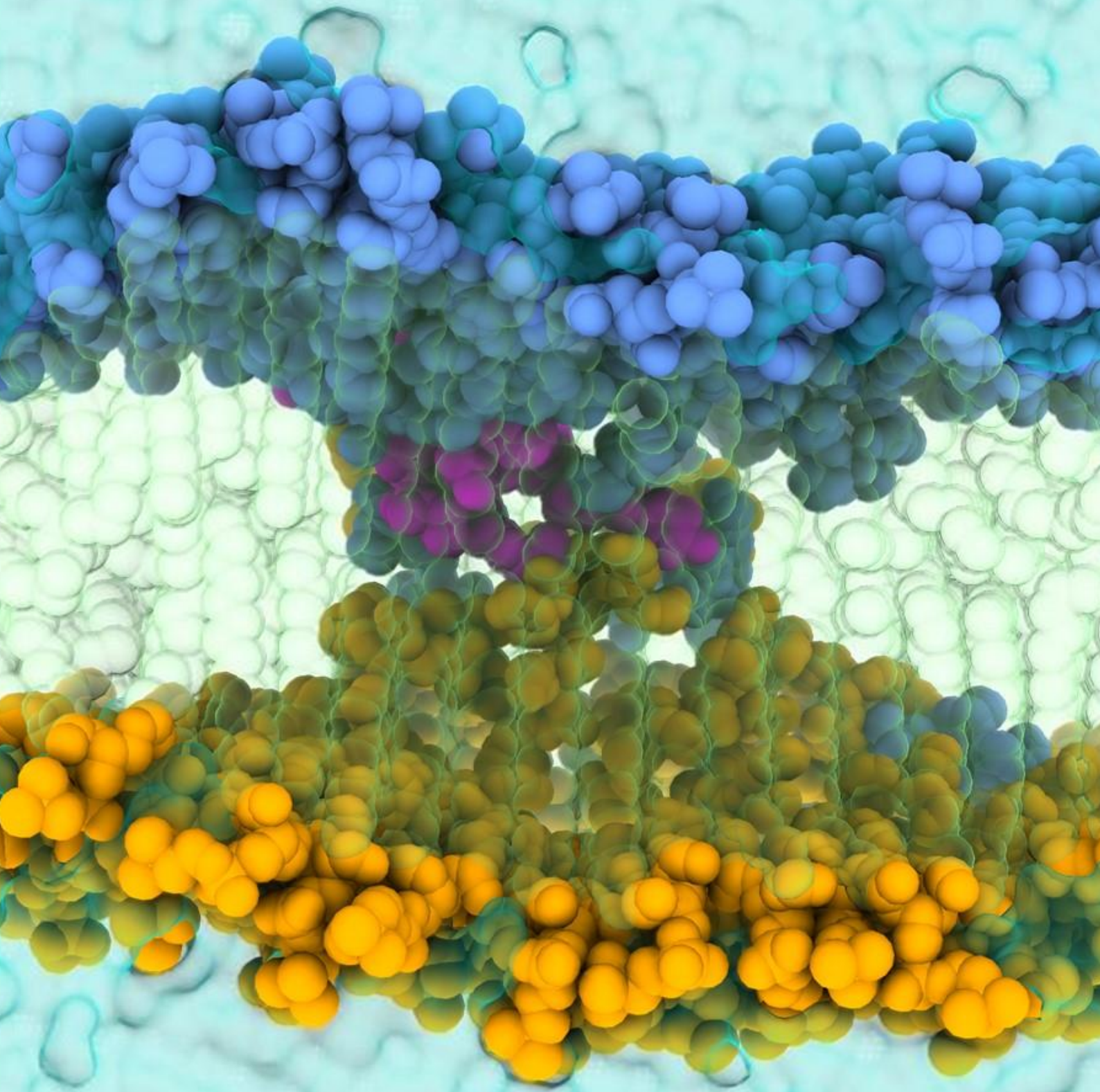
XIX Jornada Científica del Departament de Bioquímica i Biologia Molecular

Facultat de Biociències, 13 de juny de 2023



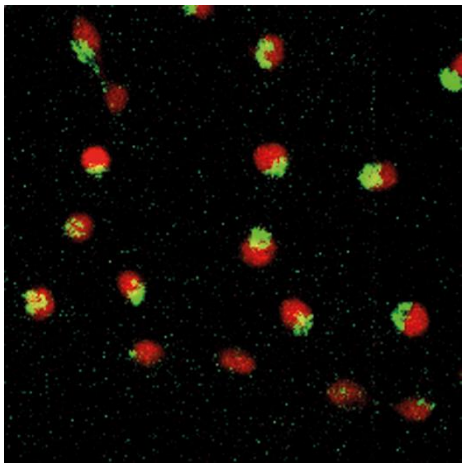
UAB Departament de Bioquímica i Biologia Molecular

Facultat Biociències UAB

Finalista: *Strawberry Fields: Green nucleoli and red nuclei.*

Saccharomyces cerevisiae strain carrying nucleolar Cdc14-GFP and histone HTB2-mCherry. Cell membranes and cytosols are not fluorescent.

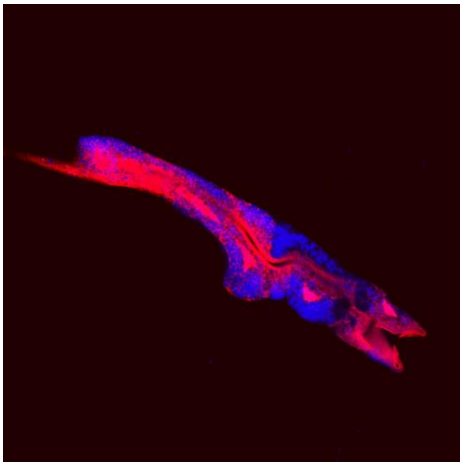
Instrument: Zeiss Spinning Disc microscope (Dundee Imaging Facility, Dundee University, Scotland.



Fortografia cedida per Alberto Zurita
Unitat de Biofísica de la UAB

Finalista: *C. elegans as a dragon*

Confocal picture of an adult specimen of the *C. elegans* CL2355 strain, a model of Alzheimer Disease with pan neuronal expression of human Aβ1-42 and intestinal expression of GFP. MitoSox staining shown in blue (mitochondrial ROS) and GPF (intestinal cells) shown in red.



Fortografia cedida per Adan Dominguez
Unitat de Biociències de la UAB

PORTADA: Formació de porus en l'estudi de la translocació d'un pèptid
En aquesta figura s'observa el resultat de l'estudi de la translocació d'un pèptid. Aquest forma un porus en la bicapa, el que pot ser un pas intermedi per la translocació. El pèptid, en forma d'esferes, està acolorit en magenta. Els caps polars dels fosfolípids (també com a esferes) de la bicapa de color blau (superior) i groc (inferior). Les cues apolars estan acolorides en verd transparent. També es representa la superfície formada per les molècules d'aigua, en color blau clar.

Fotografia cedida per Eric Catalina,
Unitat de Biofísica i Institut de Neurociències-INc de la UAB

Benvolgudes companyes i companys,

Des de la Direcció del Departament us donem la benvinguda a la **XIX Jornada Científica del Departament de Bioquímica i de Biologia Molecular**, tot agraint-vos la vostra presencia i contribució a un esdeveniment que enguany ve precedit per la celebració de la **Primera Jornada Doctoral del Programa de Doctorat en Bioquímica, Biologia Molecular i Biomedicina**, organitzada de forma conjunta amb **l'Institut de Recerca de la Vall d'Hebron**.

Així, un any més les diferents Unitats Departamentals, el personal adscrit als Instituts i Centres de Recerca, així com els doctorands del programa de Bioquímica, Biologia Molecular i Biomedicina ens tornem a retrobar en unes Jornades que ja formen part de la història del Departament. A més, enguany obrim les **Jornades de Biorecerca–*UABio***, que es continuen al llarg de tota la setmana amb la Jornada del Dept. de Genètica i Microbiologia, del Dept. de Biologia Cel·lular, Fisiologia i Immunologia, i del Dept. de Biologia Animal, Vegetal i Ecologia.

Volem que la trobada, una vegada més, sigui una plataforma efectiva per a la comunicació i debat de la nostra recerca, idees i projectes. Esperem que això serveixi per facilitar la interacció amb els companys i l'establiment de noves col·laboracions, tant amb els grups del Departament com amb altres grups de recerca de l'àmbit de Biociències i Biomedicina, tot donant una visió global del nostre potencial científic.

Desitgem que la Jornada us sigui ben profitosa!

Departament de Bioquímica i Biologia Molecular
Assumpcio Bosch, Directora
Jose Ramon Bayascas, Secretari Acadèmic
Monica Lluch, Gestora
Montserrat Godia, Secretària

Programa de Doctorat en Bioquímica Biologia Moleuclar i Biomedicina
Jordi Ortiz, Coordinador
Josep Quer, Comisionat VHIR

Bellaterra, 1 de juny de 2023

Comitè Científic de la XIX Jornada

Dr. Antonio Casamayor	*Unitat de Bioquímica, Facultat de Veterinària*
Dra. Montserrat Solé	*Unitat de Bioquímica, Facultat de Medicina*
Dr. Mario López	*Unitat de Biofísica, Facultat de Medicina*
Dra. Julia Lorenzo	*Institut de Biotecnologia i de Biomedicina (IBB)*
Dra. Verónica Jiménez	*Centre Biotecnologia Animal i Teràpia Gènica (CBATEG)*
Dr. Alfredo Miñano	*Institut de Neurociències (INc)*
Dra. Alicia Roque	*Unitat de Bioquímica, Facultat de Biociències*

Agraïm tot el suport i ajut del Dr. Joaquin Ariño en l'edició i producció d'aquest llibre d'abstracts.

1ª Jornada Doctoral Departament / Vall d'Hebron Institut de Recerca, Programa de Doctorat en Bioquímica, Biologia Molecular i Biomedicina, Universitat Autònoma de Barcelona, 12 JUNY DE 2023		
10:00	**Inauguració i presentació**	**Dr. Jordi Ortiz** - Coordinador del Programa de Doctorat
		Dra. Imma Ponte - Directora de l'Escola de Doctorat de la UAB
		Dr. Josep Quer - VHIR i Dept. Bioquímica i Biologia Molecular UAB
10:15	**Primera sessió d'exposicions orals**	Moderador: Dr. Jordi Ortiz
10:15	Hepatic oxi-inflammation and neophobia as potential liver-brain axis targets for Alzheimer's disease and aging, with strong sensitivity to sex, isolation, and obesity.	Fraile-Ramos, J *Institut de Neurociències-INc i Departament de Psiquiatria i Medicina Legal, UAB*
10:30	Dynorphin A highway to Cell-Penetrating Peptide: Adaptatively Steered Molecular Dynamics in the characterization of Dynorphin A Clinical Variants	Catalina, È *Unitat de Biofísica, Departament de Bioquímica i Biologia Molecular i Institut de Neurociències-INc, UAB*
10:45	Understanding a-synuclein aggregation propensities through endogenous cross-interactions with dynorphin peptides	Masnou-Sánchez, David *Unitat de Biofísica, Departament de Bioquímica i Biologia Molecular i Institut de Neurociències-INc, UAB*
11:00	Anti-Amyloidogenic Effects of Neurokinin A and its Analog NKAW on Amyloid-β1-42 Aggregation in Alzheimer's Disease	Habibnia M *Unitat de Biofísica, Departament de Bioquímica i Biologia Molecular i Institut de Neurociències-INc, UAB*
11:15	Purine alterations in tumoral cell lines maintained with physiological levels of folic acid	Cano-Estrada, C *Unitat de Bioquímica de Medicina, Departament de Bioquímica i Biologia Molecular i Institut de Neurociències-INc, UAB*
11:30	**Coffe break**	
12:00	**Segona sessió d'exposicions orals**	Moderador: Dr. Josep Quer
12:00	Impact of the protein corona in cellular uptake and in vivo biodistribution of targeted liposomes to improve the enzymatic replacement therapy of Fabry disease	Moltó-Abad, M *Biochemical Chemistry, Drug Delivery & Therapy, VHIR*
12:15	An epigenetic algorithm to predict radioiodine refractoriness in differentiated thyroid cancer	Rodríguez-Lloveras, H *Germans Trias i Pujol Research Institute*
12:30	p21Cip1/WAF1 and its relationship with androgen metabolism in prostate cancer primary cultures	Toscano E *Bioquímica clínica, VHIR*
12:45	Defective Viral Genomes (DVGs) in SARS-CoV-2 quasispecies as trackers for viral adaptation	Campos, C *Liver Diseases, Viral Hepatitis research group, VHIR*
13:00	How Quasispecies Studies Could Help in Viral Infections: Negative Effect of Early Ribavirin Discontinuation in a Chronically Infected HEV Patient.	Colomer-Castell, S *Liver Diseases, Viral Hepatitis, Liver Unit, VHIR*
13:15	**Dinar**	
14:15	**Tercera sessió d'exposicions orals**	Moderadora: Dra. Enea Sancho Vaello
14:15	Air pollution induces ventricular arrhythmogenesis by altering the arrhythmogenic substrate, the cardiac metabolic profile; by inducing ROS production and changes in expression of signaling enzymes	Ganse, GF *Cardiovascular Diseases Research Group, VHIR*
14:30	Functional characterization of Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC) cellular models carrying the p.G20D CLDN19 founder mutation.	Torchia, J *Renal Physiopathology Group, VHIR*
14:45	Impact of hypercapnia on the course of bacterial infection in an in vitro model of pneumonia	Campaña Duel, E *Critical Care Research Center, Parc Taulí Hospital Universitari, I3PT*
15:00	Deciphering Pseudomonas aeruginosa infections through Tandem mass spectrometry analysis	Mesas, C *Unitat de Biociències, Departament de Bioquímica i Biologia Molecular, UAB*
15:15	Harnessing alkaline pH regulatable promoters as a novel platform for heterologous protein expression in Saccharomyces cerevisiae	Zekhnini, A *Unitat de Veterinària, Departament de Bioquímica i Biologia Molecular i Institut de Biotecnología i Biomedicina-IBB, UAB*
15:30	**Coffe break**	
16:00	**Taula Rodona: Perspectives i experiències professionals**	Moderadora: Dra. Anna Bassols Teixidó
	Dra. Sancho Vaello, Enea	*Universitat Autònoma de Barcelona*
	Dr. Briansó, Ferran	*Roche Diagnostics*
	Dra. Garrido Martínez, Maria	*VHIR, Innovation, Tech Transfer and Project Management*
17:00	**Cloenda**	

XIX JORNADA CIENTÍFICA DEL DEPARTAMENT DE BIOQUÍMICA I BIOLOGIA MOLECULAR, 13 JUNY DE 2023		
09:15	**Inauguració i presentació**	**Dr. Isidre Gibert** – Degà Facultat Biociències **Dra. Assumpció Bosch** - Directora del Departament
09:30	**Primera sessió d'exposicions orals**	Moderador: Dr. Carles Arús
09:30	Bioengineered self-assembling nanofibrils for the capture and neutralization of SARS-CoV-2	Molood Behbahanipour *Unitat de Bioquímica de Biociències i Institut de Biomedicina i Biotecnologia-IBB*
09:45	Development of a highly potent transthyretin amyloidogenesis inhibitor: Design, synthesis and evaluation	Francisca Pinheiro *Unitat de Bioquímica de Biociències i Institut de Biomedicina i Biotecnologia-IBB*
10:00	The influence of immune landscape in response to therapy in glioblastoma: a preclinical example	Marta Mulero Acevedo *Unitat de Bioquímica de Biociències i Institut de Biomedicina i Biotecnologia-IBB*
10:15	MAP kinase ERK5 modulates cancer cell sensitivity to extrinsic apoptosis induced by death-receptor agonists and Natural Killer cells	Sergio Espinosa Gil *Unitat de Bioquímica de Medicina, Institut de Neurociències-INc i Vall d'Hebron Research Institute-VHIR*
10:30	**Primera sessió de pòsters**	**Coffee Break**
12:00	**Conferència Plenària *Josep Vendrell* Inauguració VIII Biojornades, Sala d'Actes**	Dra. Assumpció Malgosa - Vicerectora de Recerca Presentació: Dra. Assumpció Bosch
	How to engineer human pluripotent stem cells to understand human development and disease: from cells to organoids	**Dra. Núria Montserrat** *Institute for Bioengineering of Catalonia-IBEC*
13:00	**Fi de la sessió matinal**	
15:00	**Segona sessió d'exposicions orals**	Moderador: Dr. Enrique Claro
15:00	Altered excitatory and inhibitory hippocampal neurons correlate with memory deficits in a new model of Alzheimer's Disease	Angel Deprada Fernández *Unitat de Bioquímica de Medicina i Institut de Neurociències-INc*
15:15	Pathological and functional characterization of emotion-related neural circuits in novel Alzheimer's disease transgenic mice	Maria Dolores Capilla López *Unitat de Bioquímica de Medicina i Institut de Neurociències-INc*
15:30	Lesch-Nyhan disease fibroblasts alterations revealed under physiological culture conditions	Dra. Paula Escudero Ferruz *Unitat de Bioquímica de Medicina i Institut de Neurociències-INc*
15:45	**Segona sessió de pòsters**	**Coffee Break**
16:45	**Tercera sessió d'exposicions orals**	Moderador: Dr. Francisco Rodríguez Frías
16:45	AAV9-Mediated Expression of Secreted Klotho Reduced Several Aging-Associated Phenotypes and Increased Longevity	Joan Roig Soriano *Unitat de Bioquímica de Medicina, Institut de Neurociències-INc i Vall d'Hebron Research Institute-VHIR*
17:00	Towards hereditary spastic paraplegia type 52 (SPG52) AAV-based gene therapy strategy	Laura Rodriguez Estevez *Unitat de Bioquímica de Medicina, Institut de Neurociències-INc i Vall d'Hebron Research Institute-VHIR*
17:15	Therapeutic effect of sKL gene therapy strategy to treat aging and neurodegeneration	Dra. Beatriz Almolda Ardid *Departament de Biologia Cel·lular, Fisiologia i Immunologia, Institut de Neurociències-INc i Vall d'Hebron Research Institute-VHIR*
17:30	**Acte de cloenda i lliurament de premis**	

SESSIONS D'EXPOSSICIONS ORALS **JORNADA DOCTORAL** **DILLUNS 12 JUNY**

SESSIONS D'EXPOSSICIONS ORALS **XIX JORNADA DEPARTAMENT** **DIMARTS 13 DE JUNY**

PÒSTERS

O1-01

Hepatic oxi-inflammation and neophobia as potential liver-brain axis targets for Alzheimer's disease and aging, with strong sensitivity to sex, isolation, and obesity.

Fraile-Ramos, J; Garrit, A; Reig-Vilallonga, J; Giménez-Llort, L

Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain; Department of Psychiatry and Forensic Medicine, School of Medicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain; Department of Anatomy, School of Medicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

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Research on Alzheimer's disease (AD) has classically focused on alterations that occur in the brain and their intra- and extracellular neuropathological hallmarks. However, the oxi-inflammation hypothesis of aging may also play a role in the neuro-immuno-endocrine dysregulation and the disease's pathophysiology, where the liver emerges as a target organ due to its implication in regulating metabolism and supporting the immune system. Moreover, oxidative stress and inflammation are risk factors for chronic hepatic disease and negatively affect one another. Recent findings have implicated this organ in the pathophysiology of AD, as an association between peripheral markers of liver function and central markers associated with AD has been described in people with the disease. Preliminary results from our laboratory in 3xTg-AD mice suggested hepatic oxidative stress dysfunction at the onset of the disease. Therefore, biochemical communication between these two organs via the so-called liver-brain axis has begun to attract attention. In addition to this new perspective on the involvement of peripheral organs in AD, the classical cognitive conceptualization of this disease, centered on memory impairment at the clinical level, now embraces the disease's broad and heterogeneous spectrum of neuropsychiatric (NPS) and behavioral manifestations. Taking advantage of the conspicuous NPS-like phenotype of 3xTg-AD mice in the present work, we investigated liver dysfunction at different levels of study, from cellular oxi-inflammation and histological analysis of the functional correlates with the HPA axis (corticosterone) and the behavioral phenotype. Hepatic dysfunction was determined in 16-month-old 3xTg-AD animals presenting with hepatomegaly, acute amyloidosis and ballooning. In addition, increased oxidative stress and inflammation were shown as the dysregulation of antioxidant enzymes and increased levels of pro-inflammatory cytokines, with a sexual dimorphism. In contrast, liver steatosis was present in their male and female C57BL/6 wild-type counterparts, and in the case of males it was comorbid with obesity. Dysregulation of the HPA axis may also be involved in liver oxidative stress and inflammation, as shown by the higher corticosterone levels in 3xTg-AD females, which correlated with GSH levels. Neophobia has previously been shown to exert a clear psychological effect by increasing hyperactivity, anxiogenic patterns, and bizarre/flight behavior. In this study, neophobia correlated with oxidative stress variables in the liver. In forced isolated animals, the genotype differences in hepatic histopathology (liver damage score) and oxi-inflammation mechanisms (reduced GSH levels and increased IL-6 and TNFa) were enhanced or appeared for the first time. The liver-brain axis is an emerging research area in AD that demands further efforts to unveil the interplay of the pathways involved in the systemic component of this disease.

O1-02

Dynorphin A highway to Cell-Penetrating Peptide: Adaptatively Steered Molecular Dynamics in the characterization of Dynorphin A Clinical Variants

Catalina, È[1,2]; López, M[1,2]; Aguilella, M[3]; Perálvarez-Marín, A[1,2]

1 Unitat de Biofísica, Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona; 2 Institut de Neurociències, Universitat Autònoma de Barcelona; 3 Departament de Física, Escola Superior de Tecnologia i Ciències Experimentals, Universitat Jaume I

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Dynorphins are endogenous peptides and the canonical kappa-opioid receptor (KOR) substrate. Pathophysiological implications for dynorphins have been described owing to their cell penetrating peptide (CPP)-like behavior, as molecules capable of inducing membrane translocation via membrane destabilization and/or pore formation. Dynorphins are prohormones derived from prodynorphin (PDYN), which is cleaved into big dynorphin (BigDyn, 32 residues), and further processed into dynorphin A (DynA, 17 residues) and B (DynB, 13 residues), which are related to Alzheimer's and Parkinson's diseases (AD, PD). Furthermore, 4 clinical variants have been described for DynA: wild type (WT), L5S, R6W, and R9C. Hence, we have studied DynA clinical variants CPP activity in 3 types of membrane DPPC, DPPC:DOPC:CHOL, and DPPC:DOPC:DPPS:DOPS:CHOL, for the 4 DynA clinical variants and a control peptide (Arg9, a described CPP). For that purpose, we induced membrane translocation through adaptatively Steered Molecular Dynamics (aSMD) simulations, and we ran a conventional MD (cMD) of 100 ns to see how the system 'relaxes' after the potential addition. We analyzed the peptide distance to the lower leaflet, the potential of the mean force (PMF), the radius of the pore formed, and the residue occupancy by the lipids polar head, computed for every peptide and membrane composition. In conclusion, DPPC membrane has allowed the best CPP-like behavior characterization, since we saw the translocation of Arg9 (positive control), besides, we oberve in DynA WT, DynA L5S, and DynA R6W, but not R9C, a proteolipid pore formation, which we hypothesize is the preliminary step leading to peptide translocation.

O1-03

Understanding α-synuclein aggregation propensities through endogenous cross-interactions with dynorphin peptides

Masnou-Sánchez, D; Habibnia, M; Catalina-Hernández, È; López-Martín, M; Perálvarez-Marín, A

Biophysics Unit, Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona; Institute of Neurosciences, Autonomous University of Barcelona

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Parkinson's disease is the most common movement disorder and the second most common neurodegenerative disorder only after Alzheimer's. At the molecular level, it is characterized by the misfolding and aggregation of the protein α-synuclein, a 140 amino acids intrinsically disordered protein. Its sequence can be divided into 3 distinct regions, the N-terminal, the central hydrophobic region (known as NAC) and the highly acidic C-terminal. The NAC region constitutes the minimal zone needed for aggregation because of its hydrophobic character, thus tending to interact with those of other α-synuclein monomers and leading to the formation of β-sheet rich aggregates in the form of oligomers and, lately in the aggregation process.

Experimental evidence suggested big dynorphin (BigDyn) as a potential anti-amyloidogenic peptide. BigDyn is a 32 amino acid endogenous opioid neuropeptide belonging to the dynorphins family, constituted by some of the most positively charged peptides in our organism, thus interacting with many different partners, including α-synuclein.

Therefore, we have performed molecular dynamics simulations to examine the potential interactions between these two partners. To observe events occurring at high time scales at the molecular level, such as conformational changes or the aggregation process itself, we have decided to use enhanced sampling algorithms. The results of these simulations elucidate some of the key features of these interactions at the molecular level, which can be correlated with the experimental evidence, thus confirming the potential of BigDyn as an anti-amyloidogenic peptide.

O1-04

Anti-Amyloidogenic Effects of Neurokinin A and its Analog NKAW on Amyloid-β1-42 Aggregation in Alzheimer's Disease

Habibnia, M; Masnou-Sánchez, D; Catalina-Hernández, È; López-Martín, M; Perálvarez-Marín, A

Biophysics Unit, Biochemistry and Molecular Biology Department, Institute of Neuroscience, Universitat Autònoma de Barcelona (Cerdanyola del Vallés, Spain).

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Mammalian tachykinins (TKs) are a family of neuropeptides broadly expressed in neurons, whereas some are also produced in nonneuronal tissues. TKs receptors are vastly localized in the brain and these neuropeptides function as neurotransmitters or neuromodulators in different biological activities including pain processing, nausea, aggression, stress, neuroinflammation, emesis, hormone regulation, immune function, and memory formation. The three foremost TK members in mammalian species are substance P (SP), neurokinin A (NKA), and Neurokinin B (NKB) which consist of 10-12 amino acids, and share a conserved motif; FXGLM-NH2 in their COOH-terminal, where X is a hydrophobic residue. The tachykinergic system's functionality is altered in patients suffering from Alzheimer's disease (AD), as it's been indicated that TKs levels are decreased in the brains of AD patients, as well as in extracted postmortem AD brains. The aim of this work was to investigate and compare the potential anti-amyloid effects of NKA and NKAW (NKA's analog: Phe6 substitute with Trp) *in vitro* and *in silico* by using several techniques including ThT fluorescence assay, circular dichroism spectroscopy, dynamic light scattering, cell toxicity and computational simulations. Preliminary results indicate that NKA interacts with Aβ1-42 preventing amyloid aggregation, and using mutagenesis, we have assessed the role of NKA Phe6 in the interaction. In this research, novel work has been undertaken on NKA and Aβ1-42 interactions which collectively allows us a clearer vision of how TKs act in AD. Further studies are required to assess and understand this potential endogenous cross-interaction.

O1-05

Purine alterations in tumoral cell lines maintained with physiological levels of folic acid

Cano-Estrada, C[1,2]; de Benito-Gómez, L[3]; Escudero-Ferruz, P[1,2]; Ontiveros-Roca, N; Iglesias-Serret, D[3,4]; López-Blanco, JM[1,2]

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2. Departament de Bioquímica i Biologia Molecular, Unitat de Bioquímica, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain
3. Departament de Ciències Fisiològiques, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona-IDIBELL (Institut d'Investigació Biomèdica de Bellvitge), 08907 L´Hospitalet de Llobregat, Barcelona, Spain
4. Facultat de Medicina, Universitat de Vic-Universitat Central de Catalunya (UVic- UCC), 08500 13 Vic, Barcelona, Spain

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Purines are essential for nucleic acid synthesis, cell signaling, energy metabolism and other cellular processes. There are two pathways for purine biosynthesis, the "de novo" synthesis, which is energetically expensive and folate dependent, and the salvage synthesis, mediated by the enzime hypoxantine-guanine phosphoriboliltransferase (HGPRT).

Lesch-Nyhan disease (LND) is an X-linked recessive disorder caused by the complete deficiency of HGPRT. It is known that fibroblasts from patients have an accelerated de novo purine biosynthesis to compensate a deficiency in the salvage pathway. LND cells accumulate ZMP (an intermediate metabolite in the "de novo" pathway) when are maintained with physiological levels of FA (25 nM).

Most cancer cells also present an acceleration in the synthesis of purines to fulfil their enhanced division rate. Using cell culture media with physiological levels of folic acid (25 nM) we uncover purine alterations in several human cell lines. HEK293T, Jurkat, and A549 cells accumulate ZMP at physiological levels of folic acid, but not with the artificially high levels (2200 nM) present in regular media. Interestingly, these cell lines do not accumulate high levels of ZMP when AICAr, the precursor of ZMP, is added to the medium containing 2200 nM folate, instead ATP and GTP pools are increased. On the other hand, HeLa and EHEB cells do not accumulate ZMP at physiological levels of folic acid, but they do in a medium containing AICAr plus 2200 nM folate, indicating that these cells preferentially rely on the salvage pathway for purine biosynthesis.

Expression of SLC19A1, which encodes the reduced folate carrier (RFC), is increased in HEK293T and Jurkat cells compared with HeLa and EHEB, and it was correlated with the total purine content. These results indicate that some human cell lines preferentially use the de novo purine biosynthetic pathway and express higher levels of the folate transporter SLC19A1 than others that preferentially use the salvage pathway for their purine requirements.

O1-06

Impact of the protein corona in cellular uptake and in vivo biodistribution of targeted liposomes to improve the enzymatic replacement therapy of Fabry disease.

Moltó-Abad, M[1,2,9]; Díaz-Riascos, ZV[1,2,9];Tomsen-Melero, J[2,3,4]; González-Mira, E[2,4]; García-Prats B[1,2,9]; Corchero, JL[2,6]; Pulido, D[2,5]; Soldevila, A[7]; Royo, M[2,5]; Córdoba, A[3]; Ventosa, N[4,2]; Villaverde, A[2,6]; Canals, F[8]; Martín, L[8]; Pintos-Morell, G[1]; Schwartz, S. Jr[1,9]; Abasolo, I[1,2,9]

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Fabry disease (FD) is a lysosomal storage disorder caused by a deficient activity of the α-galactosidase A (GLA) that leads to a progressive lysosomal accumulation of glycosphingolipids, mainly in endothelial cells. The most common treatment is the enzymatic replacement therapy (ERT), but it shows a poor biodistribution and auto-antibodies generation. In order to overcome these limitations, RGD-functionalized nanoliposomes containing recombinant GLA (nanoGLA) were developed. Since intravenously administered liposomes will adsorb plasmatic proteins on their surface generating a protein corona (PC) we aimed at studying how PC composition might impact in cell internalization and in vivo biodistribution. To that end, liposomes with different targeting moiety concentrations (0, 3 and 6% of RGD) were incubated with mouse plasma and isolated using size exclusion chromatography and protein composition characterized by HLPC-MS/MS. Cell internalization and in vivo tissue biodistribution of these 3 types of nanoGLA was also studied. Regarding PC composition, results show that regardless RGD presence, all 3 nanoparticles showed a similar protein profile. However, relative abundance of certain proteins varied. For instance, different complement factors, are more abundant in RGD nanoGLAs (1.27-1.39 fold), whereas some immunoglobins are more abundant in liposomes without RGD nanoGLAs (1.12-1.25 fold). Furthermore, incubation of nanoGLA with PC reduced the effect of the RGD in endothelial cells uptake by a 37-51%, indicating that RGD moiety might be partially hidden. In vivo, biodistribution of different nanoGLAs showed significant differences of enzymatic activity among tissues depending on the quantity of RGD in the liposome. Significantly, the 0%RGD nanoGLA version showed more enzymatic activity in plasma than the 3 and 6% RGD nanoGLA 30 min post-administration. Conversely, liposomes with RGD showed higher enzymatic activity levels in liver, spleen, kidneys and heart. All nanoGLAs showed enzymatic activity in brain. Overall, these results showed that although the formation of the PC might hamper cellular uptake and induce a greater opsonization, RGD-functionalized nanoGLA are able to bring higher quantities of enzymatic activity to target tissues of Fabry disease such as kidneys and heart.

O1-07

An epigenetic algorithm to predict radioiodine refractoriness in differentiated thyroid cancer

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Differentiated thyroid cancer is the most common endocrine malignancy, with an increasing incidence over the past three decades. Most patients show a survival rate >90% at 10 years. However, around 10% of patients develop distant metastases, and 2/3 of them do not respond to radioiodine (RAI), the main treatment in thyroid cancer. In such cases, the survival rate drops to less than 10% at 5 years, and the only available therapies, multikinase inhibitors (MKIs), are non-specific, with reduced effectivity, and patients eventually develop resistance. Currently, there are no markers to predict refractoriness to RAI.

Most studies on differentiated thyroid cancer are based on genetic and transcriptomic alterations, while there is a lack of epigenetic studies. Our objective is to characterize the DNA methylome of RAI-refractory carcinomas and to identify markers that can predict RAI refractoriness. To do so we have analyzed the DNA methylome of 94 thyroid samples (including 15 normal samples, 50 RAI-avid and 29 RAI-refractory tumors) using Illumina's EPICmethylation array.

We performed a differential DNA methylation analysis and found a signature of 1247 CpGs that was differentially methylated between RAI-avid and RAI-refractory tumors. These CpGs are generally located far from CpG islands and in intergenic regions. Still, a functional enrichment analysis revealed that they are mostly associated with signal transduction. Next, we reduced the signature to 6 CpGs using a regularized random forest algorithm to generate a preliminary model, named RAI Refractoriness Score (RRS), which determines the probability to develop RAI refractoriness with a 94% accuracy. Additionally, we established quantitative assays based on bisulfite pyrosequencing to quantify the methylation of the 6 CpGs that can be easily implemented in the clinical routine.

Since RAI refractoriness tends to appear after the initial diagnosis, determining the RRS in the initial tumor sample may help stratify the patients at early stages and provide them with a more personalized treatment.

O1-08

p21$^{Cip1/WAF1}$ and its relationship with androgen metabolism in prostate cancer primary cultures

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Prostate cancer (PCa) is recognized as the second cause of cancer death in men. Although the 5-year survival rate is over 90% when the cancer is detected early, survival drops to 30% when the tumor has progressed. The major problem is the progression to castration resistant PCa (CRPC) after Androgen deprivation therapy (ADT). Several mechanisms have been proposed to explain this progression, including the change in androgen metabolism due to altered expression of steroidogenic enzymes. For an early intervention, before the resistance is imperative to develop tools to study new biomarkers that predict the patient response to ADT. Neoplastic cells, that activate several genes involved in DNA repair and cell cycle progression such cyclin-dependent kinase inhibitor p21 (CDKN1A), are able to bypass senescence through the downregulation or loss of p21, however, p21 overexpression can drive some cells to acquire a more aggressive phenotype.

In this work, to identify new biomarkers predictor of response to ADT, we developed primary tumor cultures (PTCs) resembling original tumors and studied their metabolic reprogramming and changes in major driver genes. Twelve PTCs derived from needle biopsies from hormone-naïve aggressive primary prostate tumors (gleason≥8) were established and partially characterized for their major genetic lesions, phenotype, proliferation, and resistance to chemotherapy. The expression levels of enzymes associated to androgen synthesis were also analyzed and compared with their own tissue biopsies. PCa cell lines (LNCaP and Du145), tumor biopsies from radical prostatectomies, and control tissues from benign hyperplasia.

A first screening of the expression levels of 35 genes revealed numerous changes in the PTCs and cell lines, including genes in the androgen (AR, CREB1, SRD5A1, PAPSS2), estrogen (ER1 & 2, ESRRA, GPER1, CYP19A1) and the p53 pathway (TP53, MDM2, CDKN1A). The high expression of the cyclin-dependent kinase inhibitor p21 (CDKN1A) and β-Galactosidase revealed senescence-like state of PTCs, although a sustained low proliferation was detected. Because a strong positive correlation of CDKN1A expression and a number of steroidogenic genes was observed, we performed p21 RNA-silencing in PTCs and LNCaP cells. AKR1C3 and CYP19A1 were significantly downregulated in p21 knockdown ($p<0.01$) cultures, whereas MDM2 and SRD5A1 were upregulated in at least 3 PTCs. Western blot and immunofluorescence analysis revealed the presence of p21 in the cytoplasm, suggesting p21 is oncogenic rather than tumor suppressor. These results suggest changes in androgen metabolism and de novo cholesterol synthesis that could be related to a metabolic reprogramming activated by senescence bypassing of these cells in vitro.

O1-09

Defective Viral Genomes (DVGs) in SARS-CoV-2 quasispecies as trackers for viral adaptation

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SARS-CoV-2 Omicron variant has promptly emerged showing higher transmissibility and probably higher resistance to current COVID-19 vaccines than other variants dominating the global SARS-CoV-2 pandemics. We performed a study in March 2020 involving patients with COVID-19 infection of varying severity, where we found that a portion of genomes in the SARS-CoV-2 viral population accumulated deletions at the S1/S2 cleavage site (PRRAR/S) of the spike gene, generating a frameshift and appearance of a premature stop codon. The main aim of this study was to determine the frequency of defective deletions in prevalent variants from the first to the seventh pandemic waves and discuss whether the observed changes might support epidemiological proposals.

The complete SARS-CoV-2 spike gene was deeply studied by next generation sequencing using the MiSeq Illumina platform by the methodology of overlapped amplicons. More than 148 million reads have been obtained from respiratory swab specimens of 119 COVID-19 patients mildly infected with the most relevant variants circulating in Barcelona city area during the seven pandemic waves: B.1.5, B.1.1, B.1.177, Alpha, Beta, Delta and Omicron BA.1, BA.1.1, BA.2, BA.5 and BQ.1.1 variants.

The frequency of defective genomes found in Omicron BA.1 subvariant was similar to that seen in variants dominating the first and second waves, but differed from the frequencies seen in the Alpha, Beta and Delta variants. Surprisingly, the frequency of defective deletions found in Omicron BA.1.1, BA.2, BA.5 and BQ.1.1 subvariants was identical to that found in Alpha, Beta and Delta variants.

Our results concur with findings from previous studies indicating that the S1/S2 cleavage site is an important region for the biology of the virus, affecting the capability of SARS-CoV-2 to readily infect humans. Here we discuss how defective deletions naturally occurring before S1/S2 cleavage site might have putative effects during adaptation of the virus to human infection.

O1-10

How Quasispecies Studies Could Help in Viral Infections: Negative Effect of Early Ribavirin Discontinuation in a Chronically Infected HEV Patient.

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Hepatitis E is a liver inflammation caused by the Hepatitis E Virus (HEV). According to WHO, approximately 20 million people get infected with HEV every year, out of which 3.3 million exhibit symptoms and 44,000 die due to hepatic failure. HEV is a major cause of acute viral hepatitis globally, particularly in low- and middle-income countries, and its incidence is on the rise in industrialized nations. Althought HEV mostly triggers an acute infection, it becomes chronic when patients are inmunodepressed. HEV has a single-stranded RNA genome of around 7.2kb in length, consisting of three open reading frames although a fourth ORF has been described in genotype 1. The lack of polymerase proofreading activity makes HEV to incorporate different mutations in each replication cycle, being considered as a quasispecies virus.

In our study, several samples from a chronic HEV patient treated at different stages with ribavirin –mutagen- have been collected during the course of the infection. HEV RNA has been extracted and purified using spin columns, and a conserved fragment of ORF2 amplified using consecutive RT-PCR and Nested-PCR. Finally, amplified fragments have been sequenced using NGS MiSeq platform, obtaining a high deep coverage for every sample. A new method to analyze viral quasispecies relying on haplotype fitness has been designed by dividing the quasispecies in four fractions: the master haplotype, the master haplotype, rare haplotypes (RHL) at two levels (those present at <0.1%, and those at 0.1–1%), and a fourth fraction that we term emerging haplotypes, present at frequencies >1%, but less than that of the master haplotype. Results showed that HEV quasispecies were much unstructured, being very complex at a nucleotide level. What is more, the treatment with ribavirin increased the proportion of RHL to master haplotype. However, at protein level (phenotype/functional) the pattern was the opposite, with high frequencies of dominant haplotype, meaning that most of the ribavirin-induced variability were synonymous mutations, leading to a final resistance to the drug. Taken all together, the study of quasispecies in HEV chronic disease has been shown useful to understand the virus response to a mutagenic drug, especially ribavirin resistance, with important clinical implications.

O1-11

Air pollution induces ventricular arrhythmogenesis by altering the arrhythmogenic substrate, the cardiac metabolic profile; by inducing ROS production and changes in expression of signaling enzymes

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Background: Air pollution is one of the most important risk factors of disease and death. Effects of air pollution are especially relevant in the cardiovascular system. Clinical and epidemiological studies have associated it to the genesis of cardiac arrhythmias. However, a cause-effect relationship has not been clearly demonstrated. Furthermore, mechanisms involved have not been elucidated.

Aims: To assess the effects of diesel exhaust particles (DEPs) exposure on ventricular arrhythmogenesis in isolated rat hearts perfused under normoxic conditions, and to explore the mechanisms involved.

Methods: Sprague-Dawley healthy rats were intratracheally instilled with saline containing or not diesel exhaust particles (DEPs, 3 days a week for 3 weeks; 7.5 mg/Kg, 0.375 mL/Kg). Ventricular arrhythmias were induced in isolated hearts using a protocol of electrical stimulation. Additional hearts were used to assess the mechanisms involved (conventional histology and Western Blot). Effects on cardiac metabolic profile (^{1}H-NMR) and mitochondrial function were also assessed.

Results: Hearts from rats instilled with DEPs showed enhanced duration and number (0.50±0.17 vs. 0.00±0.00 in DEP-instilled and control animals, respectively, $p<0.05$) of sustained ventricular tachyarrhythmias following 2 extrastimulus. These effects were associated with enhanced interstitial collagen deposition, reduced expression of the antioxidant enzyme thioredoxin reductase 2 and with enhanced ERK1/2 activation. NMR analysis demonstrated altered cardiac metabolic profile and isolated mitochondria showed enhanced baseline ROS production.

Conclusion: Exposure to DEPs enhanced ventricular arrhythmogenesis in healthy rats associated with modifications in the arrhythmogenic substrate and the cardiac metabolic profile and by inducing ROS production and changes in cytosolic signalling pathways.

O1-12

Functional characterization of Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC) cellular models carrying the p.G20D CLDN19 founder mutation.

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Introduction: FHHNC is an ultra-rare autosomal recessive disease that occurs in less than one person per million inhabitants (<1/1,000,000) and manifests mainly in paediatric age. FHHNC is caused by loss-of-function mutations in genes *claudin* (*CLDN*)*-16* or *CLDN19*. The pathogenesis of this disease is explained by impaired kidney paracellular Mg^{+2} and Ca^{+2} reabsorption, resulting in hypomagnesemia, hypercalciuria, nephrocalcinosis and progression to kidney failure requiring dialysis and kidney transplantation. In Spain, *CLDN19* mutations are highly prevalent and around two-thirds of patients exhibit the *CLDN19* c.59G>A (p.G20D) mutation in homozygosis. Currently there are no specific treatments for the disease, or biomarkers to predict its progression.

Objective: To characterize cellular models carrying the p.G20D mutation to identify and validate therapeutic targets/strategies for FHHNC.

Methodology: HK-2 and RPTEC/TERT1 cells stably transduced with *CLDN19* WT or p.G20D were characterized by analyzing CLDN19 sub-cellular location, cell proliferation, adhesion and differentiation, endoplasmic reticulum (ER) stress and *CLDN16* mRNA expression.

Results: CLDN19 WT or p.G20D were overexpressed in HK-2 and RPTEC cells, although their levels decreased over time. Importantly, analysis of the epithelial markers ZO-1 and E-cadherin showed that both cell lines were fully differentiated at 4-5 days post-seeding. Localization analysis confirmed that CLDN19 WT reached the cell membrane and established contacts with neighboring cells, while CLDN19 p.G20D was retained in intracellular compartments. This retention did not affect ER stress levels given that neither PERK nor IRE1 ER stress pathways were activated. Besides, no differences in cell proliferation/adhesion were found. Finally, our data showed that CLDN19 p.G20D overexpression enhances *CLDN16* mRNA levels in both HK-2 and RPTEC/TERT1 cells. Interestingly, overexpression of CLDN19 WT had different effects on *CLDN16* depending on the cell line. While it strongly increased *CLDN16* transcription in HK-2 cells, it reduced *CLDN16* levels in RPTEC/TERT1 cells.

Conclusions: Our data revealed that *CLDN16* mRNA levels represent a promising readout to screen novel FHHNC pharmacological strategies that could constitute a great advance in health care, improving the health and quality of life of patients and their families.

O1-13

Impact of hypercapnia on the course of bacterial infection in an *in vitro* model of pneumonia

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Introduction

Among the most frequent causes of pneumonia are *Streptococcus pneumoniae* (SPNE) and *Pseudomonas aeruginosa* (PA). Patients with advanced respiratory pathologies can commonly develop hypercapnia. It is vital to study the course of infection in easy-to-perform models as well as the effect of hypercapnia in this period.

Objectives

This project aims to evaluate the bacterial survival and the course of the biological response of the co-culture of alveolar epithelial cells (HPAEpiC) and macrophages as a result of infection with different types of bacteria (PA and SPNE) under different CO_2 concentrations.

Methods

Co-cultures of HPAEpiC and THP-1 cells differentiated to macrophages were separately infected with PA or SPNE for 1 hour at 37 °C under normocapnic (5% CO_2) or hypercapnic (15% CO_2) conditions. Bacterial survival was then assessed after infection.

At 1 hour or 24 hours after the onset of infection, at 5% or 15% CO_2, intracellular and extracellular proteins were analyzed by ELISA for inflammatory (IL-1β), chemoattractant (IL-8, CCL-2) and cell junction (occludin, ZO-1) mediators. Apoptosis was also assessed in these two time-points by TUNEL assay.

Results

Infection with PA or SPNE in a culture of HPAEpiC and THP-1 cells under hypercapnia increases inflammation and decreases phagocyte chemotaxis.

Hypercapnia would potentiate ZO-1 and occludin production in the co-culture after infection but not 24 hours later, showing incomplete tissue repair in respect to the control cultures. SPNE infection displayed the poorest regeneration levels.

In addition, PA-infected THP-1 cells show decreased apoptosis under hypercapnia compared to normocapnia conditions.

In terms of the clearance of extracellular bacteria, there are no differences between exposure to the different CO_2 concentrations in both infections, although the inflammatory response produced against PA succeeds in eliminating most of the initial inoculum.

Conclusions

The hypercapnic condition on bacterial-infection response could play a detrimental in the course of PA and SPN infection.

O1-14

Deciphering *Pseudomonas aeruginosa* infections through Tandem mass spectrometry analysis

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For decades, classical antibiotics have been the cornerstone of infectious disease therapy, effectively killing or limiting the growth of pathogens[1]. However, the ongoing evolution of antibiotic resistance has significantly diminished their effectiveness. This is particularly concerning in the case of *P. aeruginosa* (PA).

PA is usually a nosocomial pathogen that colonizes a variety of organs[2]. Its infections are linked to high morbidity and mortality rates in individuals with sustained trauma or weakened immune systems[3]. Cystic Fibrosis patients are particularly vulnerable to PA respiratory infections as the pathogen has propensity to form biofilms in the fibrotic respiratory milieu[4].

From the perspective of disease pathogenesis, PA infections involve complex pathogen-host molecular interactions. These interactions are predominantly established among protein complexes and modulate processes such as initial adherence to the tissue, immune evasion, exertion of virulence and dissemination to the surrounding media[5,6]. Understanding the protein-protein interaction (PPI) landscape in disease is vital for developing new effective therapies to combat the pathogen.

Interestingly, over the past decade mass spectrometry has become an essential analytical tool for studying PPIs, expression profiles, and regulatory mechanisms[7]. Here, we describe a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) based approach to study PA infections in pulmonary cells at the protein level, with a particular emphasis on identifying new pathogen effectors and host targets. Ultimately, analyzing this pathointeractome may provide a valuable resource for understanding the complexity of cystic fibrosis-associated infections and for the development of new treatments.

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O1-15

Harnessing alkaline pH regulatable promoters as a novel platform for heterologous protein expression in *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae* responds to a moderate alkalinization of the medium with extensive remodeling of gene expression, affecting hundreds of genes. These changes occur in response to the activation of diverse signaling pathways, including the conserved Rim101/PacC pathway, the calcium-activated phosphatase calcineurin, the Wsc1-Pkc1-Slt2 MAP kinase, oxidative stress-responsive pathways, and diverse nutrient-signaling pathways such as the Snf1, Pho85, and PKA kinases[1]. The transcription factors mediating these inputs are also well characterized and there are examples (such as the *ENA1* Na^+-ATPase gene) of a single promoter integrating various signals, to better shape the appropriate response. We will describe current work aiming to the generation, by both rational design and combinatorial methods, of alkaline pH-driven synthetic hybrid promoters that could serve as novel and inexpensive platforms for heterologous protein expression in *S. cerevisiae*. Over 50 different constructs, including single and multiple combinations of the calcineurin-regulatable CDRE motif, the *PHO* sequence, and a novel Stp1/2 consensus recently identified in the *ENA1* promoter[2], were made and tested for GFP production. In some cases, GFP production was increased up to 30-fold by a simple addition of KOH to the medium. A selection of these promoters are currently tested for production of industrial enzymes, and preliminary results suggest that they could match the production achieved from the GAL promoter, generally accepted as a paradigm of strong regulatable promoter..

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O2-01

Bioengineered self-assembling nanofibrils for the capture and neutralization of SARS-CoV-2

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The coronavirus disease 2019 (COVID-19) pandemic has prompted the scientific community to prioritize the development of innovative antiviral tools, such as nanotechnology-based virucides and anti-spreading materials. It is crucial to develop enhanced and highly efficient prophylactic measures. Our study demonstrates the successful utilization of "self-assembled antiviral fibers" and the strength of multivalency for the deactivation of viruses from surfaces, providing a cost-effective, multifaceted approach for addressing future pandemics or potential threats. To achieve this, we employed amyloids as self-organizing protein scaffolds, customized them to contain SARS-CoV-2 miniprotein binders, that efficiently bind and trap the virus. Our modular approach involved fusing a 20-residue-long soft amyloid core (SAC), from the Sup35 prion protein, which acted as the assembling module, with two different high-affinity spike protein minibinders, using a flexible S/G linker. Biophysical assays revealed that the embedded minibinders remained in their native folded, and active state in the fusion. Upon assembly, the biocompatible and multivalent amyloid fibrils effectively captured the spike protein, neutralizing SARS-CoV-2 with IC50 values in the low nM range. This prevented the virus from being taken up by the angiotensin-converting enzyme 2 (ACE2) receptor binding expressing cells. Furthermore, we modulated the morphology of the nanofibrils by adjusting the salt concentration during the assembly process. In addition, the nanofibrils displayed high chemical stability against chaotropic agents, such as guanidine chloride, indicating a typically longer half-life. Overall, our study presents a novel functional protein-based nanomaterial with diverse applications in biomedicine and biotechnology, including the development of diagnostic kits for SARS-CoV-2, and the creation of prophylactic agents and virus-neutralizing equipment.

O2-02

Development of a highly potent transthyretin amyloidogenesis inhibitor: Design, synthesis and evaluation

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Transthyretin misfolding and amyloid aggregation is associated with a group of fatal diseases named transthyretin amyloidosis (ATTR). Most forms of ATTR are associated with inherited mutations that destabilize the protein's native state. Yet, *wild-type* (WT) protein deposition might also occur in elderly people. Small molecules that bind to TTR act as kinetic stabilizers, preventing tetramer dissociation and thus protein aggregation. In this context, we have applied a molecular dynamics (MD) simulations aided analysis to the rational design of a collection of tolcapone derivatives, a potent TTR aggregation inhibitor, with optimized activity. This strategy crystallized in the discovery of M-23, a tolcapone congener displaying one of the highest binding affinities described for WT-TTR thus far. The crystal structure of M-23 bound to WT-TTR at 1.2 Å confirmed the formation of the new and strong protein-ligand contacts predicted by MD simulations resulting in a higher tetramer stabilizing activity both *in vitro* and in human plasma, in comparison with tolcapone. Altogether, the results of this study support the application of MD simulations in the design of TTR ligands that, as M-23, hold the potential to become potent ATTR disease-modifiers.

O2-03

The influence of immune landscape in response to therapy in glioblastoma: a preclinical example

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INTRODUCTION: Preclinical glioblastoma (GB) studies for improving therapeutic outcomes are necessary since clinical GB has no current cure. Our group has coined the expression IMS-TMZ (Immune-enhancing Metronomic Schedule Temozolomide) to describe the every-6-day administration schedule, which was successful in curing 50% of GL261 GB mice [1]. In comparison to GL261, the CT-2A model was reported to have an immunosuppressive environment and a lower mutational load, which more closely resembles the human GB condition [2,3]. Our goal is to evaluate the IMS-TMZ therapy on the CT-2A model.

METHODS: CT-2A cells were implanted in C57/BL6 mice as described for GL261 [1]. Magnetic resonance imaging and spectroscopic imaging (MRI/MRSI) acquisitions were performed in a 7T Bruker preclinical scanner with Paravision 5.1. T2w MRI and multislice MRSI parameters were as described in [4]. Postprocessing and generation of nosological images was performed with the classification system developed for GL261 GB [5].

RESULTS & DISCUSSION: Untreated CT-2A bearing mice had longer survival than GL261 (34±4 vs 21±4 days p.i., n=17 and n>100), and tend to form partially extracranial tumours. Their spectroscopic pattern showed typical GB tumour features such as decreased N-acetyl aspartate, increased choline-to-creatine ratio and mobile lipids. MRSI-based nosological images suggest that the classifier developed for GL261 GB could be robust enough to recognize CT-2A GB. IMS-TMZ produced modest results in CT-2A with lower tumour growth rate than controls, and a small, albeit significant, survival increase (35±4 for treated mice vs 28±2 days p.i, n=5 each). A new cohort is ongoing for investigating whether the MRSI-based classifier is able to recognize response to therapy in CT-2A.

CONCLUSION: GB models with different immunogenicity may respond differently to IMS-TMZ treatment. These findings highlight the relevance of testing treatment strategies in preclinical models with immunophenotypes similar to those observed in GBM patients.

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O2-04

MAP kinase ERK5 modulates cancer cell sensitivity to extrinsic apoptosis induced by death-receptor agonists and Natural Killer cells

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Death receptor ligand TRAIL is a promising cancer therapy due to its ability to selectively trigger extrinsic apoptosis in cancer cells. However, TRAIL–based therapies in humans have shown limitations, mainly due inherent or acquired resistance of tumor cells. To address this issue, current efforts are focused on dissecting the intracellular signaling pathways involved in resistance to TRAIL, to identify strategies that sensitize cancer cells to TRAIL-induced cytotoxicity. In this work, we describe the oncogenic MEK5-ERK5 pathway as a critical regulator of cancer cell resistance to the apoptosis induced by death receptor ligands. Using 2D and 3D cell cultures and transcriptomic analyses, we show that ERK5 controls the proteostasis of TP53INP2, a protein necessary for full activation of caspase-8 activation in response to TNFα, FasL or TRAIL. Mechanistically, ERK5 phosphorylates and induces ubiquitylation and proteasomal degradation of TP53INP2, resulting in cancer cell resistance to TRAIL. Concordantly, ERK5 inhibition or genetic deletion, by stabilizing TP53INP2, sensitizes cancer cells to the apoptosis induced by recombinant TRAIL and TRAIL/FasL expressed by Natural Killer cells. The MEK5-ERK5 pathway regulates cancer cell proliferation and survival, and ERK5 inhibitors have shown anticancer activity in preclinical models of solid tumors. Using endometrial cancer patient-derived xenograft organoids, we propose ERK5 inhibition as an effective strategy to sensitize cancer cells to TRAIL-based therapies and Natural Killer cells.

O2-05

Altered excitatory and inhibitory hippocampal neurons correlate with memory deficits in a new model of Alzheimer's Disease

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Memory deficits in Alzheimer's disease (AD) are associated with excitatory/inhibitory neurotransmission imbalance in memory neural circuits affected by amyloid-β and tau pathologies. However, the specific mechanisms by which these neuropathological changes induce dysfunction of excitatory and inhibitory hippocampal neurons remain poorly understood. Here, we report differential transgene effects on cognitive performance, as well as selective cell type-specific pathology in novel double APP/Tau transgenic mice expressing human familial AD-linked mutant amyloid precursor protein (APP) and microtubule-associated protein tau (Tau) genes. Histopathological analyses reveal that Aβ and hyperphosphorylated Tau are mainly present in excitatory neurons (CaMKIIα+), rather than inhibitory interneurons (Pvalb+) at 6 months of age. Additionally, we employed the RiboTag approach to analyze cell type-specific mRNAs from excitatory and inhibitory neurons in control and APP/Tau mice expressing CaMKIIα-Cre;RiboTag and Pvalb-Cre;RiboTag, confirming that human APP and Tau are specifically expressed in excitatory but not inhibitory neurons. At 6 months of age, Tau and APP/Tau mice show spatial learning and memory deficits which are associated with reduced levels of synaptonuclear factors and synaptic proteins related to excitatory neurotransmission in the hippocampus.
Interestingly, tissue clearing and 3D imaging analyses show reduced neuronal number and altered dendritic morphology of Pvalb-expressing neurons in the hippocampus of APP/Tau mice, evidencing pathology-dependent alterations in inhibitory neurotransmission in our model.
Globally, our novel APP/Tau;RiboTag mice recapitulate AD pathology at the histological, biochemical and behavioral levels, establishing this model as a valuable tool for the study of cell type-specific molecular mechanisms underlying selective neuronal vulnerability in AD.

O2-06

Pathological and functional characterization of emotion-related neural circuits in novel Alzheimer's disease transgenic mice

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Alzheimer's disease (AD) progresses with memory loss and neuropsychiatric symptoms associated with cell-specific vulnerability in memory- and emotion-related neural circuits. The abnormal connectivity within the hippocampus and amygdala, which govern the acquisition and expression of episodic and emotional memories, are thought to underlie memory loss and emotional disturbances in AD. Accumulation of amyloid plaques and neurofibrillary tangles (NFT) containing amyloid-β (Aβ) peptides and hyperphosphorylated tau protein are crucial factors influencing clinical progression to dementia. However, the crosstalk mechanisms by which Aβ and tau cause synaptic dysfunction and differential vulnerability of memory and emotional neural circuits remain largely unclear. Our results demonstrate that Tau and APP/Tau mice develop age-dependent tau pathology and spatial memory deficits associated with hippocampal accumulation of synaptic tau. By contrast, APP and APP/Tau mice of both sexes exhibit innate anxious behavior and impaired fear memory extinction linked to Aβ pathology in the basolateral amygdala (BLA). Remarkably, chemogenetic inactivation of excitatory engram neurons in the BLA ameliorates aberrant fear-related associative memory in APP mice. Importantly, tissue-level and cell-specific RNA sequencing reveal region-specific but common transcriptional changes related, among others, to synapse transmission and plasticity in response to Aβ/tau pathology. Notably, bulk transcriptional profiles show 63 orthologs of human AD risk genes identified in GWAS differentially expressed in the hippocampus and/or BLA of APP/Tau mice. Accordingly, a genetic score generated from the BLA bulk-RNA dataset of APP/Tau mice is correlated to AD pathology and emotional affect and personality traits from the ROSMAP cohort, suggesting that our mouse model exhibits transcriptional changes connected to established molecular determinants of AD development. In conclusion, these findings disentangle the region-specific pathogenic effects of Aβ and tau in excitatory neural networks governing emotional and memory processing, indicating that pathological factors and their molecular cascades should be considered in future AD preventive and treatment initiatives.

O2-07

Lesch-Nyhan disease fibroblasts alterations revealed under physiological culture conditions

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Lesch-Nyhan disease (LND) is a severe neurological disorder caused by the genetic deficiency of hypoxanthine-guanine phoshoribosyltransferase (HPRT), an enzyme involved in the salvage synthesis of purines. HPRT deficiency causes guanine, xanthine and hypoxanthine accumulation in the cells which are degraded to acid uric leading to the presence of hyperuricemia. Moreover, there is an acceleration of de novo purine biosynthetic pathway. Uric acid toxicity has been discarded since its suppression in patients by the administration of allopurinol from birth does not attenuate the neurobehavioral dysfunction. Recently, it has been shown that 5-aminoimidazole-4-carboxamide riboside 5'-monophosphate (ZMP), an intermediate of the purine biosynthesis pathway, accumulates in LND fibroblasts. ZMP accumulation in LND fibroblasts but not in controls is observed under physiological levels of FA (25nM) which strongly differs from FA levels of the vast majority of commercial media (2200nM). In order to avoid nutrient exhaustion when culturing cells, most of the commercial media contain non-physiological levels of nutrients having a great impact in cell metabolism that does not meticulously recapitulate the in vivo behavior of cells. This current work aims to evaluate differences of using commercial or physiological culture media when studying a metabolic disorder and to study the potential alterations of LND fibroblasts that may have been mask by the usage of non-physiological media. For that, we have quantified ZMP accumulation under different culture conditions and we have evaluated the activity of two known ZMP-target proteins (AMPK and ADSL), the mRNA expression of the folate carrier SLC19A1, possible mitochondrial alterations and functional consequences in Lesch-Nyhan disease fibroblast cultivated under conditions that best recapitulate the physiological situation.

O2-08

AAV9-Mediated Expression of Secreted Klotho Reduced Several Aging-Associated Phenotypes and Increased Longevity

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Advances in health care and quality of life in modern societies have led to an increase in the percentage of the population reaching advanced ages, with a projected 25 % of U.S. citizens being over the age of 65 by 2060. Aging is one of the main risk factors for a wide range of pathologies, including osteoporosis, sarcopenia, and cognitive degeneration. These conditions are accompanied by suffering, disability, and elevated economic and social costs, thus new therapies are needed to achieve healthy aging.

The protein Klotho (KL) has been identified as a promising anti-aging molecule due to its pleiotropic actions, with pro-longevity effects on pathways such as insulin/insulin-like growth factor and Wnt signaling, and inflammatory and oxidative stress modulation. Here, we explored the anti-aging potential of the secreted isoform of this protein in the SAMP8 and C57BL mice, models for accelerated and non-pathological aging, respectively. Systemic and intracerebroventricular delivery of AAV9 efficiently increased concentration of s-KL protein in serum, improving the aging phenotype in different organs analyzed. KL treatment improved fitness in behavioral tests, associated with reduced muscular fibrosis and increased muscle regeneration. Cortical and trabecular microstructural parameters in the aged bones, measured by MicroCT analysis, were also improved in treated animals. Cognitive capacities of aged animals were improved, which was accompanied by increased markers of adult neurogenesis (Ki67 and DCX), and normalization of cellular markers in the hippocampus like Iba1+ and GFAP+ cells populations, supported by histological and transcriptional analysis. Remarkably, long-term AAV-mediated expression of s-KL lead to a 20% increase in total longevity of C57BL mice.

These results show for the first time, the potential of exogenous increase in s-KL protein to treat several age-associated deficits, increasing both health and life span of wild type animals. Due to the safer pharmacological profile of s-KL compared to other KL isoforms, and being a naturally secreted protein, gene therapies increasing s-KL levels represent a promising approach to reduce the impact of the age-associated degeneration in multiple organs.

O2-09

Towards hereditary spastic paraplegia type 52 (SPG52) AAV-based gene therapy strategy

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Hereditary spastic paraplegia type 52 (SPG52) is an ultra-rare inherited neurological disorder characterized by lower limb spasticity, weakness, global developmental delay, intellectual disability, and seizures. SPG52 is caused by biallelic mutations in the AP4s1 gene, which encodes a subunit of the adaptor protein complex 4 (AP-4). Mutations in any of its 4 subunits result in complex destabilization and degradation of all subunits, leading to a shared pathophysiology and symptomatology. The function of AP-4 within the cell, although unclear, it is believed to play a key role in autophagosome generation. Currently, this disease does not have a cure nor treatment. We believe that gene therapy aimed at restoring AP4s1 expression is a rational therapeutic approach to ameliorate the disease phenotype. Thus, we have generated, characterized, and successfully treated patient-derived fibroblasts with viral vectors carrying a correct copy of the AP4s1 gene. This treatment has not only restored the expression of this specific subunit but has enabled complex expression restoration, confirming our therapeutic approach's effectiveness. To further study the effects of our gene therapy approach we have developed and characterized a disease-relevant human model by generating patient-specific induced pluripotent stem cells (iPSCs) that upon differentiation to neurons recapitulate aspects of the disease's pathophysiology and can be used to test the efficacy of our novel gene therapy strategy. In parallel to our in vitro studies, we have developed the first rodent animal model of SPG52. This animal model is a knock-out (KO) mouse generated using the CRISPR-Cas technology targeted to abolish AP4s1 expression in a C57Bl6 strain. To characterize the animal model, cognitive and physical skills batteries have been performed. KO mice show decreased motor coordination as well as muscle strength by grip strength, inverted grid, RotaRod, and clasping tests. These KO animals, based on their performance doing the Open Field and Morris Water Maze tests, also display impaired memory while having a regular exploratory behavior with no signs of anxiety. Distinctive hallmarks described in patients have been observed by magnetic resonance imaging, electrophysiology, and clasping test. In the present, we are treating AP4s1 KO animals systemically with an *in vitro* validated neurotropic AAV vector carrying a correct copy of the AP4s1 gene at different ages and assessing phenotype rescue. The generation of the hiPSC model together with the establishment of this newly generated mouse model, both with a phenotype that resembles the human pathology, will be crucial to elucidate SPG52 pathogenesis and to validate our gene therapy approach, key milestones on the road to a successful treatment for SPG52.

O2-10

Therapeutic effect of sKL gene therapy strategy to treat aging and neurodegeneration

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The gene Klotho encodes a type-I-membrane protein expressed in two forms, membrane and secreted. Inhibition of klotho induces an accelerated age phenotype whereas its over-expression extends lifespan and thus has been proposed as a promising candidate for treating age-related disorders. In the last years we have been focused on gene therapy strategies with klotho as a therapy for cognitive deficits associated with aging and neurodegeneration. Our studies demonstrated that just a single in vivo administration of AAVs expressing soluble klotho (sKL) resulted in long-lasting enhancement memory in non-pathological aged and accelerated-senescence (SAMP8) mice. Also, sKL increases bone density, reduces bone fragility and osteopenia, as well as increases muscle strength and reduces sarcopenia. Moreover, long-term expression of sKL increases longevity and rejuvenate the neuronal epigenetic profile. Considering all these data, in the present study we investigate the effects of gene-therapy with sKL in Alzheimer's disease (AD). Principal results showed that sKL overexpression tended to improve spatial learning and reference memory in the MWM in males as well as the recognition and spatial memory in the NOR and T-maze tests in females of the APP/Tau model. In this context, and with the objective to get closer to humans, we determined that sKL is the predominant klotho transcript expressed in human brains, both in healthy-aged and AD's patients. Furthermore, sKL therapy is a safety strategy and improves cognitive performance in aged non-human primates. In conclusion, we demonstrate that sKL is an attractive therapy with high potentiality to treat human brain pathologies like AD

P1-01

Protein-only Nanoparticles for T-cell Activation

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Adoptive T-cell immunotherapy (ACT) utilizing nanosized artificial antigen presenting cells (aAPCs) has emerged as a promising approach for cancer treatment. Although diverse nanomaterials have been explored as aAPC scaffolds, protein-only nanoparticles have been largely overlooked, despite their high designability and biocompatibility. In this study, we present a novel plug-and-play approach for the development of protein-only nanoparticles as aAPCs, using the self-assembling properties of ZapB coiled-coil and the Z-domain antibody-capturing ability. The resulting coiled-coil based nanoparticles (ccNPs) can be easily and rapidly functionalized with a tailored combination of antibodies, making them a versatile platform for T-cell expansion. Our results demonstrate that ccNPs decorated with anti-CD3 and anti-CD28 antibodies induce T-cell proliferation and activation at a level comparable to commercial magnetic beads, while sustaining cytokine production for an extended period. The biocompatibility, modularity, and chemistry-free surface modification of this protein-only platform offers a valuable tool for developing personalized ACT for cancer treatment.

P1-02

Exploring the cleavage and binding features of RNase 2 on tRNA

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Non-coding RNA (ncRNA) is a type of RNA that does not encode proteins but plays an important role in regulating cellular physiological activities and executing cellular functions. As one of the basic types of ncRNA, transfer RNA (tRNA) derived fragments (tRFs) have been implicated in various diseases. In our previous research, we found that human Eosinophil-Derived Neurotoxin (EDN/RNase 2) can selectively cleave tRNA molecules both under normal conditions and during viral invasion[1]. This was demonstrated through a series of experiments, including comparison of wild-type and RNase 2 knock-out THP1-derived macrophages, prediction of cleavage sites using 2,3'-cyclic phosphate (cP) RNA sequencing (cP-RNA-Seq), and screening of tRF & tiRNA array.

Significant changes in tRF population are associated to RNase 2 expression, which directly participates in cellular antiviral activity. In vitro digestion of the top 5 tRNA targets was performed by in vitro transcription (IVT) and incubation with the RNase2 recombinant protein. A specific cleavage pattern by RNase2 was identified using urea-PAGE and product amplification by RNAseq. Next, we performed biochemical validation of the terminal characteristics of cleaved fragments, and in vitro degradation of single-point mutations of tRNA and DNA/RNA hybrid hairpins with RNase2, which validated the preference of RNase2 for U/C(B1) and A(B2) as the main cleavage sites. Finally, we aimed to study RNase2 in complex with a DNA hairpin based on the anticodon loop of one of the main protein target: tRNA AspGTC and performed multiple crystallization trials. The presence of the hairpin binding was verified by EMSA and Gel-filtration chromatography but unfortunately no crystals of the complex were obtained. Alternatively, we performed docking and molecular dynamics to predict the protein-nucleotide interactions and explore the protein selective recognition at the tRNA anticodon loop. After inspection of the H-bond and van der Waals interactions in the current simulation studies, we found that residues at the protein L4 loop (Ser64, Asn65, Lys66 and Arg68) were important for the protein B2 binding in the group of hairpins (UCA, UAA, CAA) that can be cleaved by RNase 2 in vitro.

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P1-03

The structural architecture of an α-synuclein toxic oligomer

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Oligomeric species populated during α-synuclein aggregation are considered key drivers of neurodegeneration in Parkinson's disease. However, their structure and the molecular determinants driving their conversion to fibrils remain elusive. In this work, we determined the symmetry and architecture of α-synuclein oligomers, dissecting the conformational properties of individual chains within these toxic assemblies. We demonstrate that the NAC domain is insufficient to promote oligomer to fibril conversion; instead, this transition is controlled by a short α-synuclein N-terminal motif. A missense mutation causing early-onset Parkinson's disease remodels this N-terminal region conformation, which results in a population of long-lived oligomers less susceptible to disaggregation by the human Hsp70 machinery. Our results provide a structural understanding of oligomer to amyloid conversion and identify targets for therapeutic intervention.

P1-04

The histone mark H3K27me3 is associated to environmental-responsive genes in plants

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Among different epigenetic mechanisms, histone posttranslational modifications are one of the most important players of the transcriptional regulation of gene expression. There is a wide range of histone marks and, depending on the specific modification, their effect on gene activity is associated to either activation or repression of transcription. The trimethylation of the lysine 27 of the histone H3 (H3K27me3) is traditionally associated to repression of gene activity. In fact, this trimethylation is deposited by the so-called Polycomb Repressive Complex 2 (PRC2). Later, H3K27me3 attracts chromatin remodelers, such LIKE HETERECHROMATIN PROTEIN 1 (LHP1) and the PRC1 complex, that results in the stabilization and spread of the H3K27me3 and the ubiquitination of the lysine 121 of the histone H2A (H2Aub). The effect of this latter mechanisms often results in the stable repression of genes, but recently activation of genes has been also described, although still in an unclear manner. Most of the studies to understand the regulation of genes by Polycomb activity have been done studying developmental-associated genes, like the homeotic genes. For that reason, in this work we direct our attention to the H3K27me3-targeted genes which are regulated in response to changes in the environment. Based on transcriptomic and epigenomic datasets, we relate the presence of H3K27me3 and LHP1 on either activated or repressed genes in response to environmental cues. We find that not only repressed genes, but specially the highly activated genes are H3K27me3 targets and, therefore, the repression role of the Polycomb activity is not always occurring.

P2-01

Levels of vascular endothelial growth factor-A as a marker of collateral circulation in patients with acute ischemic stroke and large-vessel occlusion

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Background and aims: Vascular endothelial growth factor A (VEGF-A) is involved in angiogenesis. We aimed to study whether higher VEGF-A plasma levels are associated with better Collateral Circulation (CC) in patients with acute ischemic stroke (AIS) and large-vessel occlusion (LVO).

Methods: Prospective multicentre study of patients with anterior circulation AIS due to LVO treated with endovascular therapies within the first 24 hours after onset. Blood samples were obtained on admission before endovascular treatment. Plasma VEGF-A levels were quantified by ELISA and classified into quartiles. The primary outcome was CC at admission classified by Collateral Score (CS). The CS was assessed by automated and validated software (Brainomix®) from baseline single-phase CT angiography: poor, filling of ≤10% of the occluded MCA territory; moderate, 11-50%; good, 51-90%; excellent, >90%. We performed a shift-analysis and a multivariable ordinal logistic analysis using a backward stepwise regression approach to predict CS.

Results: We included 183 patients (mean age 72.8±13.7 years; 50.3% were women; 68.5% had a time from stroke onset to admission <6h). CS was poor in 6.0%, moderate in 17.5%, good in 43.2% and excellent in 33.3% of the patients. Better CC was associated with lower age, lower baseline NIHSS score, higher ASPECTS, longer time from stroke onset to admission and higher plasma VEFG-A levels. In the final multivariable analysis adjusted by age and baseline NIHSS, higher plasma VEGF-A levels were associated with better CS (aORx1 quartile increase= 1.30, 95%CI 1.01-1.66; p=0.042).

Conclusions: In patients with AIS due to large-vessel occlusion, higher concentrations of plasma VEGF-A were associated with better collateral circulation.

P2-02

Salt, pH and Amyloid-beta-peptide aggregation

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Amyloid-beta-peptide (AB) aggregation is considered one of the main hallmarks of Alzheimer's disease. Special attention has been given to the relationship between the changes in pH in the brain and the development of this neurodegenerative disease. Recently, altered endosomal and lysosomal pH values have been observed in AD mouse models. Also, in certain micro-environments, the pH can differ, for example, at the membrane surface, the pH is more acidic, favouring the formation of aggregates. A property that has been associated with the AB antimicrobial activity. In this context, the cells also regulate salt concentration, which forms a repulsion shield that can be modulated to control protein interactions. Moreover, amyloid conformation is pH sensitive. Accordingly, AB aggregation can be reversed by changing the pH. This suggests that, inside the cell, AB conformation could be regulated through pH and salt concentration. In this context, we systematically characterize how AB aggregation and conformation are affected by changes in pH and salt. From a molecular point of view, we aim to shed light on the different toxic and functional effects that this peptide produces on the cell.

P2-03

Assessment of the contribution of gut microbiome derived prion-like proteins in the progression of Alzheimer disease

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Our life is closely linked to microorganisms, either through a parasitic or symbiotic relationship. Regarding this symbiotic relationship, microorganisms are present in different human tissues, being the largest microbial community our gut microbiota. Microbial cells and genes outnumber human cells about 10:1 and genes about 100:1, so they constitute the largest diffuse organ system in the human body. Microbiota strongly influence host nutrititve-, innate-inmune, neuroinflammatory-, neuromodulatory- and neurotransmission-functions. In this context, understanding the link and mechanisms by which our microbiome influences our body is crucial in human health and disease. Regarding this, the present project aims to shed light into the understanding the link between modifications in gut microbiota and development and progression of Alzheimer disease. This will be achieved by deciphering the mechanisms by which the proteins of the gut microbiome trigger the aggregation of the host proteins. This aggregation can be transmitter between cells in different tissues in our organism. It has been demonstrated that alterations in microbiota are closely related with neurodegenerative diseases and it has been suggested that amyloid structures formed by microbiota may trigger host proteins aggregation. We have analyzed a group of putative prion-like proteins derived from the gut microbiome that are able to aggregate into amyloid fibrils and influence the aggregation of amyloid beta peptide. To do that, we feed our Caenorhabditis elegans model of Alzheimer disease (CL2355) and wild type C.elegans (N2) with modified *Escherichia coli* that express those peptides. We analyzed the diseases status and progression in those animals by assessing the short-term associative memory (STAM).

P2-04

Transcriptional and epitranscriptional mechanisms in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by progressive memory loss associated with neuropathological features and gene expression changes in memory neural circuits, but the molecular mechanisms linking transcriptome and pathological changes during disease progression are largely unknown. Previous studies from our lab revealed altered activity-dependent genes in the human hippocampus at early AD pathological stages. In this study, we evaluated the expression of activity-dependent genes in mouse neuronal cultures and in human AD hippocampus, to assess their implication in AD. We found that neuronal activity regulates the expression of genes mediating epitranscriptomic N6-methyladenosine (m6A) RNA methylation in primary neurons. Interestingly, specific immediate activity-dependent and m6A-related genes are altered in the human hippocampus during AD, indicating that neuronal activity and m6A RNA methylation mechanisms are altered in AD. Taken together, our results suggest that altered activity-regulated expression of m6A-related genes in the hippocampus may contribute to cognitive dysfunction in AD.

P2-05

The small aromatic compound SynuClean-D inhibits the aggregation and seeded polymerization of multiple α-synuclein strains

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Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, as well as the accumulation of intraneuronal proteinaceous inclusions known as Lewy bodies and Lewy neurites. The major protein component of Lewy inclusions is the intrinsically disordered protein α-synuclein (α-Syn), which can adopt diverse amyloid structures. Different conformational strains of α-Syn have been proposed to be related to the onset of distinct synucleinopathies; however, how specific amyloid fibrils cause distinctive pathological traits is not clear. Here, we generated three different α-Syn amyloid conformations at different pH and salt concentrations and analyzed the activity of SynuClean-D (SC-D), a small aromatic molecule, on these strains. We show that incubation of α-Syn with SC-D reduced the formation of aggregates and the seeded polymerization of α-Syn in all cases. Moreover, we found that SC-D exhibited a general fibril disaggregation activity. Finally, we demonstrate that treatment with SC-D also reduced strain-specific intracellular accumulation of phosphorylated α-Syn inclusions. Taken together, we conclude that SC-D may be a promising hit compound to inhibit polymorphic α-Syn aggregation.

P2-06

TAC-1: Advancing towards the development of small molecules to treat tauopathies

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Tauopathies are a set of neurodegenerative diseases characterized by the amyloid aggregation of an intrinsically disordered protein named tau. The most common tauopathy is Alzheimer's disease, which is the main cause of dementia worldwide and leads to a high social and economic burden on our society. Nowadays, the current therapies are only addressed to treat the symptomatology, but not the onset nor progression of these disorders. Here, we developed a novel high-throughput screening methodology to find new tau aggregation inhibitors based on the implementation and optimisation of seeded tauK18 aggregation kinetics. We obtained 97 compounds with anti-aggregating properties that subsequently were tested on aggregation kinetics with full-length tau (Tau2N4R). This two-step screening process (TauK18 and Tau2N4R) allowed us to identify TAC-1, a promising compound that inhibits the amyloid aggregation of the two variants as reported by thioflavin-T and light scattering. We found that TAC-1 is an inhibitor with concentration-dependent activity that may impact aggregate structures. Flow Induced Dispersion Analysis and Small Angle X-Ray Scattering results suggest that TAC-1 targets Th-T binding species altering the structure of tau aggregates. Taken together, we conclude that TAC-1 may constitute a promising hit compound to inhibit and modulate tau amyloid aggregation.

P2-07

Development of an LC-MS/MS method for the multiplex quantification of sphingolipids in brain tissue of Parkinson's disease mouse model.

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BACKGROUND- AIM

The GBA gene codes the lysosomal enzyme β-glucocerebrosidase (Gcase), which metabolizes glucosylceramide (HexCer) and its deacylated form glucosylsphingosine (HexSph). Mutations in GBA are the major genetic risk factor for Parkinson's Disease (PD). In PD patients Gcase activity is reduced and its substrates can accumulate and present dysfunction of the autophagy-lysosomal system. Quantification of these species can be used as a biomarkers to assess the efficacy of disease-modifying treatments in mice. Sensitive and specific methods are needed for this purpose.

The aim of the study was to develop and optimize a lipid extraction protocol and a liquid chromatography coupled to mass spectrometry (LC−MSMS) method for the multiplex analysis of HexSph and HexCer isoforms in mouse brain samples.

METHODS

Method development included pretreatment optimization, adjustment of the range of the curve, testing with different internal standards (IS) and optimization of all MS and LC parameters. The precision of the method were verified with different runs of independent samples and internal controls.

Tissue samples obtained from GBA mouse models were homogenized using a douncer. Then, 100µL of the homogenate were mixed with 120µL of H2O and 600µL of a MeOH:$CHCl_3$ (1:2) (MC) solution containing IS GluSph-d5 and C22-GluCer-d4. After centrifugation the resulting organic phases were separated, evaporated to dryness and resuspended with MC. Species were analyzed by a LCMS-8050 (Shimadzu) operated in positive ion electrospray mode using a C18 column (Teknokroma).

A binary gradient was designed with 0.1% FA in H2O (A) and 0.1% FA in IPA:MeCN 3:1 (B) at a flow rate of 0.3 ml/min. Calibration curves ranged from 0.0275 to 100 pmol. Peaks were processed using LabSolutions Software and results were normalized to the protein content.

RESULTS

MRM chromatograms showed the presence of multiple and separate target peaks during a run time of 6 min in the following elution order: HexSph and C16:0, C18:0, C20:0, C22:0, C23:0, C24:1, C24:0 isoforms.

Interassay variability was <15% for all species.

PD-GBA mouse midbrain samples showed an accumulation of HexCer and HexSph substrates (92.8 and 0.34 pmol/mg tissue respectively) and 5-10% decrease with respect to their baseline levels when mice were treated with GBA chaperones therapy.

CONCLUSION

Although it needs to be fully validated, a robust LC-MSMS method for the multiplex quantification of HexSph and HexCer is presented. The results show that this method is useful for evaluating the efficacy of treatment in brain mice and suggest that sphingolipids may be biomarkers of interest for PD.

P2-08

Anti-amyloidogenic potential of a histidine-maltose-dendrimer-lipid nano-vector in Alzheimer´s disease

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by the aggregation of β-amyloid and the presence of neurofibrillary tangles. Currently, no effective treatment has been found, although some drugs have been approved to slow down the progression of the disease by interacting with Aβ amyloid peptides. A recent study demonstrated that dendrimers (branched nano-polymers) with a histidine-maltose shell (DG4-His-Mal) have the capacity to interfere with Aβ amyloid aggregation, reduce Aβ toxicity, and delay the cognitive decline in AD transgenic mice.

One strategy for improving the delivery of dendrimers into the brain is to encapsulate them in liposomes. Previous studies by our group showed that phosphatidylcholine liposomes containing 30 % cholesterol (PC:Chol) encapsulated and retained more dendrimers than those in the absence of cholesterol. The administration of DG4-His-Mal encapsulated in PC:Chol liposomes improved the cognitive performance of 5xFAD transgenic mice in the corner test in the early stages. In this study, we investigated Aβ42 aggregation in the presence of dendrimers and dendrimers encapsulated in PC:Chol liposomes *in vitro* to better understand the interactions between DG4-His-Mal and liposomes with amyloid peptides. With this aim, ThT fluorescent dye was used to study Aβ aggregation, and Transmission Electron Microscopy (TEM) was used to analyse the morphology of the Aβ aggregates. Our results showed that dendrimers accelerated amyloid aggregation at low dendrimer/Aβ ratios and slowed down kinetics (and reduced fibril formation) at high ratios. In contrast, liposomes strongly reduced the fibril formation. Interestingly, in the presence of dendrimers encapsulated in liposomes, the effect of dendrimers (accelerating kinetics and enhancing fibril formation) prevailed at low lipid dendrimer ratios, whereas at high lipid/peptide ratios, the lipid effect (reduction of fibril formation) dominated the process.

P2-09

Microbiota prion-like proteins impact on amyloidogenesis

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Prions can adopt multiple structural conformations, usually an amyloid state, from which at least one has self-propagating properties. This conformation can be transmitted between species and, in the case of neurodegenerative diseases, between patients and from one region of the brain to another. Alterations in microbiota have been associated with the development of neurodegeneration and it has been suggested that microorganisms' amyloid structures may trigger host protein aggregation.

Understanding the link and mechanisms by which our microbiome influences our body is crucial in all aspects of our life, including health and disease. In this context, our project aims to shed light on the understanding of the link between the changes in the gut microbiome and the development of Alzheimer's disease.

We identified microorganisms, from the gut microbiome of Alzheimer's disease patients, that produce prion-like proteins with the potential to trigger cellular disorders associated with the disease. We have analyzed the group of putative prion-like proteins and showed a variety of molecules able to aggregate into amyloid fibrils and influence the aggregation of the amyloid beta peptide. Knowing these organisms and proteins will be relevant for human health, by providing useful data to open new therapeutic strategies aimed to control the burden of neurodegenerative diseases. Our results indicate that the interaction between human and microbiota amyloid proteins could be possible and more common than expected. Overall, the microbiota large numbers, the long periods of time that stay in our bodies, and the discovery of multiple amyloid proteins in the bacteria Domain outline a worrying scenario.

P2-10

How does glutamate inhibit fatty acid oxidation in astrocytes?

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Metabolic changes are vitally important for brain plasticity and cognitive functions, and fatty acid oxidative metabolism (FAO) in astrocytes is emerging as a key pathway. However, the current knowledge of the regulation of FAO and its dysregulation in disease is still very limited. In this sense, we have previously demonstrated that excitatory neurotransmission inhibits astrocytic FAO. Here we aimed to elucidate the mechanisms of glutamate regulation of FAO in culture cortical astrocytes.

Our results show that the effect of glutamate is not mediated by activation of glutamatergic receptors and requires glutamate uptake though EAAT1 and EAAT2 transporters. Glutamate does not appear to affect the synthesis of malonyl-CoA, the endogenous FAO regulator, through Acetyl-CoA Carboxylase dephosphorylation. However, glutamate decreases the overall oxygen consumption rate and disrupts mitochondrial membrane potential. Interestingly, no differences were found among astrocytes from male and female rats regarding glutamate effects on FAO.

Overall, our results demonstrate that glutamate uptake by astrocytes induces a sex-independent decrease in mitochondrial oxidative metabolism.

P3-01

A single-cell analysis approach using wild-type cells supports the existence of a replication completion checkpoint in eukaryotic cells

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The time it takes for individual eukaryotic cells to complete chromosome replication varies stochastically within a proliferating population. And yet, even the slowest replicating cells avoid the occurrence of premature chromosome segregation, thus preventing the loss of genomic integrity. However, the underlying mechanism remains, to this day, an unsettled question. One possibility is the existence of a generous set cell-cycle time frame from the onset of S phase to the onset of anaphase, sufficient even for those cells that take longer to complete DNA replication. Alternatively, cells may rely on a replication completion checkpoint that actively prevents chromosome segregation until the bulk of DNA replication concludes. Excellent previous studies have provided experimental evidence both supportive (1-2) and contrary (3) to the existence of one such checkpoint. However, in all cases, the experimental approaches involved significant cell alterations, such as adding a large artificial chromosome with a single origin of replication, or mutations in replication-relevant genes, which might alter cell behavior.

To overcome previous experimental limitations, we developed an experimental approach based on single-cell analysis of wild-type cells. We labeled cell cycle markers Sic1, Mcm4, and Pds1 with different fluorescent tags that can be monitored by time-lapse fluorescence microscopy. The duration of the S phase in individual cells was defined as the time between the loss of the Sic1 signal and the nuclear exclusion of Mcm4, whereas the time from the loss of the Sic1 signal to the loss of Pds1 signal was used to define the time from the onset of replication to the start of anaphase in individual cells.

Our data show a strong positive correlation between the duration of S phase and the time that individual cells take from the onset of S phase to the onset of anaphase, discarding a set time common to all cells. The observed correlation is consistent with the existence of a checkpoint that delays mitotic entry for as long as chromosome replication goes on.

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P3-02

MEK5-ERK5 pathway transcriptionally regulates N-Myc transcription factor, a major oncogenic driver in high-risk neuroblastoma cancer.

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Neuroblastoma (NBL) is the most common solid tumor of infancy accounting for 15% of paediatric cancer deaths. Most diagnosed children either do not respond to conventional therapies or relapse after treatment, indicating a need for more effective therapies to treat NBL. Transcription factor N-Myc is amplified in 20% of cases and serves as an oncogenic driver of high-risk NBL, correlating with NBL aggressivity and poor prognosis. Therefore, N-Myc protein is an interesting target to tackle NBL.

The MAP kinase and its upstream kinase MEK5 constitute a unique signaling pathway that controls proliferation and survival of cancer cells, and either MEK5 or ERK5 pharmacologic inhibitor have shown anticancer activity in several solid cancers. However, the role of ERK5 pathway in high-risk, N-Myc driven, NBL has not been explored.

First, and using cDNA microarray technology, we investigated the impact of ERK5 silencing (lentiviral shRNAs) in high-risk NBL, CHLA-90 and SK-N-BE(2) cell lines. Bioinformatic analysis showed that ERK5 silencing resulted in a robust and significant downregulation of *N-MYC* and *N-MYC* target genes. Here, we provide evidence showing that MEK5 kinase is a positive regulator of N-Myc and N-Myc transcriptional targets. Thus, MEK5 inhibition resulted in impaired N-Myc mRNA and protein expression in a panel of human NBL cell lines. Experiments using the proteasome inhibitor MG132 showed that MEK5/ERK5 inhibition did not affect N-Myc protein stability, indicating that MEK5 transcriptionally regulates *N-Myc* levels. Finally, we show that ERK5 or MEK5 inhibitors sensitize high-risk NBL cells to the toxicity induced by topotecan and doxorubicin, two standard chemotherapics used to treat *N-Myc* amplified high-risk NBL.

Overall, our results suggest that pharmacologic modulation of the ERK5 pathway could be of help to tackle high-risk NBL. Future work will be addressed to uncover the precise mechanism involved the transcriptional regulation of *N-Myc* exerted by MEK5, as well as to validate MEK5 target in animal models of NBL.

P3-03

Mapping the transcriptome of *Komagataella phaffii* in search of alkaline pH-responsive promoters useful for heterologous protein expression

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Komagataella phaffii (formerly called *Pichia pastoris*) is an ascomycetous yeast that has become an organism widely used for heterologous protein production. Expression of recombinant proteins in this methylotrophic organism has been classically performed using the strongly controlled alcohol oxidase *AOX1* promoter, which require methanol for induction. However, the toxic and flammable nature of methanol causes safety concerns and is considered a limitation in the process. This has led us to find a more sustainable induction system, avoiding the need of methanol, such as promoters that can be triggered by alkalinization of the culture medium. However, the adaptive mechanisms allowing survival when pH in the environment becomes moderately alkaline have not yet been examined in this organism. For this reason, we investigated the changes in the transcriptional landscape of *K. phaffii* occurring in response to sudden alkalinization of the medium. Our mRNA-Seq results revealed that alkalinization triggers fast changes in the yeast's mRNA levels, resulting in more than 400 genes induced at least 2-fold[1]. Guided by the transcriptomic data, we demonstrated the appearance of oxidative stress. We have characterized the alkaline-inducible *PHO89* promoter defining two Calcineurin Dependent Response Elements (CDRE) required for this induction, thus proving the participation of the calcineurin/Crz1 pathway in the transcriptional response to alkali. This and other alkali up-regulated promoters are being currently tested to drive the expression of several proteins of industrial interest (such as phytase, lipase and laccase).
We believe that further exploration of the *K. phaffii* transcriptional landscape in response to alkalinization may provide novel native or hybrid promoters useful to develop new and more sustainable platforms for heterologous protein expression in this organism.

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P4-01

Study of the role of Klotho in the spinal cord and peripheral nerve injuries

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Traumatic spinal cord injury (SCI) leads to a devastating pathology without effective curative treatments. After the initial event that produces an immediate mechanical disruption, a secondary injury cascade takes place. This cascade cyclically causes ischaemia, excitotoxicity, microglia activation, neuroinflammation and oxidative stress, leading to neuronal and glial death.

In a similar way, some of these processes also underlie traumatic peripheral nerve injuries (PNIs), which have a much better outcome than SCIs. However, PNIs can be very severe, they usually never recover completely, and neuromas may manifest as the nerve attempts to regenerate.

Here we propose to act synergistically on the main mechanisms activated after SCI and PNI by treating the lesion with the secreted isoform of the pleiotropic protein □-Klotho (s-KL), which is involved in the reversion of some of these events. s-KL is also known to play a crucial role in neuroprotection against age-related neurodegeneration. We expect Klotho to enhance motoneuron (MN) survival and boost axonal regrowth after spinal cord and peripheral nerve injuries. The first approach to be tested is to overexpress s-KL with a self-complementary AAV9 vector, which is already produced and proven to be infective and drive s-KL expression.

First, we have demonstrated in rat spinal cord organotypic cultures that s-KL completely protects from glutamate-induced motor neuron death. Next, to determine the main cell types expressing Klotho in the spinal cord we performed immunohistochemistry assays with tyramide signal amplification (TSA). Our results clearly show that neurons, notably ventral horn interneurons and motoneurons, are the main cells responsible for Klotho production in the mouse spinal cord. Moreover, we studied the mRNA levels of Klotho isoforms in SCI and PNI. A five-fold decrease in secreted and membrane Klotho expression has been found up to at least 28 days after SCI in the spinal cord. In brachial plexus injury, Klotho is downregulated in the atrophied forearm muscles at 28 days-post injury (dpi), whereas in the spinal cord only the secreted isoform is downregulated at 7 dpi. Similarly, at 14 dpi of sciatic nerve crush in neonatal mice, mKL is downregulated in the gastrocnemius and soleus muscles, whereas only the secreted isoform tends to be downregulated in the spinal cord.

Our results identify neurons, especially the ones in the ventral horn, as the main Klotho producing cells in the mouse spinal cord. Therefore, their massive death in SCI or in some types of PNI may lead to decreased Klotho levels, with the consequent reduced neuroprotection and increased neuronal damage. Hence, quick overexpression after injury or administration of recombinant s-KL could be good candidate therapies for SCI and PNI.

P4-02

Characterization of megalencephalic leukoencephalopathy with subcortical cysts mouse models and therapeutic strategies

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC, also known as van der Knapp disease) is a vacuolating leukodystrophy caused by mutations in either two genes expressed in glial cells, *MLC1* and *GLIALCAM* (or *HEPACAM*). Recessive mutations in *MLC1* and *GLIALCAM* cause MLC1 and MLC2A and result in an identical clinical phenotype, including macrocephaly, epileptic seizures, motor deterioration and mild cognitive decline. Dominant mutations in *GLIALCAM* cause MLC2B and are linked to a remitting phenotype. The histopathological cause underlying these manifestations is the progressive vacuolation of myelin sheaths and astrocytic endfeet, which has been associated to the dysregulation of water and ion homeostasis mechanisms putatively regulated by the MLC1/GlialCAM unit. Most clinical features of MLC are absent or have not been described in the two knockout mouse models of the disease, *Mlc1*$^{-/-}$ and *Glialcam*$^{-/-}$. Our objective is to develop an effective gene therapy strategy to tackle MLC, so it is crucial to thoroughly phenotype both models to eventually validate any treatment.

We have found that behavioural tests for motor function and magnetic resonance imaging (MRI) provide results which successfully correlate with previously described data about the cerebellar histopathology of *Mlc1*$^{-/-}$ and *Glialcam*$^{-/-}$ mice.

Our group previously developed a gene therapy strategy for MLC1 capable of recovering white matter vacuolation consisting of the intracerebellar subarachnoid administration of AAVrh10 vectors, but therapeutic gene expression was limited to the cerebellum, thus limiting its translatability to patients. Here we describe a novel gene therapy approach based on the intravenous administration of a new synthetic brain-blood barrier-penetrating AAV serotype which allows for a widespread and astrocyte-driven MLC1 expression in the mouse brain.

P4-03

A Novel Gene Therapy Approach for ALS by Overexpressing in Muscles the Pleiotropic Chronokine α-Klotho

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In Amyotrophic lateral sclerosis, muscle denervation and degeneration of motoneurons result in progressive muscle weakness and atrophy. For preserving neuromuscular function in the SOD1^{G93A} mouse model, we synergically influenced on motoneuron terminals and muscles by boosting the secretion of α-Klotho in skeletal muscles. α-Klotho is a pleiotropic chronokine with an excellent profile as a neuroprotective and myoregenerative agent.

To overexpress α-Klotho in the muscles of SOD1^{G93A} mice, AAV8 vectors were systemically administered at 3x10^{14}vg/kg at an early stage of the disease. Secretion of α-Klotho enhanced motor function and strength of the animals and delayed the onset of the disease. Neuromuscular functional improvement was reflected as increased amplitudes of the compound muscle action potentials (CMAP) and the motor evoked potentials (MEPs). α-Klotho-treated SOD1^{G93A} mice showed more surviving motoneurons and a significant reduction in neuroglial reactivity in the spinal cord. All this correlating to a higher number of occupied neuromuscular junctions and a preserved mass of the muscles.

In view of the high doses of AAV8 vectors needed to reach therapeutic efficacy, we moved to a myotropic AAV vector. With a 20-fold decrease in the dose, we achieved higher preservation of neuromuscular connectivity, motor performance and strength in SOD1^{G93A} mice. More importantly, when mice were treated at a symptomatic stage, α-Klotho slowed down the progressive decline characteristic of SOD1^{G93A} mice.

Overall, our results provide evidence that the secretion of α-Klotho by muscles can promote functional improvement in ALS and may open a new avenue for the treatment of this devastating disorder.

P5-01

ctDNA in breast milk for early detection of pregnancy associated breast cancer

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The potential of cell-free tumor DNA (ctDNA) for early tumor detection in asymptomatic patients is yet to be established. In the case of pregnancy associated breast cancer (BC), early detection is of special interest, since it is an entity of special aggressiveness due to a delay in diagnosis, along with the negative effect of mammary gland involution when BC is diagnosed during the postpartum period (PPBC). Indeed, PPBC has double metastatic risk and worst prognosis. With a potential applicability for cancer screening during breastfeeding, here we explored the presence of ctDNA in breast milk (BM) from women with BC associated to pregnancy.

Matched samples from breast tumor, plasma and BM from a cohort of 14 women diagnosed during pregnancy or breastfeeding were analyzed by droplet digital PCR and a targeted next generation sequencing panel (NGS). Thirteen patients had early-stage disease (11% Stage I, 61% Stage II and 28% Stage III) whilst one was diagnosed at advanced stage. BM harbored ctDNA, since mutations present in the tumor tissue were detected in 86% of the cases by ddPCR and in 71,4% by NGS (difference owing to technique sensitivity). Matched plasma samples had detectable ctDNA levels in only 8% of the cases. In one of the patients, a BM sample collected 18 months prior to BC diagnosis revealed the presence of a pathogenic PIK3CA mutation later detected in the surgically removed tumor.

With the ultimate goal of applying the NGS in BM as a technique for early detection of BC in the postpartum period, we have collected samples from healthy volunteers and patients at high risk of developing BC (defined as women becoming pregnant at >40 years or carriers of germ-line pathogenic variants associated with BC -ie: BRCA1, BRCA2, PALB2, RAD51C/D). The application of NGS in BM as a technique for early detection of BC in the postpartum period, identified in a high-risk woman (criteria of enrolment was the age, 46yo) an AKT1 pathogenic mutation in the right-sided BM anticipating by 6 months the radiological diagnosis of a Luminal A tumor, stage pT1bN0M0.

In summary, our data provides evidence that ctDNA in BM is highly prevalent even at initial tumor stages, and could be exploited for early breast cancer screening during breastfeeding.

P5-02

Crosstalk between the adiposity and inflammation with the development and the aggressiveness of the epithelial thyroid carcinoma

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Obesity has been identified as a significant risk factor for various adult cancers, including epithelial thyroid carcinoma. Moreover, adipose tissue, releases adipocytokines and inflammation-related molecules that play important roles in modulating physiological processes. The interplay between adiposity, adipocytokines, inflammation, and cancer development suggests their potential involvement in tumor progression. To investigate the relationship between adipose tissue surrounding epithelial thyroid carcinoma and tumor aggressiveness, we conducted a study using human adipose tissue (AT) samples obtained of the neck from patients who underwent thyroidectomy from benning and malignaciy tumors. The adipose samples were categorized into three groups from:carcinoma papillary thyroid (CPT) (n=22) and two types of benign tumors (adenomas and multinodular) (BT) (n=26). These AP were maintained O/N in culture (CM) and several cytokines: TNF-alpha, EGF, IL-6, leptin, adiponectin, and resistin were analyzed using multiplex ELISA. The findings revealed higher secretion levels of IL-6, TNF-alpha, and resistin in the CM from patients with CPT in comparison with the BT and adiponectin secretion was found to be higher in the CM from adenomas compared to multinodular and CPT examined.It is known that, adiponectin levels are associated with diminishing of proliferation and tumoral aggressiveness, being this adipokine a promise for its possible anticancer effects. Nevertheless, despite of its mechanisms of action is no complete known, some report identified its effect by act in the mTOR and AMPK pathways. Treatment with an adiponectin receptor agonist (AdipoRon) at different concentrations in two thyroid cell lines (BCPAP and TPC-1) strongly inhibited their cell proliferation, showing in the BCPAP cell line higher sensitivity to AdipoRon treatment compared to the TPC-1 cell line.In summary, our preliminary results suggest a potential relationship between the secretion of adipocytokines by adipose tissue surrounding tumors and epithelial thyroid carcinoma. The higher levels of IL-6, TNF-alpha, and resistin observed in CPT cases compared to BT, indicate their potential involvement in tumor aggressiveness. Adiponectin secretion was found to be higher in BT (adenomas) in comparison with malignant tumors (CPT). The CM derived from AT cultures with low adiponectin levels reduced significant cell proliferation in the BCPAP cell line, which was also effectively inhibited by treatment with AdipoRon.These findings highlight the potential role of adipocytokines and AdipoRon in the context of epithelial thyroid carcinoma. Further investigations are warranted to understand the underlying mechanisms and to explore the potential therapeutic implications of targeting these pathways in obesity-related thyroid cancers.

P5-03

Monitoring of Busulfan by LC-MS/MS in a clinical settings of pediatric patients: our experience in 2 years.

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Introduction. Monitoring of busulfan (Bu) during high-dose conditioning regimens before hematopoietic stem-cell transplantation (HSCT) is strongly recommended to ensure efficacy and prevent toxicity of patients undergoing HSCT. We reported a new, fully validated and cost-effective method to measure Bu in plasma by LC-MS/MS[1].

Objective. The aim of this study was to evaluate the Bu measurement into our health-care practice of a tertiary care hospital.

Patients and methods. We summarized analytical performance through accuracy (bias,%), inter-day precision (CV,%), specificity and trueness (agreement and bias,%) calculated using spiked samples and quality controls from SKML External Quality Assurance Scheme (range of 0.50–3.50 mg/L).

The second part of the evaluation was a retrospective study of 41 pediatric patients monitored in our clinical laboratory between Dec, 2020, and May, 2023. After the initial Bu dose, blood samples were collected for Bu therapeutic drug monitoring (TDM) before intravenous infusion (IV), and at 3, 4, 5, and 6 h, followed later by 3 daily doses adjusted according to the pharmacokinetics.

Results. The bias, CV and total error were calculated at three concentrations of Bu:

Spiked samples (mg/L)	N	Inter-day CV (%)	Overall bias (%)	Total error (%)
0.25	187	8.42	0.68	14.56
0.75	182	8.16	2.25	15.71
4.00	87	6.98	1.12	12.63

No interfering components were revealed in the relevant mass transitions from blank patient samples (n>100). The results of SKML controls obtained demonstrated a good agreement with the assigned values (n=12; r^2>0.97). The observed mean bias was -2.1%.

We monitored 41 pediatric patients on Bu conditioning treatment (n=705 samples; 29.3% females; median age: 3 years, range 2 months–16 years). The ratio allogeneic (alloHSCT) /autologous (autoHSCT) HSCT was 4.9 (34:7). The cumulative AUC was targeted of 55,000 to 95,000 ng/mL*h depending on the conditioning regimen. Patients included in the study were being administered Bu together with other chemotherapeutic agents like fludarabine (n=17), fludarabine and thiotepa (n=4), cyclophosphamide (n=7) or cyclophosphamide and melphalan (n=6) in alloHSCT. Patients undergoing autoHSCT were being administered Bu together with melphalan (n=7). The AUC estimated from Bu measurements was compared to the target AUC and Bu dosing was consequently adjusted. Using TDM-guided Bu dosing, dose adjustment was performed in 37/41 patients (90%), increasing or decreasing the dose. Adverse events, including graft rejection and/or death, ocurred in 7 patients after HSCT (19%) with a follow-up of at least 6 months. These results were consistent another previously reported, in which the administration of oral Bu without TDM resulted in 30% of event-free survival and 53% of survival rates, versus IV Bu based on TDM (83 and 83%, respectively)[2].

Conclusions. Bu TDM and dose adjustment are recommended in the Bu conditioning treatment, as the target AUC may not match the achieved AUC.

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2. Bartelink IH, Bredius RG, Ververs TT, et al. Once-daily intravenous busulfan with therapeutic drug monitoring compared to conventional oral busulfan improves survival and engraftment in children undergoing allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2008;14(1):88-98.

P5-04

The influence of endonuclease DFF40/CAD activation on the molecular profile of glioblastoma

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Introduction: From a clinical perspective, one of the significant challenges we face today in cancer research, specifically in glioblastoma, is the failure or the inability of the systemic therapies to completely remove residual tumour cells. Amongst the factors that may explain the limited effectiveness of conventional systemic therapies for glioblastoma, our research group has identified the inability of glioblastoma cells to undergo complete apoptotic cell death upon exposure to cytotoxic agents. This phenomenon can be attributed, in part, to an intrinsic deficiency in the expression of adequate levels of the DFF40/CAD. Despite the deficient expression of DFF40/CAD, when exposed to appropriate stimuli, they can complete the apoptotic program. However, this phenomenon is only seen in a limited number of glioblastoma cells.The intracellular factors contributing to the refractoriness of glioblastoma cells are still a subject of ongoing investigation. We propose the DNA conformation as one of the main factors. Besides its role as apoptotic endonuclease, recent results of our group also suggested that DFF40/CAD can also play a role in gene expression regulation.

Objective:1.To determine the influence of DFF40/CAD on the gene expression of cells derived from glioblastoma, under normal growth conditions or in the presence of a proapoptotic stimuli and provide potential biomarkers to predict treatment responses. **2**.To establish the importance of the DNA structure for the proper function of the endonuclease DFF40/CAD.

Methods: 1. We conducted a microarray analysis of GBM-derived LN18 cell line transfected with the DFF40/CAD cDNA or with the empty vector. We stablished three experimental groups: untreated, treated with a stp and treated with stp+ q-VD. **2**. Brain tumour cell lines were incubated with several cytotoxic drugs that interact with the DNA. The effects they exerted on the nuclear fragmentation were observed after adding stp.

Results: 1.Candidate genes were selected based on their log fold change (logFC) >±1.2 and p value <0.05. The selected genes were further validated with RT-PCR using three different commercial glioblastoma cell lines.**2.** Preliminary results suggested that the pre-treatment with agents which directly interact with nucleic acids may force DNA to adopt a conformation that impairs DFF40/CAD function.

Conclusions: As far as we know this is the first attempt that suggests a role of DFF40/CAD in gene expression regulation. We believe that DNA conformation is crucial for the proper function of DFF40/CAD.

P7-01

Analytical validation of lactoferrin and calprotectin determination in bovine fecal samples

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Dairy calves are exposed to a wide number of factors that might affect gastrointestinal functionality. Inflammation of the gastrointestinal tract may cause diarrheas, decreased absorption of nutrients and inflammatory response which causes releasing of cellular components into the blood or feces. The quantitative determination of these components may be used as diagnostic or prognostic biomarkers. Calprotectin and lactoferrin may be good candidates as protein biomarkers and measurement in fecal samples is interesting due to the non-invasive character of sample collection. In this study, two assays have been validated for measurement of calprotectin and lactoferrin in bovine feces. Calprotectin and lactoferrin fecal extractions were performed following the kit manufacturer's instructions with slight modifications in the final dilution. Calprotectin was measured using Bühlmann fCAL turbo test for human samples on a Beckman Coulter AU400 Chemistry Analyzer. Lactoferrin was measured with the Bovine Lactoferrin ELISA Kit from Bethyl Laboratories. Analytical validation was performed following the ASVCP Guidelines: Both assays kept linearity under dilution with R^2>0.978. Precision study results for calprotectin, within-run and between day CVs were under 2%, except for low concentrations wherein CVs up to 20% are acceptable. Precision study results for lactoferrin, within-run and between day CVs were under 10%. In both assays, recovery was within the acceptable range (80-120%).

In conclusion, both methods are suitable to measure bovine calprotectin and lactoferrin in fecal samples with accurate and precise results.

P7-02

Servei de Bioquímica Clínica Veterinària (SBCV)

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El **Servei de Bioquímica Clínica Veterinària** és un laboratori (LPS) que pertany al Departament de Bioquímica i Biologia Molecular, situat a la Facultat de Veterinària. Es realitzen determinacions de diferents paràmetres bioquímics (metabòlits, enzims, hormones, proteïnes, citoquines, immunoglobulines, aminoàcids, antioxidants....) en mostres procedents de diferents espècies animals, incloent petits animals, animals de renda, exòtics i de laboratori. La matriu més important és sèrum o plasma, però també s'han posat a punt tècniques en mostres de pinso, femtes, pèl, extractes de teixits, orina, líquid cefalorraquidi, calostre, saliva i llet. Es processen mostres provinents de l'Hospital Clínic Veterinari, usuaris interns de la UAB i externs (laboratoris farmacèutics, altres universitats, centres de recerca, etc.).

El Servei té àmplia experiència en analítiques en animals de laboratori, on sovint el volum de mostra és una condició limitant.

A més dels analits de rutina, es destaquen els següents paràmetres:

- Anàlisis en llet i calostre: citoquines, IgG, cortisol, lactosa, citrat, insulina.
- Citoquines: IL-1 beta, IL-6, IL-8, IL-10, IFN-gamma, TNF-alfa.
- Immunoglobulines: IgG, IgM, IgA.
- Paràmetres d'estrès oxidatiu: superòxid dismutasa, glutatió peroxidasa, glutatió reductasa, glutatió total, MDA (TBARS), TAS.
- Indicadors de l'estat nutricional: NEFA, β-hidroxibutirat.
- Proteïnes de fase aguda: Haptoglobina, SAA, CRP, Pig-MAP, etc.
- Cortisol en plasma, saliva, pèl, i llet/calostre.
- Corticosterona en femtes.
- Leptina
- Creatina-Quinasa (CK), LDH, lactat.
- Anàlisi d'aminoàcids mitjançant HPLC.
- Electroforesi capil·lar.
- Hormones: Tiroidees, Cortisol, Prolactina, Progesterona, Estradiol, Testosterona, PTH, Insulina, IGF-1.

El SBCV participa en projectes relacionats amb benestar animal i altres, on s'optimitza el disseny i validació de noves tècniques, el desenvolupament de reactius comercials per veterinària i la validació d'equips analítics pel seu ús en veterinària. També s'ofereix assessorament científic i tècnic.

Ofereix formació pràctica a alumnes de Grau, Màster i Cicles Formatius de Grau Superior.

P7-03

Establishment of a new model for the estimation of change limits(deltacheck) applied to biochemical and hematological quantities

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Background-Aim: Change limits (CL) determine if an analytical result is valid, and show if variation between current and previous results of the same patient could be due to error. The aims are to establish an individualized model to estimate CL in biochemical and hematological quantities, based on multiple previous results of each patient, and to evaluate the effectiveness of quantity combinations for the detection of erroneous laboratory reports.
Methods: Frequently requested quantities are selected: serum concentration of alanine aminotransferase, albumin, aspartate aminotransferase, bilirubin, calcium(II), creatinine (CRE), alkaline phosphatase, gamma-glutamyltransferase, glucose (GLU), potassium ion (K), sodium ion and urea, measured on cobas® c702 (Roche Diagnostics); number concentration of erythrocytes (RBC), leukocytes (WBC) and platelets (Plt), hemoglobin mass concentration (Hb), erythrocyte volume fraction (PCV) and entititic volume (MCV), measured on Sysmex XN (Sysmex). Two CL models are proposed, considering each patient's previous results, based on: 1) percentiles of the differences observed (CL_{Px}); 2) variation percentages of the minimum and maximum values ($CL_{\%X}$). Reports of inpatients are selected, from Infinity (Roche Diagnostics): 41 erroneous and 68 not erroneous reports. Results of the previous 30 days from each report are obtained. To evaluate the effectiveness, it is checked if each result of selected reports is inside/outside CL. A report is considered suspicious when at least one result is outside CL. Sensitivity (SE) and specificity (SP) are calculated for 4-quantity combinations, which individually present SE or SP >70%. It is considered acceptable if SE and SP >80%.
Results: Selected CL are $CL_{P2.5}$, $CL_{P1.25}$, CL_{P0}, $CL_{\%10}$, $CL_{\%20}$ and $CL_{\%50}$. $CL_{P2.5}$ presents SE>70% for GLU. $CL_{\%50}$ shows SP>70% for GLU, K, CRE, RBC, Hb, PCV, MCV, Plt, WBC. GLU+K+CRE+Hb, GLU+K+CRE+PCV, GLU+K+CRE+RBC reveal SE=100%, but low SP (<27%). $CL_{\%20}$ of GLU+K+CRE+MCV shows SE=85% and SP=81%.
Conclusions: The percentile-based CL allows detecting all erroneous reports, despite a low SP. $CL_{\%20}$, based on variation percentages, in GLU+K+CRE+MCV, achieves the best SE and SP combination. Plausibility control can be efficiently applied using a 4-quantity combination.

P7-04

Repurposing Disulfiram as an Antifungal Agent: Development of a New Disulfiram Vaginal Mucoadhesive Gel

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Alternative formulations need to be developed to improve the efficacy of treatments administered via the vaginal route. Mucoadhesive gels with disulfiram, a molecule that was originally approved as an antialcoholism drug, offer an attractive alternative to treat vaginal candidiasis. The aim of the current study was to develop and optimize a mucoadhesive drug delivery system for the local administration of disulfiram. Such formulations were composed of polyethylene glycol and carrageenan to improve the mucoadhesive and mechanical properties and to prolong the residence time in the vaginal cavity. Microdilution susceptibility testing showed that these gels had antifungal activity against *Candida albicans*, *Candida parapsilosis*, and *Nakaseomyces glabratus*. The physicochemical properties of the gels were characterized, and the in vitro release and permeation profiles were investigated with vertical diffusion Franz cells. After quantification, it was determined that the amount of the drug retained in the pig vaginal epithelium was sufficient to treat candidiasis infection. Together, our findings suggest that mucoadhesive disulfiram gels have the potential to be an effective alternative treatment for vaginal candidiasis.

P7-05

Metagenomics' tools to identify infectious agent genomes in Amazonian *Culex* sp. mosquitoes and sensitivity study.

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Background: Nowadays, humans are exposed to a wide range of infectious diseases, partially caused by the emergence of unrecognized viruses. Specifically, the interaction between humans and animals can facilitate the virus's transmission, leading to human infection. Metagenomics based on Next-Generation-Sequencing (NGS) allows the identification of microbial communities and characterization of infectious agents.
The study aimed to develop high-throughput metagenomic tools using mosquito samples by performing deep-sequencing metagenomics, implementing a study of sensitivity, and developing a bioinformatics pipeline for data analysis.
Methods: *Culex* sp. mosquitoes captured from the Peruvian Amazonia were crushed and pooled, amplified using random hexamers and deep-sequenced. A sensitivity study was performed using a Hepatitis C Virus (HCV) clone, diluted to $3x10^6$, $1.5x10^5$, $3x10^5$, $3x10^4$, $3x10^3$ molecules, and mixed with pooled mosquitoes duplicates. Data preprocessing was performed with Trimmomatic, and non-targeted sequences were removed. Taxonomic profiling was performed with kaiju.
Results: A total of 9,706,882 reads were obtained after preprocessing and removal of unwanted sequences. Most removed unwanted sequences belonged to mosquito and human, showing animal-human interaction. Moreover, 2,101,031 reads matched with a known genome, 2.6% to viruses. Interestingly, 45,483 reads were classified as Bunyavirales, which has been associated to hemorrhagic fever in humans. The sensitivity study confirmed that minority genomes are underrepresented after amplification and NGS sequencing.
Conclusion: Overall, metagenomics and the bioinformatics pipeline allow the identification of diverse genomic entities and identify potentially zoonotic viruses. The sensitivity study has proved that minority genomes are underrepresented, suggesting that these minority genomes cannot be trusted quantitatively.

P7-06

Proteasome-dependent degradation of histone H1 is mediated by its C-terminal domain.

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Histone H1 is involved in chromatin compaction and dynamics. In human cells, the H1 complement is composed of different amounts of somatic H1 variants, H1.0-H1.5 and H1X. The amount of each variant depends on the cell type, the cell cycle phase, and the time of development and can be altered in disease. However, the mechanisms regulating H1 protein levels have not been described. We have analyzed the contribution of the proteasome to the degradation of H1 variants in human cells, using two different inhibitors, MG132 and bortezomib. H1 variants accumulate upon treatment with both drugs indicating the role of the proteasome in the regulation of H1 protein levels. We found that the global increase in histone H1 caused chromatin rearrangements and H1 accumulation in the cytoplasm, presumably to prevent alterations in the nuclear processes. We analyzed the pathway of H1 degradation by the proteasome. Our results suggest that H1 degradation is mostly ubiquitin-independent, whereas the whole protein and its C-terminal domain can be degraded directly by the 20S proteasome. Our findings revealed a new regulatory mechanism for histone H1 degradation, where its basicity and intrinsically disordered domains are responsible for its targeting and degradation by the 20S proteasome.

P7-07

Production, purification and characterization of tagged and untagged GCase

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β-glucocerebrosidase (GBA) is a lysosomal enzyme that catalyzes the hydrolysis of the glucose from substrates such as glucosylsphingosine and glucosylceramide. Deficiency of its activity leads to substrate accumulation which leads to an impaired lysosomal activity, thus causing Gaucher's Disease. Enzyme replacement therapy, consisting of the periodic intravenous administration of a recombinant GBA is the standard of care for GD. *GBA1* gene variants are also the most common genetic risk factors of Parkinson's Disease. Therefore, some research lines to treat PD are focused on the GBA activity restorage.

To date, three recombinant GCase are approved by the Food and Drug Administration, Imiglucerase, Taliglucerase alfa and Velaglucerase alfa. However, the cost of their production and purification are extremely elevated, so there is an actual need for a cheaper and effective design to express and purify this protein.

Within this work, a new strategy for both tagged and untagged GBA production and purification is set up based on the expression and secretion of GBA in mammal HEK 293F cells. Supplementation of the expression media with a GBA small pharmacological chaperone significantly enhances the production of active GBA. To purify them, the conditioned media underwent a three-step purification process combining different consecutive chromatographic principles obtaining in both cases a high purification yield.

Although containing a different glycosylation pattern than other commercial recombinant GBA, our His-tagged and untagged GBA display the same catalytical parameters regarding the affinity with artificial substrate resorufin β-d-glucopyranoside, optimal pH and temperature and thermal stability.

P7-08

Measurement of the serum concentration of the soluble fraction of the ACE2 receptor in patients with SARS-CoV-2 infection: follow-up one year later

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BACKGROUND AND AIM. The SARS-CoV-2, the causal agent of Coronavirus Disease 19 (COVID-19) was shown to infect human cells through the interaction of the viral spike protein with the Angiotensin Converting Enzyme 2 (ACE2) expressed on the surface of human cells. The Metalloproteinase-17 has the property to cleave ACE2 and release the soluble polypeptide (sACE2) which maintains its affinity for the viral spike protein and may compete with membrane bound ACE2. We studied the serum concentration of sACE2 in COVID-19 surviving patients admitted to the Intensive Care Unit (ICU). Our aim is to verify whether increased serum levels of sACE2 are associated with the resolution of disease.
PATIENTS AND METHODS. A cohort of 31 patients (18 men and 13 women) with severe COVID-19 admitted to ICU was selected. The admission analysis was associated with a positive result (POS) in the Reverse Transcription Polymerase Chain Reaction (RT-PCR) analysis of a nasopharyngeal sample. Then these patients had two additional follow-up analyses. The first, before hospital discharge, was related to a RT-PCR with negative result (NEG-DIS), and the second, one year after infection (NEG-POST), was associated with a negative RT-PCR result or there was not record of reinfection in their clinical history.
The sACE2 serum concentration was measured with the *RayBio® Human ACE-2 ELISA kit.* For statistical analyses, the *STATA®* v.14 program was employed to perform a comparison of medians (Me) with the U-Mann-Whitney test (RankSum Test) and percentile calculation.
RESULTS. Medians comparison: The Me (IQR) of the sACE2 concentration in POS was 0.03 (0.05) ng/mL; for NEG-DIS was 0.04 (0.13) ng/mL and for NEG-POST was 0.11 (0.21) ng/mL. Statistically significant differences were observed for the Me in patients POS and NEG-POST, p=0.012. Percentile calculation: The p50, p75 and p95 were different between POS, NEG-DIS and NEG-POST. For patients POS, the results were 0.03 (p50), 0.08 (p75) and 6.60 (p95) ng/mL; for patients NEG-DIS, 0.04 (p50), 0.16 (p75) and 8.58 (p95) ng/mL; and for patients NEG-POST, 0.11 (p50), 0.25 (p75) and 9.33 (p95) ng/mL.
CONCLUSION. We have evaluated the value of sACE2 as a novel biomarker for COVID-19 disease. The presence of lower sACE2 concentration in SARS-CoV-2 infected patients suggests that it could be associated with the viral activity. We have found that sACE2 concentration increases with the disease resolution, suggesting that the measurement of sACE2 concentration may be a disease prognostic biomarker.

P7-09

Antimicrobial and immunomodulatory properties of recombinant bovine β-defensins

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Host Defense Peptides (HDPs) are short and cationic peptides from the innate immune system. They are a promising alternative to conventional antibiotics for treating infections due to their vast variety of functions, from microbicidal activities to the modulation of immune responses, including the regulation of cytokine secretion and the neutralization of free endotoxins. This study aims to investigate the ability of recombinant bovine HDPs to regulate intestinal infections by directly killing *Escherichia coli*, binding to lipopolysaccharides (LPS), and inducing innate immunity in intestinal cells. To accomplish this, three bovine β-defensins (BNBD-1, BNBD-2, and BNBD-3) were selected and fused to a carrier protein (Green Fluorescent Protein-GFP-) to be recombinantly produced in *Lactococcus lactis*. All three β-defensins were found to have potent antimicrobial activity against *E. coli* and bind to soluble lipopolysaccharides (LPS) of *E. coli* O111:B4. BNBD-1 showed the strongest affinity for the tested endotoxin (EC50= 0.04 μM), whereas the bactericidal activity was similar in all cases. Proteins did not show toxicity on CACO-2 but induced a slight increase of IL-8 and IL-4 secretion upon β-defensins treatment. Specifically, BNBD-1 and BNBD-3 predominantly induced IL-8, whereas BNBD-2 enhanced the secretion of IL-4. These results demonstrate that recombinant BNBD-1, BNBD-2, and BNBD-3 can tackle gastrointestinal infections caused by *E. coli* with multiple modes of action, including the direct target of the bacteria, endotoxin binding, and possible neutralization and regulation of innate immunity. Because these proteins not only target the bacteria but also the immune system of the host, they have a valuable potential to reduce two major complications related to infectious diseases: the development of drug resistance and the hyperstimulation of the immune system.

P7-10

Polycomb activity has an important role in the adaptation of plants to environmental changes

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Plants have evolved mechanisms that gives them the ability to adapt to rapid changes in the environment. Among others, this developmental plasticity is generated by the epigenetic regulation of transcriptional activity. Using Arabidopsis as a model plant, we address the essential role of the histone modifications settled by the Polycomb group proteins, with special interest on the histone H3 lysine 27 tri-methylation (H3K27me3) and the activity of the Polycomb-related protein LHP1 (LIKE HETEROCHROMATIN PROTEIN 1). We have seen that the *lhp1* mutant has a defective response phenotype in both elevated temperatures and plant proximity shade conditions. Accordingly, gene expression of environmental-responsive genes is severely affected in this mutant. Interestingly, temperature and shade signaling pathways have a common master transcription factor, the PHYTOCHROME INTERACTING FACTOR 7 (PIF7). It has been reported that many epigenetic mechanisms influence PIF7 activity and vice versa, but so far has not been related to Polycomb activity. By analyzing the chromatin status of downstream target genes of PIF7, we have seen that LHP1 and H3K27me3 have an important role on the regulation of their transcriptional activity.

P7-11

H3K27me3 dynamics in Arabidopsis plants exposed to shade

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Plants grow in natural and agricultural settings surrounded by other plants, often in high densities. In this situation, that is known as plant proximity or shade conditions, a significant competition for resources occurs. To become more competitive in these conditions, plants have developed a set of adaptive responses. The signal that triggers these responses is a decrease in red:far-red light (R:FR) ratio, which is a characteristic light condition that occurs in plant proximity and canopy shade. This low R:FR ratio is perceived by the phytochromes, that activate a signaling cascade that results in the change of expression of thousands of genes. Particularly in Arabidopsis thaliana, shade triggers a rapid induction of positive and negative regulators of the response, such as YUC8 and HFR1, respectively. To understand the transcriptional regulation of these key regulatory genes, we analyze the repressive mark histone H3 lysine 27 trimethylation (H3K27me3), present in both HFR1 and YUC8 locus. When Arabidopsis seedlings are exposed to shade, H3K27me3 decreases at HFR1, in accordance with the overexpression of this gene. However, this change appears to be specific to HFR1, as this does not occur in YUC8, despite its shade-induced expression. Interestingly, when plants return to grow under control light conditions, HFR1 overexpression is repressed but the H3K27me3 levels continues low at least for two days more. After 1 h of re-exposure to shade, transcriptional induction of HFR1 was enhanced in comparison to the first shade exposure, leading us to consider H3K27me3 as an epigenetic memory mark. Importantly, the results presented have opened further questions: other epigenetic marks need to be explored as regulation of YUC8 expression is independent of H3K27me3; moreover, how H3K27me3 levels are rapidly reduced in shade is still unknown, as the main H3K27me3 demethylases described in Arabidopsis seems to be not involved in this process.

P7-12

NT-PROBNP as a biomarker of maternal complications in first trimester of pregnancy

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BACKGROUND-AIM
Preeclampsia (PE) is associated with a 2- to 4-fold increase risk of developing cardiovascular disease (CVD) in the short and long term. When PE is accompanied by small for gestational age (SGA) and/or preterm birth (PTB), the adjusted risk for CVD is increased by 45% for the next 5 years post-partum. Also, PE and CVD share common risk factors like hypertension, renal disease and Diabetes Mellitus. CVD biomarkers have been proposed as useful tools in this context. Natriuretic peptides, particularly type B (BNP, NT-proBNP) are sensitive to cardiac alterations existing in PE and are also predictors of its severity.
The aim of the study is to determine if NT-proBNP could predict different pregnancies complications (PE, SGA, PTB) when measured in first trimester.
METHODS
This was a retrospective case-control study of first trimester pregnancies. The case group consisted of 210 pregnant women who developed PE, SGA and / or PTB. The control group consisted of 208 pregnant women without these complications.
NT-proBNP was measured in maternal blood samples during first trimester of pregnancy using an electrochemiluminescence immunoassay.
RESULTS
NT-proBNP concentrations did not correlate with gestational weeks (r Spearman=0.043, p=0.383) or maternal age (r Spearman=-0.092, p=0.060).
Maternal age and gestational weeks showed no statistical significant differences between women who developed PE,
SGA or PTB and controls (p=0.407 and p=0.897, respectively). Significant decreased serum concentrations of NT-proBNP (p<0.001) were seen in PE, SGA and PTB women (median 36.80 ng/L, 55.78 ng/L and 44.79 ng/L, respectively) compared to controls (median 68.71 ng/L). ROC curves were performed to determine whether NT-proBNP could discriminate pregnancy complications. NT proBNP cut-off value for the prediction of PE was 27.4 ng/L (area under the curve [AUC] 0.730; sensitivity 31.4%; specificity 89.9%), 27.4 ng/L for SGA (AUC 0.600; sensitivity 19.6%; specificity 90.7%) and 26.7 ng/L for PTB (AUC 0.670; sensitivity
22.0%; specificity 89.4%).
CONCLUSIONS
NT-proBNP concentrations are a moderate predictor of PE, SGA, and PTB. The lower concentrations of NT-proBNP in the first trimester of pregnancy could promote the development of placental and/or hypertensive complications in pregnant women.

P7-13

Bioinspired Antibacterial Catechol-Amine Coating

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The use of catechols as functionalizing agents or ligand in the formation of bioinspired materials has been increasing during the past years due the outstanding properties offered by these molecules [1]. Besides, the constant search of new antimicrobial surfaces and fabrics has increased the use of antibiotics and metallic nanoparticles, among others. However, the functionalization of coatings with them usually implies drawbacks like the leaching (with its consequent loss of activity), as well as the possibility of being toxic for humans.

In this study, combinations of catechol-derivatives (specially Pyrocatechol and Caffeic acid), with diamine/triamine ligands were performed to obtain coatings on broadly used fabrics (cellulose, cotton and polypropylene) and band-aids, creating and homogeneous and hydrophilic thin-layer over them. The antibacterial properties of these materials were determined by counting the colony forming units (CFU) decrease of several phatogenic bacteria, mostly found in hospital environments, achieving reductions near to an outstanding 99,999%. Furthermore, this antimicrobial activity has been confirmed also by fluorescence and Scanning electron microscopy. Is suggested that ROS (reactive oxygen species) generation could play a main role in this property. Worth to mention, the catechol-based coatings are obtained through an affordable, straightforward and environmentally-friendly production, avoiding the use of harmful solvents, metals or antibiotics and being compatible with a broad range of materials. Therefore, this universal and bioinspired platform could be an excellent choice to tune the properties of different fabrics and increase the range of their applications.

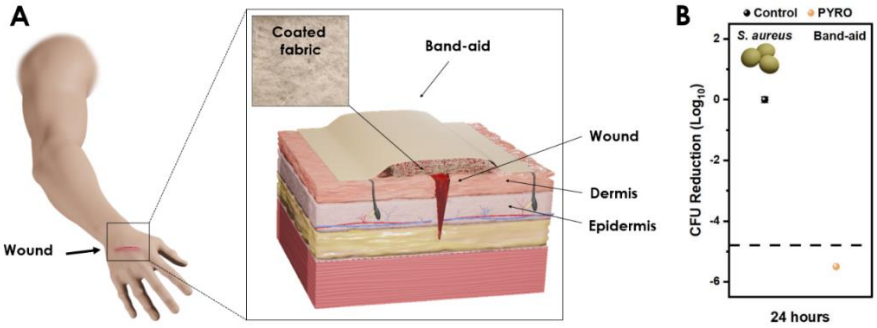

Figure 1: Catechol-amine coated Band-aids. a) Schematic proposed application in wound healing. b) CFU reduction obtained with Staphylococcus aureus.

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P7-14

Unitat d'Animals Transgènics (UAT-CBATEG)

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The Transgenic Animals Unit is a technological platform of the CBATEG in the Universitat Autònoma de Barcelona.
The objective of UAT-CBATEG is the application and development of mouse genome alteration technologies. The main objective is to offer the scientific community the generation of genetically modified murine models and technologies related to the establishment and management of transgenic mouse and rat colonies.
The services offered are:
- Transgenic mouse and rat models by DNA pronuclear microinjection.
- *Genome Edition* in mice and rats: Knockout / in models by microinjection or electroporation of CRISPR/Cas system.
- Knockout / in mouse models by gene targeting in embryonic stem cells.
- Embryo, Sperm and Ovaries Cryopreservation.
- Models Revitalization from cryopreserved embryos, sperm (IVF) or ovaries.
- Health Rederivation by embryo transfer. .
- Genotype analysis.
- Scientific-technical advice on animal transgenesis tecnologies and management of colonies.

P7-15

Viral Nervous Necrosis Virus (VNNV) Inclusion Bodies: A Promising Vaccine Approach for Fish Populations

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Nowadays, antiviral prophylactic tools against viruses have become essential in the aquaculture industry. The viral nervous necrosis virus (VNNV) is a major viral pathogen that infects a wide range of fish species such as seabass, seabream, and turbot. In this context, vaccination is one of the main methods for controlling and preventing viral diseases in aquaculture, and the development of novel approaches to vaccination is a major focus of fish vaccinology. We designed a new modular oral antiviral vaccine platform based nanostructured viral protein antigens prepared as inclusion bodies (IBs) presented as nanopellets (NPs). The use of NPs to develop vaccines provides a cost-effective and safe way to stimulate an immune response in fish. It has several advantages, such as low cost, high yield, and increased stability. Additionally, NPs are stable under different environmental conditions, making them easy to store and transport. Here, we present one versions of NPs formed by antigenic proteins from relevant virus affecting farmed fish as a proof-of-concept for oral administration: viral nervous necrosis virus (VNNV) coat protein combined with an effector molecule, the interferon gamma (IFNy). In the present study, we performed *in vitro* assays to characterize the morphology and functionality of modular VNNV-IFN and to explore its role in immune responses. As a proof of concept for oral delivery, we have verified that the NPs are successfully internalized by both sea bream Sab-1 and zebrafish ZFL cells. Encouragingly, analysis of gene expression suggests this NPs evoke an antiviral innate immune response in Sab-1 and ZFL cells. Summarizing, NPs has become a promising platform for the development of prophylactic aquafeed protection in seabream against VNN virus.

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