Hernández University (UMH), 03202, Elche, Alicante, Spain.

E-mail: rfgines@iib.uam.es

Non-alcoholic steatohepatitis (NASH/MASH) and liver fibrosis are common chronic liver conditions that impair liver function and currently lack fully established, highly effective treatments. Targeting the transcription factor NRF2 (Nuclear factor erythroid 2-related factor 2; gene name *NFE2L2*) has emerged as a promising strategy to address multiple underlying mechanisms of NASH. However, commonly studied NRF2 activators that act on its primary repressor KEAP1 are linked to significant off-target effects. This study introduces PHAR, a novel disruptor of the NRF2/ β -TrCP interaction, as an alternative method for NRF2 activation. PHAR was evaluated across various liver cell types and in preclinical models for its impact on NASH and fibrosis progression. It demonstrated strong NRF2 activation in hepatic tissue. In mouse models of NASH, chronic intraperitoneal PHAR treatment significantly improved key pathological features, including fat accumulation, inflammation, and fibrosis. Additionally, advancements in oral administration enhance its clinical applicability. These findings position PHAR as a potential therapeutic breakthrough, offering a novel approach to treating liver disorders beyond existing options.



Raquel Fernández-Ginés completed her Biochemistry degree and Immunology master degree at the Complutense University of Madrid in 2015 and 2016, respectively. In 2022, she completed her PhD in Molecular Biosciences at the Autonomous University of Madrid (UAM), where her research focused on the discovery of novel modulators of the NRF2 transcription factor, under the supervision of Dr. Antonio Cuadrado and Dr. Ana Isabel Rojo Sanchís. Currently, she serves as a Project Manager at Servatrix Biomed S.L., working in Dr. Antonio Cuadrado’s lab on the application of NRF2 modulators for the treatment of chronic liver diseases, such as NASH and fibrosis.

S4.03 Unlocking the power of NRF2 Biomarkers, at crossroad of disease and therapy

Christina Morgenstern

Medical University of Vienna, Vienna Airway Lab, Department of Otorhinolaryngology, Waehringer Guertel 18-20, 1090 Vienna, Austria

Email: christina.morgenstern@meduniwien.ac.at

Identifying reliable NRF2-related biomarkers is crucial for monitoring disease progression and therapeutic outcomes in patients. This presentation highlights the contributions of BenBedPhar Working Group 3 – Translational Medicine – in refining the role of NRF2 in redox metabolism, inflammation, and proteostasis across non-communicable diseases (NCDs). We have recently identified a panel of robust NRF2 biomarkers, including GCLC, GCLM, HMOX1, NQO1, SRXN1, and TXNRD1, consistently regulated across various cell types. We further explore how these biomarkers can advance disease monitoring and prevention strategies while guiding the development of novel NRF2 modulators for clinical applications. This work enhances the NRF2 research toolbox, aiming to bridge the gap between preclinical findings and clinical development of NRF2-targeted therapies, driving innovative solutions for patient care.



Christina Morgenstern completed her PhD in Developmental Genetics at the Cancer Research UK London Research Institute, focusing on transcriptional regulation of Notch signaling. She has experience as a science communicator, running her own business, and as a biology lecturer at the University of Graz and the University College of Teacher Education Carinthia. Driven by a strong passion for biological data, she earned a master's degree in data science with a specialization in Computational Biology. Since 2023, Christina has been a full-time bioinformatician and data scientist at the Medical University of Vienna within the Vienna Airway Lab. She also leads the Translational Medicine Working Group within the COST-Action BenBedPhar.

S5.01 Biomarkers of NRF2 activation in cancer

Anna-Liisa Levonen

University of Eastern Finland, Kuopio, Finland

Email: anna-liisa.levonen@uef.fi

Dysregulation of NRF2 is frequent in non-small cell lung cancer (NSCLC), often via somatic mutations of NRF2 or KEAP1. Aberrant activation of NRF2 leads to aggressive disease resistant to common chemo- and radiotherapy approaches and more advanced targeted therapies. Therefore monitoring NRF2 activation or activating mutations in tumors has both prognostic and predictive significance. Blood-based circulating cell-free tumor DNA (ctDNA) is a minimally invasive alternative for tissue biopsies allowing assessment of mutational status throughout the disease course. Herein, we utilized circulating cell-free tumor DNA to characterize the clinicopathological features and risk factors of inoperable NSCLC patients with oncogenic NRF2 activation in a Finnish cohort. We show that NRF2 pathway mutated NSCLC is a smoking associated high-risk molecular subtype with frequent co-occurring mutations in SMARCA4 gene, cumulatively worsening disease outcome. In addition to mutational profiling, NRF2 activation can be characterized by assessing immunohistochemical staining of both AKR1B10 and AKR1C1, that are primarily expressed in epithelial cells. Moreover, while NRF2 pathway mutated tumors often have lower cytotoxic lymphocyte infiltration, high tumor mutational burden is associated with higher T lymphocyte density independently of NRF2 activation. Our data shows that sequencing of ctDNA provides a viable option to assess mutational status of lung tumors, and that AKR1B10 and AKR1C1 protein expression can be used to verify the functionality of rare variants of KEAP1 in diagnostic pathology.



Anna-Liisa Levonen, MD, PhD, is currently Professor and Vice Dean of Research at the University of Eastern Finland, Faculty of Health Sciences. She earned her MD and PhD degrees from the University of Helsinki, Finland in 1994 and 2000, respectively, followed by a fellowship at the University of Alabama at Birmingham (UAB). Upon her return to Finland, she was recruited to the University of Kuopio (currently University of Eastern Finland), where she has established a research program focusing on the gene regulatory mechanisms activated by oxidative and electrophilic stress, particularly via the redox-activated transcription factor NRF2. She has studied the mechanism of activation of the KEAP1-NRF2 pathway and its role in disease, particularly cardiovascular diseases and cancer, and has published highly cited original and review articles on the topic.

S5.02 Thiosulfate as modulator of oxidative stress through NRF2 signaling

Harry van Goor

Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, the Netherlands

Email: h.van.goor@umcg.nl

Thiosulfate is a naturally occurring compound. The metabolism of thiosulfate in humans is mostly based on mitochondrial sulfurtransferase (Rhodenase) activity. Thiosulfate is derived from the hydrogen sulfide pathway and further processed to sulfite and sulfate after which it is excreted in the urine. However, thiosulfate can also be converted back to hydrogen sulfide, which, in the form of polysulfides is believed to be the protective mediator in (patho)-physiologicval processes. The thiosulfate – thiosulfate sulfur transferase pathway is important in cyanide detoxification, modulation of complex I-IV activity of the mitochondrial respiratory complex (mitochondrial fitness), sulfane sulfur donation of ROS scavengers (like glutathione) (antioxidant effect), and organic sulfur metabolism. Interrupting this pathway in animals leads to increased oxidative stress at least in part driven by aberrant Nrf2 signaling. It is thought that polysulfides can prevent the binding between Keap1 and Nrf2 leading to translocation of the latter to the nucleus in which it associates with the ARE element leading to the upregulation of antioxidant genes. Indeed, in of our recent studies in humans with a cardiac infarction, thiosulfate treatment led to a reduction in oxidative stress. It is not known yet whether this was caused by a direct antioxidant effect or through Nrf2 regulation. In vitro studies in endothelial cells have however shown that there is a direct effect of thiosulfate on NRF2 signaling since it upregulates various antioxidant genes associated with Nrf2. Other protective mechanisms associated with thiosulfate may be its vasodilatory characteristic.

Until now oral treatment with thiosulfate in humans was hampered by the fact that the oral bioavailability is low (8%) and highly variable (0,8-26%), and that it is degraded by hydrochloric acid (stomach) to sulphur and sulphur dioxide: $\text{Na}_2\text{S}_2\text{O}_3(\text{aq}) + 2\text{HCl}(\text{aq}) \rightarrow 2\text{NaCl}(\text{aq}) + \text{H}_2\text{O}(\text{l}) + \text{SO}_2(\text{g}) + \text{S}(\text{s})$. Also, the dose needed is high (600 -1800 mg per administration) and immediate release of the entire dose in the intestines could cause cramps and diarrhoea. An obvious enteric coating is prone to dose-dumping because of a damaged coating. Therefore, we developed a tablet from which the core is able to control release in spite of high salt concentrations. Also, the combination of excipients in the core and the coating prevents premature dose-dumping even when de coating is damaged. Furthermore, the synergistic effect of the core and the coating slow down drug release rate. This tablet has now been patented and an SME has been set up (TSVascular) recently. The first clinical studies are planned in May 2025.



Professor Harry van Goor is a fundamental researcher and a translational scientist at the University Medical Center Groningen, the Netherlands. He completed his PhD program at the University of Groningen and a post-doctoral fellowship at Penn State University, Hershey, PA, USA. He became a full professor in 2010 at the University of Groningen, the Netherlands. He is currently involved in various projects focusing on the role of the reactive species interactome i.e. ROS, NO and H₂S in COVID-19, aging, neurodegeneration, cardiovascular disease and the metabolic syndrome. In

recent years he started studying the role of NRF2-KEAP1 signaling in relation to reactive species stress in various conditions. Since sulfane sulfurs, such as thiosulfate, are thought to activate Nrf2 signaling pathways through the structural change of Keap1 protein and phosphorylation of AKT, he has now patented an oral formula for thiosulfate, which may pave the way for therapeutic interventions. An SME has been set up to further develop this product for human applications.

He has published over 450 scientific papers in peer reviewed journals and has a citation index of almost 100. His current research group consists of >10 MD/PhD students and PhD students from different nationalities and backgrounds. Forty PhD students already acquired their PhD under his supervision in recent years. Harry van Goor has received several research grants from the Dutch Kidney Foundation. He has served for three years as President of the International society of Antioxidants, Nutrition and Health with yearly meetings in Paris, France. He has been a member of the organizing committee for 4 years of the World Congress on Hydrogen Sulfide in Biology and Medicine. He currently serves as Management team member of the Cost Action on "Bench to bedside transition for pharmacological regulation of NRF2 in non-communicable diseases (BenBedPhar)" representing the Netherlands.

S5.03 Vascular function in rat models of prehypertension with and without hypertriglyceridemia: can dimethyl fumarate therapy be beneficial?

Iveta Bernatova, Aybuke Bozkurt, Andrea Micurova, Michal Kluknavsky, Peter Balis

Department of Experimental Hypertension, Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Email: Iveta.Bernatova@savba.sk

We investigated the effect of dimethyl fumarate (DMF) in two rat models of prehypertension that differ in the presence of comorbid hypertriglyceridemia. We used adult male genetically borderline hypertensive rats (BHR) and genetically hypertriglyceridemic (HTG) rats to which we orally administered DMF. BHR and HTG rats had comparable systolic blood pressure (BP) at approximately 140 mmHg. HTG rats had significantly higher plasma triglycerides (TAG) and atherosclerotic index (AI) than BHR. DMF did not affect BP in either genotype of rats, but significantly reduced plasma TAG and AI in HTG rats. We examined vascular wall function using Mulvany wire myography in the femoral and mesenteric arteries. In BHR, DMF did not affect acetylcholine (ACh)-induced and sodium nitroprusside (SNP)-induced relaxations, nor did noradrenaline (NA)- and serotonin-induced constrictions in both the femoral and mesenteric arteries. Similarly, in HTG, DMF did not affect the abovementioned parameters of vascular wall function in the femoral artery. However, DMF significantly reduced NA-induced contraction in the mesenteric artery. The results indicate that the DMF treatment might be beneficial in the presence of hypertriglyceridemia due to the improvement of metabolic disorders and reduction of NA-induced constriction despite no effect on BP.



Iveta Bernatova is a senior scientist, and head of the Department of Experimental Hypertension at the Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia. She studied Biochemistry (1991), holds a PhD in Chemistry (1997) and a title Doctor of Sciences (D.Sc.) in the field of Animal Physiology (2009). Her research is focused on the regulatory mechanisms of blood pressure in various experimental models of (pre)hypertension and the ways of prevention and treatment of high blood pressure with special attention paid to the role of nitric oxide and oxidative stress. A significant part of her research is focused on the vascular effects of various

natural substances in the prevention and treatment of hypertension and endothelial dysfunction. The most recent studies are focused on the research of the role of NRF2-activator dimethyl fumarate (DMF) in the cardiovascular system. She is the author of approximately 120 peer-reviewed publications in extenso with more than 2000 citations.

S6.01 Aberrant BACH1/Nrf2 axis in Down Syndrome brain contributes to early onset Alzheimer-like neuropathology

Marzia Perluigi

Department of Biochemical Sciences, Faculty of Pharmacy and Medicine, Sapienza University of Rome, Rome (Italy)

Email: marzia.perluigi@uniroma1.it

Several studies support the implication of aberrant redox phenotype in the brain of people with Down Syndrome (DS). Indeed, by mapping chromosome 21 several genes, such as SOD-1, BACH1, APP, CBR and S100B, are involved in the over-production of ROS in DS individuals. In particular, we investigated the role of BACH1 in the brain and its implication in the failure of the antioxidant response, in human autopsic cases and in a mouse model of DS (Ts2Cje). Our results revealed that BACH1 overexpression impairs the BACH1/NRF2 ratio in the nucleus as well as the induction of antioxidant response genes ultimately resulting in the accumulation of oxidative damage. This process acts in concert with a substantial loss of proteostasis, including defective autophagy. Our study supports the hypothesis that BACH1 triplication in DS subjects is implicated in the alteration of redox homeostasis that might contribute to accelerated neurodegeneration, ultimately resulting in early onset Alzheimer disease.



Marzia Perluigi is Professor of Biochemistry at the Department of Biochemical Sciences, Medical School Sapienza University of Rome". The major research interest is the study of the role of oxidative stress in Down Syndrome (DS) and Alzheimer Disease (AD). Projects involve both the analysis of post-mortem brains, biological fluids and cellular and animal models of the diseases. In particular, current projects focus on the identification of trisomic genes that are involved in the "oxidative stress phenotype" of DS individuals. Among these triplication of BACH1 may offer the opportunity to understand the role of Nrf2 in the neurodegenerative process ultimately leading to early onset AD.

S6.02 Serendipity as a strategic tool to expand omaveloxolone's medical landscape

Gerasimos P. Sykiotis

Lausanne University Hospital and University of Lausanne; Service EDM-CHUV, Ave de la Sallaz 8, CH-1011 Lausanne, Switzerland;

Email: gerasimos.sykiotis@chuv.ch

Drug repurposing means developing new uses for approved or experimental drugs. In addition to several purposeful strategies, serendipitous repurposing has also led to blockbuster indications. For example, sildenafil and minoxidil, originally developed for hypertension, showed unexpected effectiveness in erectile dysfunction and stimulating hair growth, respectively. Common aspects in “serendipitous repurposing” include the expression of the target beyond the tissue of interest for the initial indication, the systematic documentation of side-effects during clinical trials, and the successful engagement of experts from different medical specialties. Nrf2, with its ubiquitous expression and global antioxidant and tissue-specific actions, offers potential for repurposing. After several failed trials of Nrf2 modulators for various indications, a breakthrough was achieved with the regulatory approval of omaveloxolone for Friedreich’s ataxia (FA), a rare genetic neurological disorder characterized by increased oxidative stress due to mitochondrial dysfunction. Interestingly, patients with FA present several comorbidities, including gastrointestinal, endocrine and cardiovascular diseases. Outside FA, Nrf2 is considered a therapeutic target for several of these diseases, due to the involvement of oxidative stress in their pathogenesis. Therefore, systematically monitoring the evolution of comorbidities in FA patients during omaveloxolone treatment is a strategy to facilitate the “serendipitous” repurposing of the drug for other oxidative stress-related indications. To increase the likelihood of the “strategic serendipity” approach for omaveloxolone’s repurposing, it is important to raise awareness among decision-makers involved in the currently unfolding clinical use of the drug. This encompasses the Biogen leadership; FDA, EMA and country-level regulators; neurologists; endocrinologists, gastroenterologists, cardiologists and other experts treating FA comorbidities; and FA patient associations.



I am a physician-scientist specialized in clinical and basic endocrinology with a particular focus on thyroid physiology and thyroid diseases, including thyroid cancer. Since 2015, I am responsible for the thyroid clinic at the Endocrinology Service of the CHUV (Lausanne University Hospital). My clinical research focuses on the needs of patients with thyroid and parathyroid diseases and neuroendocrine tumors, including medical imaging for endocrine malignancies and quality-of-life issues among thyroid cancer patients and survivors. My basic and translational research, funded primarily by the Swiss National Science Foundation, focuses on the roles of cellular antioxidant response systems in thyroid physiology and pathophysiology. Using mouse models with increased or decreased Nrf2 activity, we have

characterized several roles of the Keap1/Nrf2 antioxidant response system in the thyroid gland in relation to nutrition, autoimmunity, goiter, etc. By collaborating with diverse groups with access to respective patient cohorts, we have validated our main findings also in humans. Our work over the last ten years has identified the thyroid as a main tissue of relevance for Keap1/Nrf2 signaling and its pharmacological modulators, including both as a target organ for disease prevention and treatment as well as a potential tissue to monitor for unwanted side-effects.