UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

SOCIETÀ ITALIANA DI FARMACOLOGIA

RAREDISEASEDAY.ORG

Convegno monotematico
Gruppo di Lavoro «Malattie Rare e Farmaci Orfani» SIF4RARE

INNOVAZIONE E MALATTIE RARE: DALLA RICERCA PRECLINICA AL PAZIENTE

23-24 Febbraio 2024
Bari, Camera di Commercio

Comitato Scientifico

Giuseppe Cirino (Presidente SIF)
Pier Luigi Canonico (Novara)
Annalisa Capuano (Napoli)
Emilio Clementi (Milano)
Annamaria De Luca (Bari)
Maurizio Taglialatela (Napoli)
Gianluca Trifirò (Verona)

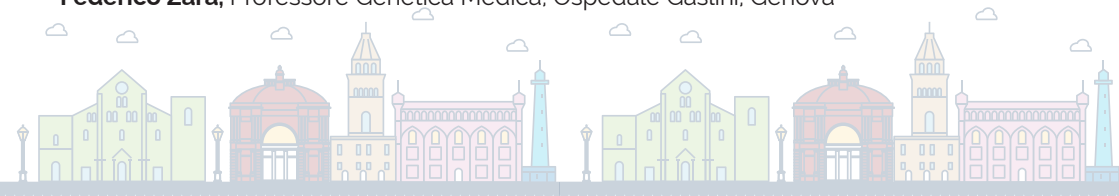
Comitato organizzatore locale

Unità di Farmacologia, Dipartimento di Farmacia – Scienze del Farmaco
Università Aldo Moro Bari
Annamaria De Luca, Domenico Tricarico, Sabata Pierno, Antonella Liantonio, Paola Imbrici, Giulia Maria Camerino, Michela De Bellis, Antonietta Mele, Ornella Cappellari, Elena Conte, Paola Mantuano, Brigida Boccanegra

Il convegno nasce con l'obiettivo principale di promuovere la ricerca traslazionale delle malattie rare, in particolare quelle di origine genetica non oncologiche. Le malattie rare rappresentano un importante "unmet medical need" ad elevato impatto socio-sanitario e sono numerosi gli sforzi mirati a sviluppare farmaci orfani, passando dal riposizionamento di farmaci all'identificazione di nuove molecole e terapie avanzate in grado di modificare significativamente il decorso delle patologie. Importanti traguardi sono stati raggiunti, ma numerose sono ancora le sfide aperte. È necessario identificare strategie per colmare le numerose incertezze su efficacia e sicurezza di nuovi agenti terapeutici mediante un confronto su approcci innovativi di ricerca farmacologica preclinica e clinica nonché di raccolta e analisi di dati in real-life per meglio affrontare le conseguenti problematiche di accesso e sostenibilità. In tal senso, e alla luce delle opportunità offerte dal nuovo Piano Nazionale delle Malattie Rare, è importante il dialogo da implementare, anche con il presente convegno, tra i diversi stakeholders, tra cui politici, decisori, associazioni dei pazienti, clinici e ricercatori.

Relatori e Moderatori su invito

Annemieke Aartsma-Rus, Professor of Translational Genetics, LUMC, The Netherland
Giuseppina Annicchiarico, Coordinatrice CoReMaR_ AReSS Puglia
Maria Rosaria Bucci, Professore Farmacologia, Università Federico II Napoli
Pier Luigi Canonico, Professore Farmacologia, Università del Piemonte Orientale
Annalisa Capuano, Professore Farmacologia, Università della Campania Vanvitelli
Giuseppe Cirino, Presidente SIF, Professore Farmacologia, Università Federico II Napoli
Emilio Clementi, Professore Farmacologia, Università Statale Milano
Diana Conte, Professore Farmacologia, Università Aldo Moro Bari
Jean- Francois Desaphy, Professore Farmacologia, Università Aldo Moro Bari
Giuseppe d'Orsi, Direttore UOC Neurologia, IRCCS Casa Sollievo della Sofferenza (Fg)
Juanma Fernandez Costa, Senior Researcher, IBEC, BIST, Spain
Loretta Ferrera, Ricercatore Sanitario, Ospedale Gaslini, Genova
Fabrizio Gardoni, Professore Farmacologia, Università Statale Milano
Armando Genazzani, Professore Farmacologia, Università di Torino
Mattia Gentile, Direttore Laboratorio di Genetica Medica, Ospedale Di Venere, Bari
Marcello Gemmato, Sottosegretario alla Salute con delega alle Malattie Rare
Loreto Gesualdo, Presidente FISM, Professore di Nefrologia, Università Aldo Moro Bari
Maria Grazia Grilli, Professore Farmacologia, Università del Piemonte Orientale
Andrea Marcellusi, Presidente ISPOR Rome – Italy
Monica Montagnani, Professore Farmacologia, Università Aldo Moro Bari
Colin Nichols, Director of CIMED, Washington University St. Louis, USA
Enrico Piccinini, Coordinatore Focus Group Malattie Rare - Farmindustria
Patrizia Popoli, Direttore Centro Nazionale per la Ricerca e la Valutazione dei Farmaci, ISS - Roma
Annalisa Scopinaro, Presidente UNIAMO
Antonio Torsello, Professore Farmacologia, Università Milano Bicocca
Luigia Trabace, Professore Farmacologia, Università di Foggia
Gianluca Trifirò, Professore Farmacologia, Università di Verona
Federico Zara, Professore Genetica Medica, Ospedale Gaslini, Genova



SCIENTIFIC PROGRAM — 23rd February 2024

12:00-13:15 Registration

13:15-14:00 WELCOME AND INTRODUCTION

- Giuseppe Cirino, *Presidente Società Italiana Farmacologia (SIF)*
- Annamaria De Luca, *Coordinatrice Gruppo di Lavoro SIF4RARE*
- Stefano Bronzini, *Magnifico Rettore Università degli Studi di Bari Aldo Moro*
- Marcello Gemmato, *Sottosegretario alla Salute con delega Malattie Rare*
- Michele Emiliano, *Presidente Regione Puglia*
- Filippo Anelli, *Presidente Ordine dei Medici Chirurghi e Odontoiatri (OMCeO) di Bari e Federazione Nazionale OMCeO*
- Luigi D'Ambrosio Lettieri, *Presidente Ordine Interprovinciale Farmacisti di Bari e Barletta-Andria-Trani*
- Francesco Leonetti, *Direttore Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari Aldo Moro*

14:00-15:30 ROUND TABLE

Therapy of rare diseases and innovation: science and politics at the service of patients

Chairs Pier Luigi Canonico, Annamaria De Luca

Panel Giuseppina Annicchiarico, Diana Conte, Marcello Gemmato, Armando Genazzani, Mattia Gentile, Andrea Marcellusi, Enrico Piccinini, Patrizia Popoli, Annalisa Scopinaro, Gianluca Trifirò

15:30-16:00 OPENING LECTURE

Chair Giuseppe Cirino

Antisense oligonucleotides in rare diseases: lessons learned from neuromuscular disorders to N-of-1 treatment in brain diseases

Annemieke Aartsma-Rus

16:00-16:15 Coffee break

SESSION 1
PATIENT-CENTERED DATA: FROM CLINICAL PRACTICE TO BASIC RESEARCH AND BACK
Chairs **Annalisa Capuano, Emilio Clementi**

16:20-16:40 *Invited talk* **Autoimmune-based renal disorders: the link between basic research and clinic**
Loreto Gesualdo

16:40-16:55 *Focus on* **Orphan drugs and advanced therapies: the value of patient-centered innovation and open challenges**
Patrizia Popoli

Oral Communications

17:00-17:10 **Epidemiological analyses through machine learning approaches to accelerate rare diseases diagnosis**
Ingrasciotta Y, Crisafulli S, Trifirò G *(Verona)*

17:10-17:20 **Safety aspects of the gene therapy for SMA, Zolgensma®: a retrospective analysis of the European pharmacovigilance database Eudravigilance**
Ruggiero R, Balzano N, Mascolo A, di Napoli R, Capuano A *(Napoli)*

17.20-17:30 **The therapeutic value of treatment for metachromatic leukodystrophy: analysis of health technology assessments of 3 European Countries**
Gozzo L, Romano GL, Longo L, Vitale DC, Drago F *(Catania)*

17:30-17:40 **Exploiting routine laboratory test to identify patients with familial chylomicronemia syndrome (FCS) patients in a large Italian hospital**
Pavanello C, Pazzucconi F, Parolini M, Turri M, Mombelli GG, Castiglione S, Alberti A, De Maria R, Calabresi L *(Milano)*

17:40-17:50 **Inborn errors of immunity: lessons learned from an ever evolving paradigm**
Martire B *(Barletta)*

17:50-18:00 **The use of real world data for regulatory purposes in the rare diseases setting**
Giannuzzi V, Stoyanova-Beninska V, Hivert V *(Bari)*

18:00-18:20 **Discussion**

18:20-18:30 *Insight* **The program of European Certified Pharmacologists (EuCP)**
Mariagrazia Grilli

POSTER VIEW AND DISCUSSION - *wine and cheese*

18:30-20:30

Poster topic 1
MUSCULAR DYSTROPHIES AND NEUROMUSCULAR DISORDERS
Chairs Maria Rosaria Bucci, Giulia Maria Camerino, Sabata Pierno

Poster topic 2
CHANNELOPATHIES
Chairs Paola Imbrici, Loretta Ferrera, Michela De Bellis

Poster topic 3
NEUROLOGIC, RENAL, METABOLIC AND OTHER RARE DISEASES
Chairs Antonella Liantonio, Antonietta Mele, Antonio Torsello

Poster topic 4
NEW MODELS AND METHODOLOGIES IN RARE DISEASES
Chairs Ornella Cappellari, Monica Montagnani, Domenico Tricarico

SESSION 2
ADVANCEMENT IN DIAGNOSIS AND PRECISION MEDICINE IN RARE DISEASES
Chairs **Jean-Francois Desaphy, Armando Genazzani**

8:30-8:50 *Invited talk* **Progresses in diagnostic-therapeutic pathways for precision medicine in rare epilepsies**
Federico Zara

8:50-9:05 *Focus on* **Glycogen storage disorders and drug repurposing: the example of Lafora disease**
Giuseppe d'Orsi

Oral Communications

9:10-9:20 **Fluoxetine as a precision-medicine approach for epileptic encephalopathies caused by KCNT1 variants**
Puzo D, Mosca I, Freri E, Ambrosino P, Belperio G, Granata T, Canafoglia L, Ragona F, Solazzi R, Filareto I, Castellotti B, Messina G, Gellera C, Trivisano M, De Dominicis A, Specchio N, DiFrancesco JC, Soldovieri MV, Taglialatela M *(Napoli)*

9:20-9:30 **Innovative therapeutic strategies for Krabbe disease: nanoparticle-mediated enzyme replacement and autophagy induction**
Del Grosso A, Carpi S, Gagliardi M, De Sarlo M, Scaccini L, Colagiorgio L, Alabed HBR, Pellegrino RM, Tonazzini I, Emiliani C & Cecchini M *(Pisa)*

9:30-9:40 **Fenamates as ClC-1 choride channels modulators for a potential repurposing in myotonia congenita**
Saltarella I, Laghetti P, Campanale C, Ninni I, Altamura C, Desaphy JF *(Bari)*

9:40-9:50 **ClC-39Na: a beacon of hope in the fight against Tubular Aggregate Myopathies**
Pessolano E, Genazzani AA *(Novara)*

9:50-10:00 **Reintroducing synthetic SIL1 protein to treat Marinesco-Sjogren Syndrome**
Bellia F, Amodei L, Viele M, Potenza F, Ruggieri AG, Dufrusine B, Federici L, Sallese M *(Chieti-Pescara)*

10:00-10:10 **Heme oxygenase 1 a new possible target therapy against inflammation in squamous cell carcinoma in Epidermolysis Bullosa patients: a pre-clinical pilot study**
Cicco G, Lospalluti L, De Marco A, Murciano M, Annicchiarico G *(Bari)*

10:10-10:30 **Discussion**

10:30-11:00 Coffee break

SESSION 3
INNOVATIVE PRECLINICAL PLATFORMS: DRUGGABLE MECHANISMS AND VALIDATION OF NEW THERAPIES
Chairs **Fabrizio Gardoni, Luigia Trabace**

11:00-11:20 *Invited talk* **Translational research in rare diseases: old and new models**
Colin Nichols

11:20-11:40 *Focus on* **Patient-derived 3D skeletal muscle organoids: new platforms for studying personalized therapies in rare neuromuscular disorders**
Juanma Fernandez Costa

Oral Communications

11:45-11:55 **Exploiting pharmacological predictive fruit fly models for the identification of promising molecules in future Hereditary Spastic Paraplegia therapy**
Guarato G, Vantaggiato C, Dianin F, Rossato R, Gumeni S, Bassi MT, Orso G *(Padova)*

11:55-12:05 **Phosphodiesterases S-sulfhydration contributes to human Malignant Hyperthermia**
Smimmo M, Vellecco V, Panza E, Bibli SI, Casillo GM, Villani R, Fleming I, Cirino G, Bucci M *(Napoli)*

12:05-12:15 **Is the D2-mdx mouse a better preclinical model for Duchenne muscular dystrophy? Insights from growth hormone secretagogues studies**
Boccanegra B, Mantuano P, Cappellari O, Tulimiero Li, Mele A, Cristiano E, Marinelli M, Conte E, Trisciuzzi D, Bresciani E, Torsello A, Denoyelle S, Nicolotti O, Liantonio A, De Luca A *(Bari)*

12:15-12:25 **A novel integrated pharmacological/antioxidant approach for Duchenne Muscular Dystrophy**
Perrotta C, Prata C, Zecchini S, Ottria R, Cervia D, Hrelia S, De Palma C, Clementi E *(Milano)*

12:25-12:35 **Drug repurposing strategy to identify novel activators of lysosomal Ca2+ channels via autophagy regulation in a preclinical model of amyotrophic lateral sclerosis**
Tedeschi V, Sisalli MJ, Ciancio R, Sapienza S, Castaldo A, Pannaccione A, Secondo A *(Napoli)*

12:35-12:45	**Testing reliability of in vitro neuronal cultures in pharmacological treatment of a genetic form of migraine** Barbieri R, Misurale F, Alloisio S, Freilinger Tobias, Pusch M, Gavazzo P (*Genova)*
12:45-13:00	**Discussion**
13:00-13:30	**Award ceremony for best oral communication and poster (under 38 SIF members)** *Award presentation* Giuseppe Cirino, Fabrizio Gardoni **Closing remarks** Giuseppe Cirino, Annamaria De Luca

Poster topic 1
MUSCULAR DYSTROPHIES AND NEUROMUSCULAR DISORDERS
Chairs Maria Rosaria Bucci, Giulia Maria Camerino, Sabata Pierno

1. Characterization of the effects of JMV2894, a synthetic growth hormone secretagogue, in a cellular model of Duchenne muscular dystrophy
Bresciani E, Rizzi L, Meanti R, Cappellari O, Mantuano P, Conte E, Sanarica F, Boccanegra B, Cerchiara AG, Liantonio A, Cantel S, Denoyelle S, Fehrentz J-A, Locatelli V, De Luca A, Torsello A

2. SRT2104, a new SIRT1 activator, is an effective metabolic enhancer that promotes muscle recovery in DMD
Giovarelli M, Zecchini S, Casati S, Clerici G, Mollica L, Cattaneo MG, Brunetti D, Banfi C, Perrotta C, De Palma C

3. SRT2104 effects on human muscle cell model of Duchenne muscular dystrophy, as a SIRT1 highly selective activator
De Santis C, Quarta R, Cristiano E, Cerchiara AG, Zecchini S, Barile SN, Lasorsa FM, Mouly V, De Palma C, Clementi E, De Luca A, Giovarelli M, Cappellari O

4. LKB1 as a novel diagnostic and therapeutic player in Duchenne muscular dystrophy: new insights from cellular and murine dystrophic models
Boccanegra B, Mantuano P, Conte E, Cerchiara AG, Tulimiero L, Quarta R, Forino M, Spadotto V, Cappellari O, Fossati G, Steinkühler C, De Luca A

5. Erucin, a natural hydrogen sulfide (H2S) donor, improves DMD-induced SKM dysfunction
Smimmo M, Casale V, Bello I, Panza E, Bonomo M, Brancaleone V, Cirino G, Bucci M, Vellecco V

6. Dasatinib as a booster of mutation-specific molecular therapies in Duchenne muscular dystrophy: first assessment of safety in murine and human cell models
Cristiano E, Quarta R, De Santis C, Cerchiara AG, Cappellari O, Boccanegra B, Conte E, Mantuano P, De Luca A

7. Class I selective HDAC inhibitors as new potential treatment for DMD: in vivo and ex vivo readouts in D2-mdx mouse model
Tulimiero L, Boccanegra B, Licandro S, Decio A, Mantuano P, Cappellari O, De Luca A, Steinkühler C

8. Gut microbiota-endocannabinoid interplay in rare skeletal muscle myopathies: an intricate relationship that must be taken into consideration
Di Martino E, Pagano E, Panza E, Ercolano G, Silvestri C, Piscitelli F, di Marzo V, Iannotti FA

9. Targeting unfolded protein response reverts ER stress and ER Ca2+ homeostasis in cardiomyocytes expressing the pathogenic variant of Lamin A/C R321X
Pietrafesa G, De Zio R, Scorza SI, Armentano MF, Pepe M, Forleo C, Procino G, Gerbino A, Svelto M, Carmosino M

10. Potential application of Growth Hormone Secretagogues (GHS) for Amyotrophic Lateral Sclerosis (ALS) treatment: mechanisms of action and neuroprotective effects in human SH-SY5Y SOD1G93A cells
Meanti R, Rizzi L, Bresciani E, Licata M, Molteni L, Omeljaniuk RJ, Fehrentz J-A, Denoyelle S, Locatelli V, Torsello A

11. Effects of irisin treatment on the expression of genes associated with myogenesis, inflammation, mitochondrial metabolism, and neuroprotection in an Amyotrophic Lateral Sclerosis "in vitro" model
Carbone G, Canfora I, Conte E, Tarantino N, Camerino GM, Pierno S

12. Preclinical study showing a gender specific protective properties of conjugated linoleic acid (CLA) for amyotrophic lateral sclerosis
Bacchetti F, Bonifacino T, Torazza C, Balbi M, Ferramosca A, Tessitore S, Boccanegra B, Pierno S, Bonanno G, Bergamo P, Milanese M

13. In-vitro and in-vivo pre-clinical evidence unveiling the mGlu5 receptor as a promising pharmacological target for ALS clinical treatment
Milanese M, Bacchetti F, Bonifacino T, Torazza C, Provenzano F, Ravera S, Balbi M, Tessitore S, Ferrando S, Bonanno G

14. New therapeutic intervention for amyotrophic lateral sclerosis: analysis of the effects of acetazolamide on the biophysical properties of skeletal muscle in a transgenic mouse model
Canfora I, Tarantino N, Mantuano P, Cappellari O, Conte E, Camerino GM, Dobrowolny G, Musarò A, De Luca A, Pierno S

15. Gene therapy for the treatment of childhood SMA1: Onasemnogene abeparvovec. Observations of results of early Zolgensma administration thanks to newborn screening
Ferrante MP, Dell'Aera M, Console V, Tornabene A, Gagliardi D, Canzio E, Storelli S, Attolini E, Annicchiarico G

16. KIF5A, a protein involved in axonal transport, represents a new druggable target in a mouse model of spinal muscular atrophy
Valsecchi V, Kolici X, Baklou M, Laudati G, Brancaccio P, Pignataro G

Poster topic 2
CHANNELOPATHIES
Chairs Paola Imbrici, Loretta Ferrera, Michela De Bellis

17. Biallelic inheritance of two novel SCN1A variants results in loss of Nav1.1 channel function and developmental and epileptic encephalopathy
Dinoi G, Conte E, Palumbo O, Benvenuto M, Coppola MA, Palumbo P, La Stella P, Boccanegra B, Di Muro E, Castori M, Carella M, Sciruicchio V, de Tommaso M, Liantonio A, De Luca A, La Neve A, Imbrici P

18. SCN2A A1659V loss-of-function variant causes early infantile onset encephalopathy
Ferrera L, Ludovico A, Riva A, Morinelli L, Albini M, Bianchi A, Sterlini B, Lombardo G, Madia F, Lesca G, Falsaperla R, Corradi A, Zara F

19. Automated patch clamp for assessing the effects of mexiletine and its pyrroline derivative on Nav1.4 and Nav1.5: towards anti-myotonic drugs with improved safety profile
Cerchiara AG, Becker N, Fertig N, Cappellari O, Okeyo G, De Bellis M, Carocci A, Lentini G, Rolland J-F, Imbrici P, De Luca A

20. Combined in silico and in vitro approaches to repurpose drugs towards Kv1.1 and Kv1.2 potassium channels for epileptic encephalopathy and ataxia pharmacological treatment
Tondo AR, Trisciuzzi D, Siragusa L, D'Adamo MC, Liantonio A, De Luca A, Nicolotti O, Imbrici P

21. Structure-based identification and characterization of novel inhibitors of KNa1.1 potassium channels
Miceli F, Carotenuto L, Mosca I, Soldovieri MV, Ambrosino P, Carleo G, Iraci N, Ostacolo C, Campiglia P, Taglialatela M

22. De novo variants in KCNA3 cause developmental and epileptic encephalopathy
Belperio G, Soldovieri MV, Ambrosino P, Mosca I, Servettini I, Pietrunti F, Syrbe S, Taglialatela M, Lemke JR

23. Potassium channels and TRPV1 modulators on SU-DIPG-36 and SU-DIPG-50 cells: in vitro effects on cell proliferation and channel currents characterization
Di Turi A ǂ, Antonacci M ǂ, Miciaccia M, Maqoud F, Perrone MG, Scilimati A, Tricarico D

24. Antiproliferative effects of tyrosine kinases (tk) inhibitors staurosporin/midostaurin on SU-DIPG cells and on cation currents: role of K+ channels and TRPV1 channels as drug targets
Antonacci M ǂ, Di Turi A ǂ, Miciaccia M, Maqoud F, Perrone MG, Scilimati A, Tricarico D

25. Kir6.1- and SUR2-dependent KATP overactivity caused intestinal tight junction protein alterations in the intestinal epithelium in murine models of Cantú syndrome
Maqoud F, Orlando A, Tricarico D, Nichols CG, Antonacci M, Russo F

26. New insights into the involvement of rare genetic variants in CLCN6 and CLCN7 associated with neurological diseases: a functional in vitro study
Coppola MA, Imbrici P, Liantonio A, Gavazzo P, Fong P, Pusch M

27. Preclinical evaluation of safinamide as an antimyotonic drug in myotonic ADR mouse model
Canfora I, Altamura C, Desaphy J-F, Vailati S, Caccia C, Padoani G, De Luca A, Pierno S

28. Functional and pharmacological characterization of sodium and chloride channel mutations in Italian families affected by non-dystrophic myotonias
Altamura C, Campanale C, Laghetti P, Ninni I, Imbrici P, Saltarella I, Desaphy J-F

Poster topic 3
NEUROLOGIC, RENAL, METABOLIC AND OTHER RARE DISEASES
Chairs Antonella Liantonio, Antonietta Mele, Antonio Torsello

29. Fingerprinting cardiolipin in leukocytes by MALDI-TOF mass spectrometry as a screening tool for Barth Syndrome
Lobasso S

30. The Keap1/Nrf2/ARE pathway as a potential therapeutic target for the treatment of Huntington's disease
Pruccoli L, Sita G, Pagliarani B, Morroni F, Tarozzi A

31. A clinical case of corpus callosum agenesia: a neuroscience multidisciplinary evaluation
Galletta D, de Bartolomeis A, Zeppetella Del Sesto FS, Marzullo A, Flace P

32. A clinical case report of cerebellar vermis hypoplasia related to deletion 15q21.3-22.31
Flace P, de Bartolomeis A, Gelato M, Zeppetella SF, Marzullo A, Galletta D

33. Calretinin in the human brain: a light microscopy immunohistochemical study
Flace P, Galletta D, Marzullo A

34. Genetic variants and inborn errors of immunity in bone marrow failure: novel potential drug targets for precision medicine
Desantis V, Andriano A, Marasco C, Pappagallo F, Di Marzo L, Ingravallo G, Di Paola R, Tabares P, Beilhac A, Vacca A, Solimando AG, Montagnani M

35. Pharmacological block of prokineticin system and microglia inhibition counteract pain in a murine model of Fabry-Anderson disease
Galimberti G, Franchi S, Amodeo G, Magni G, Riboldi B, Ceruti S, Sacerdote P

36. Exploring the link between GALC and GPR65: implications for Krabbe disease
Carpi S, Ferrero G, Del Grosso A, De Sarlo M, Colagiorgio L, Scaccini L, Battini R, Santorelli FM, Cutrupi S, Tonazzini I, Cecchini M

37. Phosphodiesterase 5 inhibitors as a new treatment for maternally inherited Leigh syndrome
Pedrotti G, Zink A, Santanatoglia C, Henke M-T, Di Donfrancesco A, Brunetti D, Decimo I, Adamo A, De Sanctis F, Tiranti V, Schuelke M, Prigione A, Bottani E

38. The β3-AR agonist BRL37344 ameliorates the main symptoms of X-linked nephrogenic diabetes insipidus in the mouse model of the disease
Milano S, Saponara I, Gerbino A, Carmosino M, Svelto M, Procino G

39. Dual targeting of the G protein-coupled receptors CaSR and V2R for treating autosomal dominant polycystic kidney disease (ADPKD)
Di Mise A, Venneri M, Ferrulli A, Centrone M, Ranieri M, Caroppo R, Tamma G, Pellegrini L, Torres VE, Valenti G

40. What is hidden in patients with unknown nephropathy? The genetic screening could represent the missing link in the diagnosis and management in kidney transplantation
Mitrotti A, Giliberti M, di Bari I, Franzin R, Conserva F, Stea ED, Rossini M, Fiorentino M, Castellano G, Pontrelli P, Gesualdo L

41. Characterization of lipid and lipoprotein profile in Alagille syndrome
Ossoli A, Cananzi M, Turri M, Pavanello C, Belotti L, Gomaraschi M, Vidal E, Calabresi L

42. Riboflavin transporter deficiency type 2 (RTD2, OMIM #614707): a focus on the endoplasmic reticulum responses
Tolomeo M, Console L, Nisco A, Magliocca V, Persichini T, Compagnucci C, Barbaro R, Colella M, Bertini E, Massey K, Indiveri C, Barile M

43. Off-label use of liraglutide counteracts the immune dysregulation associated with Wolfram syndrome
Panfili E, Gargaro M, Orabona C, Mondanelli G, Fallarino F, Pallotta MT

44. Anti-yo mediated paraneoplastic cerebellar degeneration: a case report
Flace P, Galletta D, Stucci LS, De Caro M, Mesto C, Pascazio L, Livrea P, Marzullo A, Liaci G

45. CD90-TGFβ1 co-expression promotes chemoresistance in intrahepatic cholangiocarcinoma
Pizzuto E, Mancarella S, Gigante I, Serino G, Dituri F. Giannelli G

Poster topic 4
NEW MODELS AND METHODOLOGIES IN RARE DISEASES
Chairs Ornella Cappellari, Monica Montagnani, Domenico Tricarico

46. Human cellular preclinical models to understand congenital myopathies: from pathogenic mechanism to new therapeutical targets in TAM and SEPN1-RM diseases
Conte E, Imbrici P, Dinoi G, Maggi L, De Luca A, Liantonio A

47. In vitro 3D-model of mitochondrial myopathy human skeletal muscle
Di Leo V, Tejedera A, Fernández-Garibay X, Ramón-Azcón J, Gorman GS, Russell OM, Vincent AE, Fernández-Costa J

48. Optogenetic skeletal muscle-on-chip as a drug screening and disease modelling platform for rare neuromuscular disorders.
Quarta R, Han M, De Santis C, Arduino I, Denora N, Fiermonte G, Gaio N, Rossini N, De Bellis M, De Luca A, Cappellari O

49. Characterization of cellular differentiation in an in vitro model of neuromuscular junction using a co-culture system: a preliminary study of a drug testing platform.
Canfora V, Carbone G, Pierno S, Camerino GM

50. Urine-derived stem cells and derived skeletal muscle cells as a functional model to study calcium homeostasis perturbation in neuromuscular diseases.
Talmon M, Lecchi G, Fresu LG

51. Patient-specific neuronal stem cells as an in vitro model for screening drug safety and efficacy for Aicardi-Goutières pediatric patients
Pugnetti L, Braidotti S, Ferraro RM, Irshad M, Franca R, Marinozzi V, Tommasini A, Lucafò M, Decorti G, Giliani S, Stocco G

52. Impaired bioenergetic profile in neuron progenitor cells from iPSCs of patients affected by AGC1 deficiency
Barile SN, Magnifico MC, Palmieri L, Distelmaier F, Viggiano L, Pignataro A, Petralla S, Hentschel J, Porcelli V, Poeta E, Pisano I, Fiermonte G, Anderson SA, Monti B, Lasorsa FM

53. A novel renal collecting duct model to study secondary nephrogenic diabetes insipidus associated to cystinosis
Ferrulli A, Gijsbers R, Di Mise A, Cairoli S, Van den heuvel LP, Levtchenko E, Valenti G

54. A molecular docking-based virtual screening study to tackle the cure for Lafora disease
Trisciuzzi D, Imbrici P, Gambacorta N, Dinoi G, Conte E, Mantuano P, Palumbo O, Nicolotti O, Carella M, De Luca A, d'Orsi G, Altomare CD, Liantonio A

55. Computational screening for Lafora disease: repurposing of approved drugs as a novel treatment strategy
Gambacorta N, Imbrici P, Trisciuzzi D, Dinoi G, Conte E, Mantuano P, Palumbo O, Bisulli F, Nicolotti O, De Luca A, Carella M, d'Orsi G, Altomare CD, Liantonio A

56. Innovative research methodologies in the EU regulatory framework: an analysis of EMA qualification procedures in the perspective of rare diseases
Torretta S, Giannuzzi V, Bertolani A, Reggiardo G, Toich E, Bonifazi D, Bonifazi F, Ceci A on behalf of the European Paediatric Translational Research Infrastructure (EPTRI)

57. Target therapy for high grade neuroblastoma treatment: integration of regulatory and scientific tools is needed
Ceci A, Conte R, Didio A, Landi A, Ruggieri L, Giannuzzi V, Bonifazi F

58. Academic development of a paediatric formulation of Budesonide for the treatment of eosinophilic esophagitis to cover a high unmet medical need
Spennacchio A, Cristofori F, Lacassia C, Giannuzzi V, Bonifazi F, Ceci A, Landi A, Conte R, Lopedota AA, Lopalco A, Francavilla R, Denora N

59. Distinction of drug flows for rare disease and monitoring of expenditure through optimization of IT systems used in study area
Ferrante MP, Di Pietro G, Storelli S, Attolini E, Annicchiarico G

60. Axicabtagene ciloleucel (axi-cel) for relapsed/refractory (R/R) large B cell lymphoma beside efficacy: data analysis of EudraVigilance database
Rafaniello C, Liguori V, Zinzi A, Gaio M, Falco A, Di Costanzo L, Gargano F, Trimarco V, Cataldi M, Capuano A

INFORMATION

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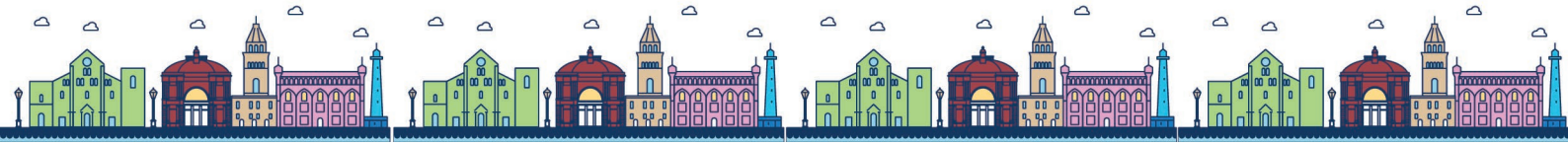
INNOVAZIONE E MALATTIE RARE: DALLA RICERCA PRECLINICA AL PAZIENTE

23-24 febbraio 2024
Bari, Camera di Commercio

abstract book



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Maurizio Taglialatela (Napoli)
Gianluca Trifirò (Verona)

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Elena Conte
Paola Mantuano
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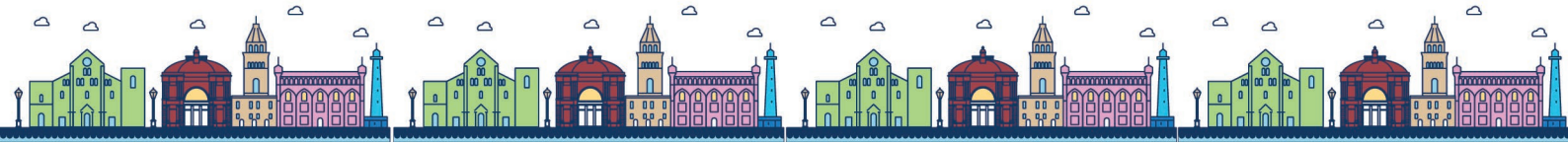


SCIENTIFIC PROGRAM 23rd February 2024

12:00-13:15 Registration

13:15-14:00 WELCOME AND INTRODUCTION

- Giuseppe Cirino, *Presidente Società Italiana Farmacologia (SIF)*
- Annamaria De Luca, *Coordinatrice Gruppo di Lavoro SIF4RARE*
- Stefano Bronzini, *Magnifico Rettore Università degli Studi di Bari Aldo Moro*
- Marcello Gemmato, *Sottosegretario alla Salute con delega Malattie Rare*
- Michele Emiliano, *Presidente Regione Puglia*
- Filippo Anelli, *Presidente Ordine dei Medici Chirurghi e Odontoiatri (OMCeO) di Bari e Federazione Nazionale OMCeO*
- Luigi D'Ambrosio Lettieri, *Presidente Ordine Interprovinciale Farmacisti di Bari e Barletta-Andria-Trani*
- Francesco Leonetti, *Direttore Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari Aldo Moro*

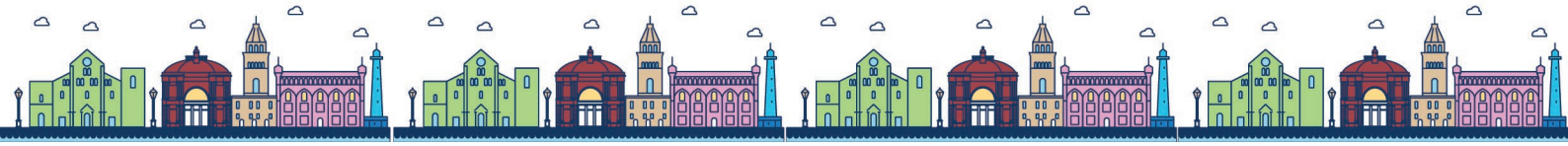
14:00-15:30 ROUND TABLE
Therapy of rare diseases and innovation: science and politics at the service of patients

Chairs Pier Luigi Canonico, Annamaria De Luca
Panel Giuseppina Annicchiarico, Diana Conte, Marcello Gemmato, Armando Genazzani, Mattia Gentile, Andrea Marcellusi, Enrico Piccinini, Patrizia Popoli, Annalisa Scopinaro, Gianluca Trifirò

15:30-16:00 OPENING LECTURE

Chair Giuseppe Cirino
Antisense oligonucleotides in rare diseases: lessons learned from neuromuscular disorders to N-of-1 treatment in brain diseases
Annemieke Aartsma-Rus

16:00-16:15 Coffee break

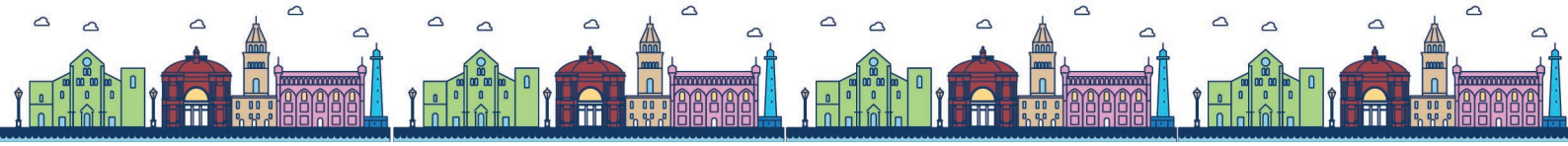


SESSION 1
PATIENT-CENTERED DATA: FROM CLINICAL PRACTICE TO BASIC RESEARCH AND BACK
Chairs Annalisa Capuano, Emilio Clementi

16:20-16:40	*Invited talk* **Autoimmune-based renal disorders: the link between basic research and clinic** Loreto Gesualdo
16:40-16:55	*Focus on* **Orphan drugs and advanced therapies: the value of patient-centered innovation and open challenges** Patrizia Popoli

Oral Communications

17:00-17:10	**Epidemiological analyses through machine learning approaches to accelerate rare diseases diagnosis** Ingrasciotta Y, Crisafulli S, Trifirò G *(Verona)*
17:10-17:20	**Safety aspects of the gene therapy for SMA, Zolgensma®: a retrospective analysis of the European pharmacovigilance database Eudravigilance** Ruggiero R, Balzano N, Mascolo A, di Napoli R, Capuano A *(Napoli)*
17.20-17:30	**The therapeutic value of treatment for metachromatic leukodystrophy: analysis of health technology assessments of 3 European Countries** Gozzo L, Romano GL, Longo L, Vitale DC, Drago F *(Catania)*
17:30-17:40	**Exploiting routine laboratory test to identify patients with familial chylomicronemia syndrome (FCS) patients in a large Italian hospital** Pavanello C, Pazzucconi F, Parolini M, Turri M, Mombelli GG, Castiglione S, Alberti A, De Maria R, Calabresi L *(Milano)*
17:40-17:50	**Inborn errors of immunity: lessons learned from an ever evolving paradigm** Martire B *(Barletta)*
17:50-18:00	**The use of real world data for regulatory purposes in the rare diseases setting** Giannuzzi V, Stoyanova-Beninska V, Hivert V *(Bari)*
18:00-18:20	**Discussion**
18:20-18:30	*Insight* **The program of European Certified Pharmacologists (EuCP)** Mariagrazia Grilli



POSTER VIEW AND DISCUSSION - *wine and cheese*

18:30-20:30

Poster topic 1
MUSCULAR DYSTROPHIES AND NEUROMUSCULAR DISORDERS
Chairs Maria Rosaria Bucci, Giulia Maria Camerino, Sabata Pierno

Poster topic 2
CHANNELOPATHIES
Chairs Paola Imbrici, Loretta Ferrera, Michela De Bellis

Poster topic 3
NEUROLOGIC, RENAL, METABOLIC AND OTHER RARE DISEASES
Chairs Antonella Liantonio, Antonietta Mele, Antonio Torsello

Poster topic 4
NEW MODELS AND METHODOLOGIES IN RARE DISEASES
Chairs Ornella Cappellari, Monica Montagnani, Domenico Tricarico

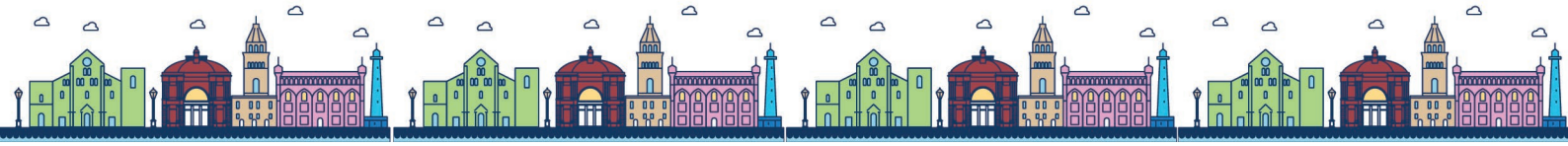
SCIENTIFIC PROGRAM 24th February 2024

SESSION 2
ADVANCEMENT IN DIAGNOSIS AND PRECISION MEDICINE IN RARE DISEASES
Chairs Jean-Francois Desaphy, Armando Genazzani

8:30-8:50	*Invited talk* **Progresses in diagnostic-therapeutic pathways for precision medicine in rare epilepsies** Federico Zara
8:50-9:05	*Focus on* **Glycogen storage disorders and drug repurposing: the example of Lafora disease** Giuseppe d'Orsi

Oral Communications

9:10-9:20	**Fluoxetine as a precision-medicine approach for epileptic encephalopathies caused by KCNT1 variants** Puzo D, Mosca I, Freri E, Ambrosino P, Belperio G, Granata T, Canafoglia L, Ragona F, Solazzi R, Filareto I, Castellotti B, Messina G, Gellera C, Trivisano M, De Dominicis A, Specchio N, DiFrancesco JC, Soldovieri MV, Taglialatela M *(Napoli)*
9:20-9:30	**Innovative therapeutic strategies for Krabbe disease: nanoparticle-mediated enzyme replacement and autophagy induction** Del Grosso A, Carpi S, Gagliardi M, De Sarlo M, Scaccini L, Colagiorgio L, Alabed HBR, Pellegrino RM, Tonazzini I, Emiliani C & Cecchini M *(Pisa)*
9:30-9:40	**Fenamates as ClC-1 choride channels modulators for a potential repurposing in myotonia congenita** Saltarella I, Laghetti P, Campanale C, Ninni I, Altamura C, Desaphy JF *(Bari)*
9:40-9:50	**ClC-39Na: a beacon of hope in the fight against Tubular Aggregate Myopathies** Pessolano E, Genazzani AA *(Novara)*
9:50-10:00	**Reintroducing synthetic SIL1 protein to treat Marinesco-Sjogren Syndrome** Bellia F, Amodei L, Viele M, Potenza F, Ruggieri AG, Dufrusine B, Federici L, Sallese M *(Chieti-Pescara)*
10:00-10:10	**Heme oxygenase 1 a new possible target therapy against inflammation in squamous cell carcinoma in Epidermolysis Bullosa patients: a pre-clinical pilot study** Cicco G, Lospalluti L, De Marco A, Murciano M, Annicchiarico G *(Bari)*
10:10-10:30	**Discussion**
10:30-11:00	Coffee break



SESSION 3
INNOVATIVE PRECLINICAL PLATFORMS: DRUGGABLE MECHANISMS AND VALIDATION OF NEW THERAPIES
Chairs **Fabrizio Gardoni, Luigia Trabace**

11:00-11:20 *Invited talk* **Translational research in rare diseases: old and new models**
Colin Nichols

11:20-11:40 *Focus on* **Patient-derived 3D skeletal muscle organoids: new platforms for studying personalized therapies in rare neuromuscular disorders**
Juanma Fernandez Costa

Oral Communications

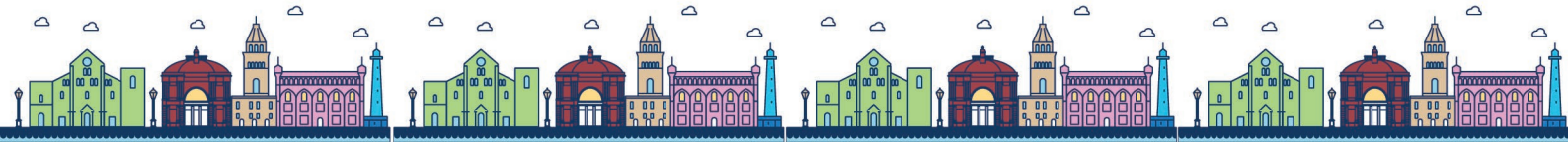
11:45-11:55 **Exploiting pharmacological predictive fruit fly models for the identification of promising molecules in future Hereditary Spastic Paraplegia therapy**
Guarato G, Vantaggiato C, Dianin F, Rossato R, Gumeni S, Bassi MT, Orso G *(Padova)*

11:55-12:05 **Phosphodiesterases S-sulfhydration contributes to human Malignant Hyperthermia**
Smimmo M, Vellecco V, Panza E, Bibli SI, Casillo GM, Villani R, Fleming I, Cirino G, Bucci M *(Napoli)*

12:05-12:15 **Is the D2-mdx mouse a better preclinical model for Duchenne muscular dystrophy? Insights from growth hormone secretagogues studies**
Boccanegra B, Mantuano P, Cappellari O, Tulimiero Li, Mele A, Cristiano E, Marinelli M, Conte E, Trisciuzzi D, Bresciani E, Torsello A, Denoyelle S, Nicolotti O, Liantonio A, De Luca A *(Bari)*

12:15-12:25 **A novel integrated pharmacological/antioxidant approach for Duchenne Muscular Dystrophy**
Perrotta C, Prata C, Zecchini S, Ottria R, Cervia D, Hrelia S, De Palma C, Clementi E *(Milano)*

12:25-12:35 **Drug repurposing strategy to identify novel activators of lysosomal Ca2+ channels via autophagy regulation in a preclinical model of amyotrophic lateral sclerosis**
Tedeschi V, Sisalli MJ, Ciancio R, Sapienza S, Castaldo A, Pannaccione A, Secondo A *(Napoli)*



12:35-12:45	**Testing reliability of in vitro neuronal cultures in pharmacological treatment of a genetic form of migraine** Barbieri R, Misurale F, Alloisio S, Freilinger Tobias, Pusch M, Gavazzo P (*Genova)*
12:45-13:00	**Discussion**
13:00-13:30	**Award ceremony for best oral communication and poster (under 38 SIF members)** *Award presentation* Giuseppe Cirino, Fabrizio Gardoni **Closing remarks** Giuseppe Cirino, Annamaria De Luca

POSTER

Poster topic 1
MUSCULAR DYSTROPHIES AND NEUROMUSCULAR DISORDERS
Chairs Maria Rosaria Bucci, Giulia Maria Camerino, Sabata Pierno

1. Characterization of the effects of JMV2894, a synthetic growth hormone secretagogue, in a cellular model of Duchenne muscular dystrophy
Bresciani E, Rizzi L, Meanti R, Cappellari O, Mantuano P, Conte E, Sanarica F, Boccanegra B, Cerchiara AG, Liantonio A, Cantel S, Denoyelle S, Fehrentz J-A, Locatelli V, De Luca A, Torsello A

2. SRT2104, a new SIRT1 activator, is an effective metabolic enhancer that promotes muscle recovery in DMD
Giovarelli M, Zecchini S, Casati S, Clerici G, Mollica L, Cattaneo MG, Brunetti D, Banfi C, Perrotta C, De Palma C

3. SRT2104 effects on human muscle cell model of Duchenne muscular dystrophy, as a SIRT1 highly selective activator
De Santis C, Quarta R, Cristiano E, Cerchiara AG, Zecchini S, Barile SN, Lasorsa FM, Mouly V, De Palma C, Clementi E, De Luca A, Giovarelli M, Cappellari O

4. LKB1 as a novel diagnostic and therapeutic player in Duchenne muscular dystrophy: new insights from cellular and murine dystrophic models
Boccanegra B, Mantuano P, Conte E, Cerchiara AG, Tulimiero L, Quarta R, Forino M, Spadotto V, Cappellari O, Fossati G, Steinkühler C, De Luca A

5. Erucin, a natural hydrogen sulfide (H2S) donor, improves DMD-induced SKM dysfunction
Smimmo M, Casale V, Bello I, Panza E, Bonomo M, Brancaleone V, Cirino G, Bucci M, Vellecco V

6. Dasatinib as a booster of mutation-specific molecular therapies in Duchenne muscular dystrophy: first assessment of safety in murine and human cell models
Cristiano E, Quarta R, De Santis C, Cerchiara AG, Cappellari O, Boccanegra B, Conte E, Mantuano P, De Luca A

7. Class I selective HDAC inhibitors as new potential treatment for DMD: in vivo and ex vivo readouts in D2-mdx mouse model
Tulimiero L, Boccanegra B, Licandro S, Decio A, Mantuano P, Cappellari O, De Luca A, Steinkühler C

8. Gut microbiota-endocannabinoid interplay in rare skeletal muscle myopathies: an intricate relationship that must be taken into consideration
Di Martino E, Pagano E, Panza E, Ercolano G, Silvestri C, Piscitelli F, di Marzo V, Iannotti FA

9. Targeting unfolded protein response reverts ER stress and ER Ca2+ homeostasis in cardiomyocytes expressing the pathogenic variant of Lamin A/C R321X
Pietrafesa G, De Zio R, Scorza SI, Armentano MF, Pepe M, Forleo C, Procino G, Gerbino A, Svelto M, Carmosino M

10. Potential application of Growth Hormone Secretagogues (GHS) for Amyotrophic Lateral Sclerosis (ALS) treatment: mechanisms of action and neuroprotective effects in human SH-SY5Y SOD1G93A cells
Meanti R, Rizzi L, Bresciani E, Licata M, Molteni L, Omeljaniuk RJ, Fehrentz J-A, Denoyelle S, Locatelli V, Torsello A

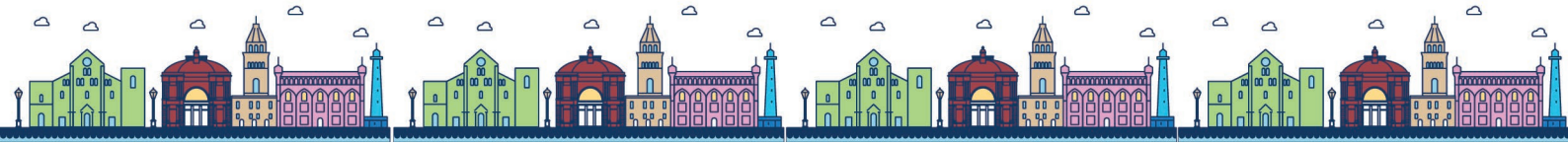
11. Effects of irisin treatment on the expression of genes associated with myogenesis, inflammation, mitochondrial metabolism, and neuroprotection in an Amyotrophic Lateral Sclerosis "in vitro" model
Carbone G, Canfora I, Conte E, Tarantino N, Camerino GM, Pierno S

12. Preclinical study showing a gender specific protective properties of conjugated linoleic acid (CLA) for amyotrophic lateral sclerosis
Bacchetti F, Bonifacino T, Torazza C, Balbi M, Ferramosca A, Tessitore S, Boccanegra B, Pierno S, Bonanno G, Bergamo P, Milanese M

13. In-vitro and in-vivo pre-clinical evidence unveiling the mGlu5 receptor as a promising pharmacological target for ALS clinical treatment
Milanese M, Bacchetti F, Bonifacino T, Torazza C, Provenzano F, Ravera S, Balbi M, Tessitore S, Ferrando S, Bonanno G

14. New therapeutic intervention for amyotrophic lateral sclerosis: analysis of the effects of acetazolamide on the biophysical properties of skeletal muscle in a transgenic mouse model
Canfora I, Tarantino N, Mantuano P, Cappellari O, Conte E, Camerino GM, Dobrowolny G, Musarò A, De Luca A, Pierno S

15. Gene therapy for the treatment of childhood SMA1: Onasemnogene abeparvovec. Observations of results of early Zolgensma administration thanks to newborn screening
Ferrante MP, Dell'Aera M, Console V, Tornabene A, Gagliardi D, Canzio E, Storelli S, Attolini E, Annicchiarico G



16. KIF5A, a protein involved in axonal transport, represents a new druggable target in a mouse model of spinal muscular atrophy
Valsecchi V, Kolici X, Baklou M, Laudati G, Brancaccio P, Pignataro G

Poster topic 2
CHANNELOPATHIES
Chairs Paola Imbrici, Loretta Ferrera, Michela De Bellis

17. Biallelic inheritance of two novel SCN1A variants results in loss of Nav1.1 channel function and developmental and epileptic encephalopathy
Dinoi G, Conte E, Palumbo O, Benvenuto M, Coppola MA, Palumbo P, La Stella P, Boccanegra B, Di Muro E, Castori M, Carella M, Sciruicchio V, de Tommaso M, Liantonio A, De Luca A, La Neve A, Imbrici P

18. SCN2A A1659V loss-of-function variant causes early infantile onset encephalopathy
Ferrera L, Ludovico A, Riva A, Morinelli L, Albini M, Bianchi A, Sterlini B, Lombardo G, Madia F, Lesca G, Falsaperla R, Corradi A, Zara F

19. Automated patch clamp for assessing the effects of mexiletine and its pyrroline derivative on Nav1.4 and Nav1.5: towards anti-myotonic drugs with improved safety profile
Cerchiara AG, Becker N, Fertig N, Cappellari O, Okeyo G, De Bellis M, Carocci A, Lentini G, Rolland J-F, Imbrici P, De Luca A

20. Combined in silico and in vitro approaches to repurpose drugs towards Kv1.1 and Kv1.2 potassium channels for epileptic encephalopathy and ataxia pharmacological treatment
Tondo AR, Trisciuzzi D, Siragusa L, D'Adamo MC, Liantonio A, De Luca A, Nicolotti O, Imbrici P

21. Structure-based identification and characterization of novel inhibitors of KNa1.1 potassium channels
Miceli F, Carotenuto L, Mosca I, Soldovieri MV, Ambrosino P, Carleo G, Iraci N, Ostacolo C, Campiglia P, Taglialatela M

22. De novo variants in KCNA3 cause developmental and epileptic encephalopathy
Belperio G, Soldovieri MV, Ambrosino P, Mosca I, Servettini I, Pietrunti F, Syrbe S, Taglialatela M, Lemke JR

23. Potassium channels and TRPV1 modulators on SU-DIPG-36 and SU-DIPG-50 cells: in vitro effects on cell proliferation and channel currents characterization
Di Turi A ǂ, Antonacci Mǂ, Miciaccia M, Maqoud F, Perrone MG, Scilimati A, Tricarico D

24. Antiproliferative effects of tyrosine kinases (tk) inhibitors staurosporin/midostaurin on SU-DIPG cells and on cation currents: role of K+ channels and TRPV1 channels as drug targets
Antonacci M ǂ, Di Turi Aǂ, Miciaccia M, Maqoud F, Perrone MG, Scilimati A, Tricarico D

25. Kir6.1- and SUR2-dependent KATP overactivity caused intestinal tight junction protein alterations in the intestinal epithelium in murine models of Cantú syndrome
Maqoud F, Orlando A, Tricarico D, Nichols CG, Antonacci M, Russo F

26. New insights into the involvement of rare genetic variants in CLCN6 and CLCN7 associated with neurological diseases: a functional in vitro study
Coppola MA, Imbrici P, Liantonio A, Gavazzo P, Fong P, Pusch M

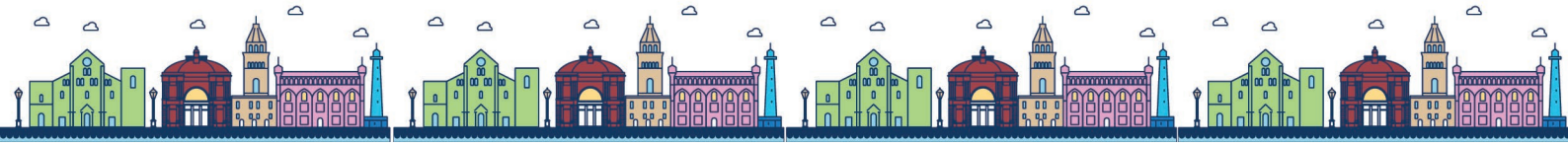
27. Preclinical evaluation of safinamide as an antimyotonic drug in myotonic ADR mouse model
Canfora I, Altamura C, Desaphy J-F, Vailati S, Caccia C, Padoani G, De Luca A, Pierno S

28. Functional and pharmacological characterization of sodium and chloride channel mutations in Italian families affected by non-dystrophic myotonias
Altamura C, Campanale C, Laghetti P, Ninni I, Imbrici P, Saltarella I, Desaphy J-F

Poster topic 3
NEUROLOGIC, RENAL, METABOLIC AND OTHER RARE DISEASES
Chairs Antonella Liantonio, Antonietta Mele, Antonio Torsello

29. Fingerprinting cardiolipin in leukocytes by MALDI-TOF mass spectrometry as a screening tool for Barth Syndrome
Lobasso S

30. The Keap1/Nrf2/ARE pathway as a potential therapeutic target for the treatment of Huntington's disease
Pruccoli L, Sita G, Pagliarani B, Morroni F, Tarozzi A



31. A clinical case of corpus callosum agenesia: a neuroscience multidisciplinary evaluation
Galletta D, de Bartolomeis A, Zeppetella Del Sesto FS, Marzullo A, Flace P

32. A clinical case report of cerebellar vermis hypoplasia related to deletion 15q21.3-22.31
Flace P, de Bartolomeis A, Gelato M, Zeppetella SF, Marzullo A, Galletta D

33. Calretinin in the human brain: a light microscopy immunohistochemical study
Flace P, Galletta D, Marzullo A

34. Genetic variants and inborn errors of immunity in bone marrow failure: novel potential drug targets for precision medicine
Desantis V, Andriano A, Marasco C, Pappagallo F, Di Marzo L, Ingravallo G, Di Paola R, Tabares P, Beilhac A, Vacca A, Solimando AG, Montagnani M

35. Pharmacological block of prokineticin system and microglia inhibition counteract pain in a murine model of Fabry-Anderson disease
Galimberti G, Franchi S, Amodeo G, Magni G, Riboldi B, Ceruti S, Sacerdote P

36. Exploring the link between GALC and GPR65: implications for Krabbe disease
Carpi S, Ferrero G, Del Grosso A, De Sarlo M, Colagiorgio L, Scaccini L, Battini R, Santorelli FM, Cutrupi S, Tonazzini I, Cecchini M

37. Phosphodiesterase 5 inhibitors as a new treatment for maternally inherited Leigh syndrome
Pedrotti G, Zink A, Santanatoglia C, Henke M-T, Di Donfrancesco A, Brunetti D, Decimo I, Adamo A, De Sanctis F, Tiranti V, Schuelke M, Prigione A, Bottani E

38. The β3-AR agonist BRL37344 ameliorates the main symptoms of X-linked nephrogenic diabetes insipidus in the mouse model of the disease
Milano S, Saponara I, Gerbino A, Carmosino M, Svelto M, Procino G

39. Dual targeting of the G protein-coupled receptors CaSR and V2R for treating autosomal dominant polycystic kidney disease (ADPKD)
Di Mise A, Venneri M, Ferrulli A, Centrone M, Ranieri M, Caroppo R, Tamma G, Pellegrini L, Torres VE, Valenti G

40. What is hidden in patients with unknown nephropathy? The genetic screening could represent the missing link in the diagnosis and management in kidney transplantation
Mitrotti A, Giliberti M, di Bari I, Franzin R, Conserva F, Stea ED, Rossini M, Fiorentino M, Castellano G, Pontrelli P, Gesualdo L

41. Characterization of lipid and lipoprotein profile in Alagille syndrome
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46. Human cellular preclinical models to understand congenital myopathies: from pathogenic mechanism to new therapeutical targets in TAM and SEPN1-RM diseases
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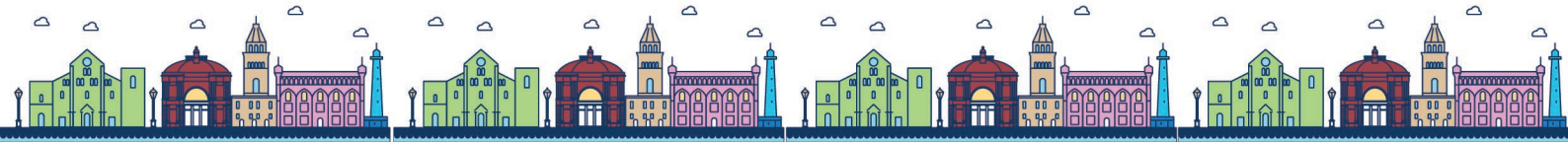

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55. Computational screening for Lafora disease: repurposing of approved drugs as a novel treatment strategy
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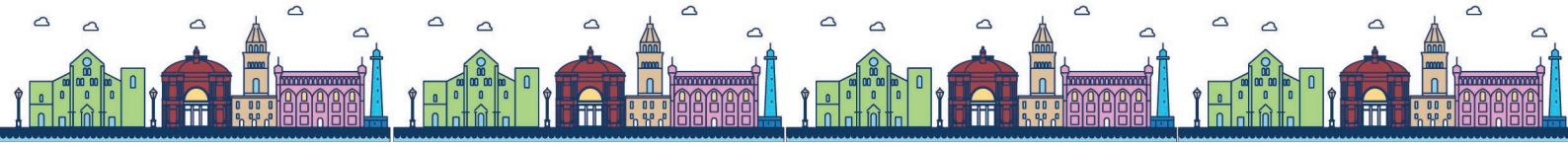
Sessione 1

Oral Communications



Epidemiological analyses through machine learning approaches to accelerate rare diseases diagnosis

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Aim: The diagnostic odyssey leading rare patients to the correct diagnosis is made up of numerous diagnostic exams, hospitalizations, and specialist visits in different centers, which lead to an average delay in diagnosis of about 5 years. This can result in a large amount of real-world data that can be useful to study rare diseases as well as to generate evidence to support the regulatory authorization processes of orphan drugs. The development of innovative algorithms, using claims/GPs databases/disease registries through different combinations of specific criteria (e.g., causes of hospitalization/prescription of specific drugs/ co-payment exemption codes/specialist visits) to predict rare diseases diagnosis, may help to reduce their diagnostic delay. Two studies were conducted to develop and internally validate predictive algorithms' application performance for the early diagnosis of acromegaly and X-linked hypophosphatemia (XLH) using the claims databases of Sicily Region (2011-2018) and the Italian paediatric general practice research database PediaNet (2007-2020), respectively.
Methods: In each study, patients identified by the algorithm with the best diagnostic accuracy were matched with controls by age and sex to evaluate the association between any potential predictor of diagnosis and the presence of the disease through logistic regression models or machine learning techniques, providing an estimate of an individual's likelihood of having the disease under study and a list of potential predictors of diagnosis that may help physicians anticipate diagnosis.
Results: The target population identified in Sicilian claims databases during the study period consisted of 533 acromegalic patients matched to 5,255 controls; the number of immunosuppressants-related pharmacy claims was the only diagnosis predictor selected by all models and algorithms. While, among 431,021 patients registered in PediaNet database, 8 XLH cases and 60 matched controls were included in the analysis. Overall, the XLH predictors were the number of vitamin D prescriptions, the number of recorded diagnoses of acute respiratory infections, the number of prescriptions of antihistamine for systemic use, the number of prescriptions of X-ray of the lower limbs and pelvis and the number of allergology visits.
Conclusions: Findings from both studies showed that machine learning models may play a prominent role for the prediction of the diagnosis of both rare diseases.

Keywords: X-linked hypophosphatemia, acromegaly, epidemiology, rare diseases, machine learning

Safety aspects of the gene therapy for SMA, Zolgensma® : a retrospective analysis of the European pharmacovigilance database Eudravigilance

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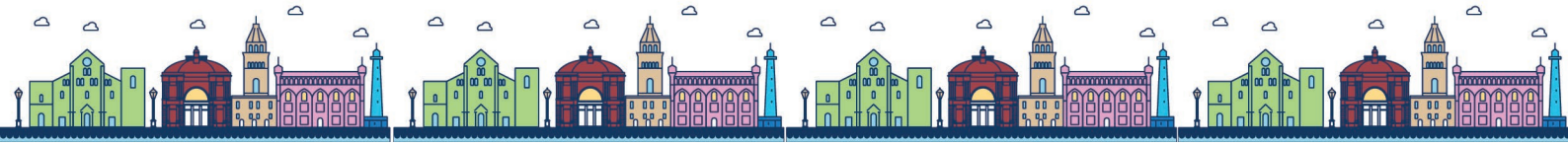
Aim: To describe the safety profile of Zolgensma® in clinical practice .
Methods: We analyzed the Individual Case Safety Reports (ICSR) referred to Zolgensma® and collected in the European pharmacovigilance database Eudravigilance (EV). Our study period was 1 January 2019 – 22 September 2023. We conducted a descriptive analysis, verifying the reporting annual trend, the distribution of the reports by gender and age group, reporter type, concomitant or other suspected drugs. The suspected adverse drug reactions (ADRs) were analyzed in terms of seriousness, outcome and type of events, categorizing them according to the reference MedDRA System Organ Classes (SOC). We focused on the most representative SOC, and on the events belonging to "Hepatobiliary disorders" and "Cardiac disorders" SOCs.
Results: We found 661 ICSRs related to Zolgensma®, with a growing trend of the annual reporting. Majority of the reports was referred to females and patients aged between 2 months-2 years, reported only Zolgensma® as suspected drug (92.1%) and were send by healthcare professionals (88.5%). Prednisolone and nusinersen were the two others most frequently reported suspected drugs. The 56.9% of the ADRs was serious. The 21.4% caused or prolonged hospitalization and the 6.9% of ADRs did not resolve. Out of a total of 2744 reported ADRs, pyrexia, vomiting, aspartate and alanine aminotransferase increased and thrombocytopenia were the most reported ones. "Investigations", "General disorders and administration site conditions" and "Gastrointestinal disorders" were the most representative SOCs. Alterations of the heart rhythm and acute hepatic failure and hepatic cytolysis emerged among the cardiac and hepatic disorders, respectively.

Keywords: safety; pharmacovigilance; SMA; gene therapy; Zolgensma



The therapeutic value of treatment for Metachromatic leukodystrophy: analysis of Health Technology Assessments of 3 European Countries

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Aim: Metachromatic leukodystrophy (MLD) is a rare inherited disorder caused by mutations in the Arylsulfatase A (ARSA) gene, leading to loss of motor and cognitive functions and early death. Available treatments are symptomatic, and none can reverse the fatal outcome.
Methods: This study aims to provide a comparative analysis of HTA recommendations issued by France, Germany and Italy following EMA approval of Libmeldy® (atidarsagen autotemcel) for MLD. A direct comparison was possible in terms of added therapeutic value (ATV) a measure included in all the available assessments.
Results: France deems that the clinical benefit is insufficient to justify the public funding cover in symptomatic children with the early juvenile (EJ) form, and substantial for asymptomatic children with late infantile (LI) or EJ forms; for these patients the Committee considers that Libmeldy® provides a moderate ATV (III). Germany recognizes the 'Hint of a major additional benefit' (I) for asymptomatic patients with LI or EJ forms and the 'Hint for a non-quantifiable additional benefit' (IV) for children with the EJ form who still can walk, before the onset of cognitive decline, since data does not allow a quantification. For Italy the ATV is 'Important' (II) considering the effect on clinically relevant outcomes and the substantial modification of the natural history of the disease, especially in the pre-symptomatic group.
Conclusions: Our results show a lack of agreement on the ATV of the drug approved for MLD. Despite the differences in terms of assessment, the access has been guaranteed but with different limitations.

Keywords: Metachromatic leukodystrophy; rare disease; added therapeutic value; health technology assessment

Exploiting routine laboratory test to identify patients with familial chylomicronemia syndrome (FCS) patients in a large Italian hospital

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Familial chylomicronemia syndrome (FCS) is a rare recessive inherited disorder of lipid metabolism characterized by elevated fasting triglycerides (TG), due to impaired chylomicron clearance. FCS has an estimated prevalence of 1:1,000,000 and frequently goes underdiagnosed. However, FCS can lead to life-threatening complications including acute pancreatitis. The timely and correct diagnosis has important prognostic implications since FCS is refractory to standard TG-lowering treatments. Searching for severe hypertriglyceridemia by using laboratory data may represent an effective strategy for early identification and referral to lipid clinic of suspected primary hypertriglyceridemic patients, and to grant access to novel effective therapies. We retrospectively retrieved laboratory tests performed at Niguarda Hospital in Milan (2016-2018). Among 143,615 valid TG values, 160 were above 885 mg/dL (0.111%), corresponding to 116 unique subjects. After excluding secondary causes of hypertriglyceridemia, 15 suspected FCS were referred to a lipid clinic for detailed clinical, biochemical, and genetic evaluation. Enrolled patients (7 females and 8 males) had a mean age of 43.9 ± 14.7 years. Nine had a history of acute pancreatitis and half reported chronic abdominal pain. All patients were already taking TG-lowering drugs. Eight patients were diagnosed as very likely FCS by applying FCS Score. Molecular analysis found at least one variant in FCS putative genes in 9 patients and 6 mutations were found in biallelic status. Following referral, 3 patients started treatment with volanesorsen, an orphan drug recently approved for the treatment of FCS. Systematic search through laboratory data extraction may increase identification of patient with suspected FCS, who may benefit from a referral to a lipid clinic for proper diagnosis and appropriate treatment.

Keywords: Severe hypertriglyceridemia, familial chylomicronemia syndrome, electronic health records, laboratory tests, volanesorsen

Inborn errors of immunity: lessons learned from an ever evolving paradigm

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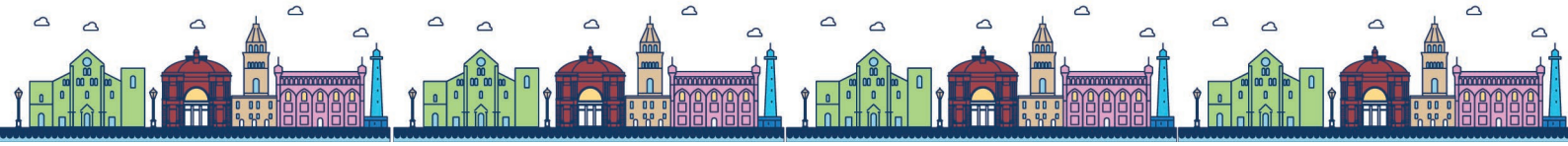
Il paradigma dei difetti innati dell' immunità ha subito una profonda revisione negli ultimi 20 anni. Da un lato, sono state identificate forme caratterizzate da una suscettibilità selettiva ad infezioni sostenute da un solo tipo di patogeno (suscettibilità mendeliana a infezioni da micobatteri) , dall' altra sono stati identificati difetti dell'immunita intrinseca ad altri tipi cellulari, oltre che a cellule del tessuto ematopoietico, encefaliti erpetiche secondarie a difetti nella produzione di interferoni di tipo I da parte di cellule del SNC. Ancora più importante il riconoscimento che molti quadri si associano ad una elevata incidenza di manifestazioni atopiche, autoimmunitarie, autoinfiammatorie e neoplastiche, espressione di un più ampio disordine dei meccanismi di immunoregolazione. Grazie al sequenziamento del genoma umano e con lo sviluppo di metodiche di studio del DNA sempre piu rapide e sofisticate , abbiamo assistito ad un aumento esponenziale delle forme di immunodeficit geneticamente determinate, attualmente oltre 500. Lo sviluppo di tecniche di analisi del numero di copie di DNA di TREC (T-cell receptor excision circle,) consente oggi di riconoscere alla nascita i neonati con linfocitopenia T grave. In campo terapeutico è ormai entrato nella fase di applicazione clinica l' utilizzo di molecole inibitorie ("small molecules") nonché di anticorpi monoclonali, che interferendo in modo specifico con i processi biochimici alla base del quadro patologico consentono di mettere in atto una vera medicina di precisione, in particolare nel trattamento delle patologie da immunodisregolazione e autoinfiammatorie. Allo stesso tempo, progressi nello sviluppo di vettori virali piu sicuri si sono associati ad un miglioramento degli interventi di terapia genica, che attualmente si avvale di nuove tecnologie come quella di gene editing (CRISPR) basate sull'inserimento nel genoma di una o piu copie aggiuntive di un gene terapeutico. Nell'ambito delle terapie cellulari, importanti progressi sono stati compiuti nella riprogrammazione di cellule somatiche in elementi staminali e nella conversione di questi ultimi in specifici tipi cellulari. Inoltre, è già divenuto possibile ricreare in laboratorio organoidi che mimano l'organizzazione cellulare e funzionale di diversi tessuti.

Keywords: (IEI) difetti innati dell' immunità , TREC . small molecules , CRISPR



The use of real world data for regulatory purposes in the rare diseases setting

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Aim: state background and specific objective of study
The use of Real World Data (RWD) for health purposes to complement traditional health care data sources is of particular interest for rare diseases, as the scarcity of individuals living with a particular condition as well as the heterogeneity of the disease are just some of the challenges making the traditional clinical research difficult and lengthy.
This work was dedicated to RWD generated for regulatory purposes in the rare diseases setting.
Methods: We invited scientists, economists and experts in social sciences to propose their research and opinions describing the use of RWD in the orphan medicines R&D and picture the possible economic, social and political impact.
Results: Seven manuscripts were successfully submitted and published in a FRONTIERS in Pharmacology Editorial (doi: 10.3389/fphar.2022.1089033): 2 original research articles, 2 reviews, 1 systematic review, 1 policy and practice review, 1 perspective article.
Conclusions: Quality assurance of sources, interoperability, ethical, legal, and social issues, appropriate governance for data processing and ownership are still the main issues to address.
To fully leverage the potential of RWD for regulatory decision-making, several actions have been proposed:
- Regulatory advice and guidance during the development of sources and tools
- Feasibility analysis and quality management with rigorous methods and validation
- Use common data models complying with the FAIR (findable, accessible, interoperable, and re- usable) principles
- Involvement of experts, including data curators and managers
- Engagement with patients, industry and registry holders
- Public-funded population-based registries.

Keywords: RWD, rare diseases, orphan medicines, medicine regulation, RWE

Sessione 2

Oral Communications

Fluoxetine as a precision-medicine approach for epileptic encephalopathies caused by kcnt1 variants

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Aim: KCNT1 gene encodes for K+ channels composed by six transmembrane segments (S1-S6) and both N- e C-termini at the intracellular level. The C-terminus contains two domains able to regulate K+ conductance (called RCK1 and RCK2) through Na+- and NAD-dependent mechanisms. Mutations in KCNT1 are associated with a wide spectrum of severe epilepsies. The aims of this study are:
• to evaluate the presence of mutations in patients affected by drug-resistant and severe epilepsy;
• to characterize functional and pharmacological properties of KCNT1 channels incorporating each variant;
• to test the ability of selected clinically-approved drugs to counteract variant-induced functional effects.
This work was dedicated to RWD generated for regulatory purposes in the rare diseases setting.
Methods: Genetic analysis were performed by next-generation sequencing approaches and the identified variants were inserted in plasmids for mammalian expression by quick-change PCR. Wildtype or mutant channels were expressed by transient transfection in CHO cells. Currents were measured using patch-clamp technique in the whole-cell configuration.
Results: Four de novo variants in KCNT1 were identified in five unrelated patients. Among them, one localized in the RCK2 domain (S937G) results novel, while the others, localized between the S6 segment and the RCK1 domain (R356W, R398Q and R428Q), have been previously reported. Electrophysiological experiments reveal that all variants prompt gain-of-function (GoF) effects compared to wild-type channels and that the antidepressant drug fluoxetine is able to counteract variant-induced functional effects. Notably, add-on therapy with fluoxetine in two patients results in the disappearance of seizures and cognitive/behavioural improvements.
Conclusions: Fluoxetine is a precision-medicine approach effective in patients with severe epilepsies caused by GoF variants in KCNT1 channels

Innovative therapeutic strategies for Krabbe disease: nanoparticle-mediated enzyme replacement and autophagy induction

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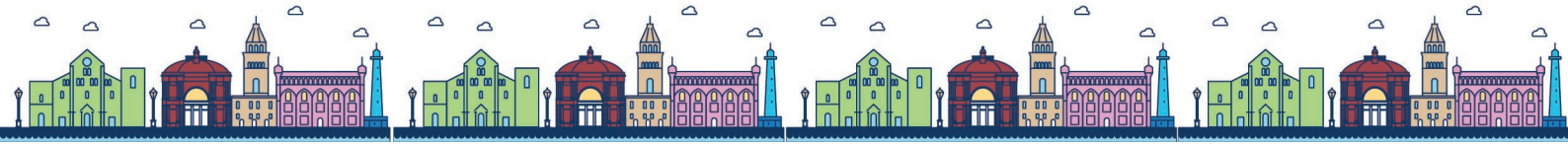
Krabbe disease (KD) is a rare leukodystrophy resulting from a deficiency in the lysosomal enzyme galactosyl-ceramidase (GALC), leading to the accumulation of the cytotoxic sphingolipid psychosine (PSY) in the nervous system. Currently, no resolutive cure exists for KD, necessitating symptomatic and supportive treatment.
Enzyme replacement therapy (ERT) holds promise for KD treatment; however, the blood-brain barrier impedes GALC translocation into the central nervous system (CNS). To address this challenge, we encapsulated GALC as cross-linked enzyme aggregates in poly(lactic-co-glycolic acid) nanoparticles (NPs) functionalized with CNS targeting ligands (g7, Angiopep-2, and Tf2 peptide). Our NPs, primarily taken up in lysosomes, restored enzymatic activity in KD cellular models and the Twitcher (TWI) mouse, a spontaneous KD mouse model.
Despite correcting GALC deficiency, recent literature suggests that additional supportive therapies targeting secondary disease aspects are crucial for an optimal therapeutic outcome. The growing consensus on LSDs being linked to autophagy disorders is reflected in our research, which unveils dysregulations in autophagy pathways contributing to KD pathogenesis. Notably, we observed p62-tagged protein aggregates in KD mouse brains and elevated p62 levels in the KD sciatic nerve.
Building on these findings, we explored the autophagy inducer Rapamycin (RAPA) in-vitro and in-vivo to eliminate unwanted cellular products from KD cells. RAPA demonstrated the partial reinstatement of the wild-type phenotype in KD cells and TWI mice, suggesting its potential inclusion in KD clinical trials. Finally, we discuss these results in the context of combining autophagy modulation with GALC-deficiency correcting therapy for a comprehensive phenotypic rescue in KD.

Keywords: Krabbe disease, nanoparticles, enzyme replacement therapy, autophagy, Rapamycin



Fenamates as ClC-1 choride channels modulators for a potential repurposing in myotonia congenita

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Aim: Myotonia congenita (MC) is a rare genetic disease caused by loss-of-function mutations of the skeletal muscle chloride channel, hClC-1. To date there are no drugs able to target ClC-1 mutations in MC patients. Previously, we demonstrated that the NSAID niflumic acid is a reversible ClC-1 inhibitor that acts as pharmacological chaperone able to restore chloride currents in traffickingdefective ClC-1 mutants. In this study, we investigated the effect of fenamates mefenamic (MFA), meclofenamic (MCFA), tolfenamic (TFA), flufenamic (FFA) acids on ClC-1 channel activity.This work was dedicated to RWD generated for regulatory purposes in the rare diseases setting.
Methods: HEK293 cells were transfected with the pRc/CMV plasmids containing the wild-type hClC-1 or the defective-trafficking isoform, hClC-1p.A531V. The acute effect of fenamates was evaluated by patch clamp technique in whole-cell configuration. The chaperone effect was tested on the hClC-1p.A531V mutant after incubation with FFA for 24 hours followed by patch clamp analysis.
Results: Dose-response relationships of fenamates (10, 30, 100 and 300 μM) demonstrated that TFA and FFA were the most potent fully reversible inhibitors of hClC-1 able to reduce the steadystate chloride currents at -90 mV. Experiments with the protein kinase C (PKC) inhibitor, chelerythrine, showed that the inhibitory effects of fenamates did not involve the Ca-dependent PKC pathway. Experiments of incubation of HEK293 expressing the hClC-1 p.A531V variant with FFA are currently performed to verify its possible chaperone effect able to rescue chloride currents.
Conclusions: Fenamates may act as pharmacological chaperones of hClC-1 with a potential repurposing for the treatment of trafficking-defective MC patients.
Supported by University of Bari “Horizon Europe Seeds”-project Medineuropa.

Keywords: myotonia congenita, chloride channel, fenamates, drug repurposing

CIC-39Na: a beacon of hope in the fight against Tubular Aggregate Myopathies

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Store-Operated Ca2+-Entry (SOCE) is a cellular mechanism that governs the replenishment of intracellular stores of Ca2+ upon depletion caused by the opening of intracellular Ca2+- channels. Gain-of-function mutations of the two key proteins of SOCE, STIM1 and ORAI1, are associated with several ultra-rare diseases clustered as tubular aggregate myopathies (TAM), with no valid treatment available at present.
Our group has previously demonstrated that a mouse model bearing the STIM1 p.I115F mutation recapitulates the main features of the disorder: thrombocytopenia and muscle weakness.
We evaluated the effect of CIC-39Na, a SOCE negative modulator, both in in vivo and ex vivo models of tubular aggregate myopathies.
KI-STIM1I115F mouse were treated with CIC-39Na (60 mg/Kg/daily 56days) in constant infusion, using micro-pumps intraperitoneally, to test the ability of the drug to reverse the symptoms of tubular aggregate myopathy.
CIC-39Na was found to restore platelet number, platelet clearance, counteract abnormal bleeding and re-establish functional motor performance in KI-STIM1I115F mice. Moreover, the compound also restored basal Ca2+ levels to normal levels both in platelets and myotubes.
Concerning the disruptions in Ca2+ homeostasis within KI-STIM1I115F myotubes, we have observed a significant impairment of Ca2+-signalling and a concomitant activation of ERstress and mitochondrial dysfunction, leading to the formation of tubular aggregates in muscles, which are hallmarks of the pathology.
Upon treatment with CIC-39Na, for 56 days, we observed a physiological restoration of Ca2+ transport, structural and functional improvement of compromised organelles in the KISTIM1I115F mouse model.
This finding paves the way to a pharmacological treatment strategy for TAM patients.

Keywords: Store-Operated Ca2+-Entry; Tubular Aggregate Myopathies; CIC-39Na; STIM1 p.I115F mutation; Ca2+ homeostasis

Reintroducing synthetic SIL1 protein to treat Marinesco-Sjogren Syndrome

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Aim: Marinesco-Sjogren Syndrome (MSS) is a rare autosomal recessive disease caused by a mutation in the SIL1 gene, encoding a nucleode exchange factor for the ER chaperone BiP (Antonen et al. 2005). Individuals with MSS display cerebellar ataxia, early-onset congenital cataracts, and progressive myopathy amongst other symptoms. A spontaneous recessive mutation in the mouse Sil1 gene results in cerebellar ataxia and muscular atrophy, with the woozy (wz) mouse representing a valid preclinical model to investigate MSS and explore pharmacotherapeutic approaches (Zhao et al. 2005). We here analysed the administration of an engineered human SIL1 protein in woozy mice, investigating whether the treatment can act on the involved tissues and slow down the disease course.
Methods: Engineered human SIL1 protein was isolated from E. coli and purified for affinity. The purified protein was intraperitoneally injected in 8-week-old woozy mice for 3 consecuve days. Aer 3 hours since the last injecon, mice were sacrificed through cervical dislocation, and stisues immediately frozen and stocked at -80°C for molecular analysis. Western blot was used to determine the presence of SIL1.
Results: Western blot analysis revealed the presence of SIL1 in quadriceps of treated mice, confirming the ability of the purified protein to reach the peripheral tissues.
Conclusions: The presence of SIL1 in the mice quadriceps confirmed the good nature of the engineered protein, and its ability to reach tissues affected in the syndrome. Further studies are needed to deepen the pharmacokinecs of the engineered protein and whether this treatment may act by regressing the affected phenotype or slowing down the course of MSS.

Keywords: Marinesco-Sjogren Syndrome; Woozy mice; SIL1

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Heme oxygenase 1 a new possible target therapy against inflammation in squamous cell carcinoma in Epidermolysis Bullosa patients: A pre-clinical pilot study

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Aim: Recessive dystrophic epidermolysis bullosa is a highly disabling genodermatosis characterized by skin and mucosal fragility and blistering. Cutaneous squamous cell carcinoma (cSCC) is one of the most devastating complications, having a high morbidity and mortality rate. Patients with recessive dystrophic epidermolysis bullosa were reported to have up to a 70-fold higher risk of developing cSCC than unaffected individuals. Immune cells play a role in cancer evolution. Heme oxygenase 1(0H-1) appears to have a great anti-inflammatory power, therefore the aim of our study is to evaluate this molecule to search for new treatment targets in these patients.
Methods: A retrospective study was made of 24 consecutive cases: 12 cases of cSCC in patients affected by severe RDEB (Group 1) were compared with 12 consecutive cases of primary cSCC in non-RDEB patients (Group 2). For each one was performed Immunohistochemistry evaluation using antibodies against the following markers: FOXP-3 (markers of lymphocytes T reg), OH-1. The HO-1 and FoxP3 values, as well as the age of onset, were parametric and analysed by Student's t-test. Significance was set at $P < 0.05$.
Results: A significantly reduced immunohistochemical expression of HO-1 was evident in the tumoral microenvironment of cSCC-RDEB (2.58 ± 0.26 signal/mm2 in Group 1) as compared to primary cSCC (3.25 ± 0.13 in Group 2), p = 0,031. The immunohistochemical expression of Foxp3 is higher in group 2 (3.54 ± 1.65 cells/mm2 than group 1 (1.76 ± 0.51), P value 0,02.
Conclusions: OH-1 and Fox P3 have a direct correlation, as one increases, the other increases. This may be the basis of the aggressiveness of the skin tumor in Epidermolysis compared to the primary tumor.

Keywords: Cutaneous squamous cell carcinoma, Epidermolysis Bullosa, OH-1, T-reg,Inflammation

Sessione 3

Oral Communications

Exploiting pharmacological predictive fruit fly models for the identification of promising molecules in future Hereditary Spastic Paraplegia therapy

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Aim: SPG15 and SPG11 (Hereditary Spastic Paraplegia, HSP) are two forms of a rare and incurable neurodegenerative disorder marked by progressive degeneration of corticospinal tracts (1). Patient-derived cells and Drosophila model showed lysosome enlargement and autophagic lysosomal reformation (ALR) defects. Our previous pharmacological screen identified SMER28, the most effective molecule able to reactivate ALR in SPG15 (2). Here we compared Tideglusib, Naringenin, and Miglustat, already used in clinical trials, with SMER28 as potential modulators of lysosomal regeneration (3, 4) and to assess the pharmacological predictiveness of Drosophila models for HSP therapy (5).
Methods: Drosophila tubulin-Gal4 and RNAi-Dspastizin lines were used for loss-of-function studies. Eclosion and climbing assays revealed developmental and locomotor differences. Confocal microscopy assessed autophagy and lysosomal defects. SMER28, Tideglusib, Miglustat and Naringenin (complexed with hydroxypropyl--cyclodextrin) were added to the food. One-way ANOVA with Dunnett's or Tukey's multiple comparisons were conducted (P-values <0.05 as significant).
Results: Miglustat yielded no effects across all parameters, as already observed in SPG11 patients (NCT04768166). Conversely, Tideglusib and Naringenin significantly enhanced autophagosome maturation ($p=0.0003$) and autolysosome degradation ($p<0.0001$). However only Naringenin successfully rescued lysosome defects by reducing their size ($p<0.0001$) and number ($p<0.0001$), similarly to SMER28. Indeed, Naringenin reactivated the ALR by extending lysosomal tubulations ($p<0.0001$) and fully restored locomotor function in adult flies.
Conclusions: We established the predictiveness of our SPG15/SPG11 Drosophila models for in vivo effects of bioactive compounds, showcasing their utility for future screenings. Notably, Naringenin and SMER28 emerged as promising candidates for treating HSP forms with ALR defects.

Keywords: Hereditary Spastic Paraplegia, Drosophila, pharmacology, Naringenin, SMER28

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Phosphodiesterases S-sulfhydration contributes to human Malignant Hyperthermia

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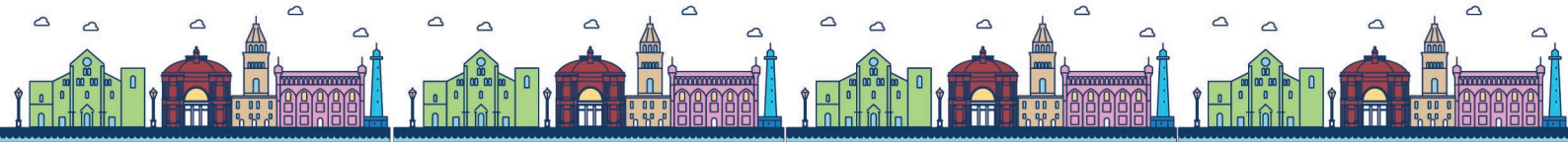
Aim: Human Malignant Hyperthermia (MH) is a rare pharmacogenetic disorder of skeletal muscle (SKM) triggered by volatile anaesthetics and depolarizing muscle relaxants. Characterized by prolonged opening of RyR1 channels, leads to uncontrolled release of calcium, sustained muscle rigidity, hyperthermia, and rhabdomyolysis. We have previously shown increased levels of H2S within the SKM of MH susceptible patients. Inhibition of phosphodiesterases (PDEs) activity is one of H2S recognized mechanisms of action, resulting in a slower rate of cyclic nucleotide degradation, deeply influencing downstream signal transduction. To better define the molecular basis of H2Srelated MH hypercontractility, here we investigated the role of cAMP-cGMP/PDEs axis in MH.
Methods: The study was performed on 28 MH susceptible (MHS) and 69 MH negative (MHN) biopsies. Western blot of PDE4 and PDE5 proteins, cAMP/cGMP determination and persulfidation of PDEs were evaluated. In vitro contracture test (IVCT) was also performed.
Results: MHN and MHS biopsies displayed a similar expression of PDE4 and PDE5, however the cAMP and cGMP resulted strongly increase in MHS (cAMP: 2.27±0.45 vs. 1.27±0.12; cGMP: 0.09±0.01 vs. 0.005±0.0005 pmol/mg of tissue *p<0.05). The IVCT revealed an anomalous contractility of MHS biopsies following exposure to rolipram (336 ± 110 vs. 17.5 ±17.5 increase in tension; * p<0.05), and sildenafil (207.3 ± 51 vs. 4.6 ±2.3 increase in tension; * p<0.05). Persulfidation of PDEs was significantly higher in MHS compared to MHN.
Conclusions: In MHS biopsies, inhibition of PDEs operated by increased levels of H2S, by raising both cAMP and cGMP, leads to the sustained muscle contraction.



Is the D2-mdx mouse a better preclinical model for Duchenne muscular dystrophy? Insights from growth hormone secretagogues studies

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Growth hormone secretagogues (GHSs) are emerging as an attractive pharmacotherapeutic opportunity for Duchenne muscular dystrophy (DMD) – a rare, devastating genetic muscle disorder [1] – in virtue of their broad spectrum of activity and reported benefits in other muscle-wasting conditions[2]. In our recent multidisciplinary study[3], a selected GHS – JMV2894 – was proven to be beneficial on muscle function, inflammation and fibrosis in classic mdx mice. In silico, we also predicted its interaction with metalloproteinases ADAMTS-5 and MMP-9, overactivated in DMD. This pushed us to test JMV2894 in the emerging D2.B10-Dmdmdx/J (D2-mdx) mouse model, featuring a severe profibrotic genetic background.
4-week-old D2-mdx mice were treated with JMV2894 at two doses (640 and 1280 µg/kg/d, s.c.) for 6 weeks. In vivo, JMV2894 partially improved hind limb plantar flexor torque (recovery scores, R.S. up to 20%), ultrasound volume (R.S. up to 118%), and fibrosis-associated echodensity in gastrocnemius (GC) muscle (R.S. 25% and 34%). Diaphragm (DIA) echodensity was also partly reduced by both doses (R.S. 20% and 15%), while DIA amplitude was ameliorated only by the lower dose (R.S. 62%). Ex vivo, however, JMV2894 neither improved DIA muscle force, nor DIA and GC muscle histopathology (i.e. % of unhealthy tissue, fibrosis, calcifications). Importantly, pharmacokinetic analyses confirmed a limited drug exposure in mice muscle tissue. Further molecular biology experiments and in silico docking simulations are in progress to better define the effects/targets of JMV2894 in DMD. Our overall findings confirm the interest in newly disclosed actions of GHSs in dystrophic settings, possibly influenced by genotype, and support the need for novel formulations to optimize their bioavailability [AFM-Téléthon Grant #22199].

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A novel integrated pharmacological/antioxidant approach for Duchenne Muscular Dystrophy

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Aim: Inflammation and oxidative stress are two hallmarks of Duchenne Muscular Dystrophy (DMD). Here, we tested the possibility to counteract these processes by administering a selective serotonin reuptake inhibitor known to have anti-inflammatory properties and antioxidants to dystrophic mice, alone or in combination.
Methods: The experiments were performed in mdx mice C57BL/10ScSnDmdmdx/J. One month old mice (n = 8-10 mice) were treated for 3 months with the following drugs or antioxidants: Fluoxetine (5mg/kg/day), Sertraline (5mg/kg/day) and N-Acetylcysteine (NAC) (1,5g/kg/day) administered in the drinking water; Plumbagin (30mg/kg/day) and Quercetin (50mg/kg/day) administered in the diet. The two most efficient drugs of each group were then combined, so that they might work in a synergistic manner.
Results: The drugs improved the phenotype of mdx mice in terms of muscular performance whole body tension = Top 10 FTP: vehicle =7.99 ± 0.38; Fluoxetine = 11.06 ± 1.13**; Sertraline = 9.87 ± 0.76; NAC = 10.88 ± 0.60*; Quercetin: 10.72 ±0.47*; Plumbagin = 12.02 ± 0.56***; $^{*}p<0.05$, $^{**}p<0.001$, $^{***}p<0.0001$ vs vehicle) and treadmill and alleviated oxidative stress and inflammation. The two most efficient drugs (Fluoxetine, 1mg/kg/day and Plumbagin, 30mg/kg/day) combined significantly improved muscular performance in vivo by reducing inflammation and oxidative stress.
Conclusions: Our data demonstrate the efficacy of a combination strategy targeting inflammation and oxidative stress. Our approach is safe and time- and cost-saving, as it is based on antioxidants, available as dietary supplements and with few side effects, and SSRIs, already on the market and available for drug repurposing.

Is the D2-mdx mouse a better preclinical model for Duchenne muscular dystrophy? Insights from growth Drug repurposing strategy to identify novel activators of lysosomal Ca2+ channels via autophagy regulation in a preclinical model of amyotrophic lateral sclerosis

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Lysosomal Ca2+ dysfunction has recently emerged as an important pathological change leading to autophagy impairment and neuronal loss in amyotrophic lateral sclerosis (ALS). A key role in the regulation of autophagy is played by the lysosomal Ca2+ channels TRPML1 (Transient Receptor Potential Mucolipin1) and TPC2 (Two-Pore Channel2) [1-2]. However, their contribution in ALS pathogenesis still remains unknown.
Aim: In this study, pharmacological repurposing was used to identify new regulators of autophagy able to pharmacologically stimulate TRPML1 and TPC2 channels in a neuronal model of ALS/Parkinson-dementia complex, reproduced by exposing motor neurons to the neurotoxin beta-methylamino-L-alanine (L-BMAA).
Methods: Protein expression levels were measured by Western blotting experiments. Intracellular Ca2+ concentration was detected by Fura-2- video-imaging. Neuronal viability was measured by MTT assay and cell death markers expression. Statistical analysis was performed using one-way analysis of variance followed by Newman-Keuls test.
Results: In L-BMAA-treated motor neurons both TRPML1 and TPC2 expression levels were downregulated. The early stimulation of TRPML1 by its synthetic agonist ML-SA1, which activates the channel in a dose-dependent manner, rescued cells from L-BMAA toxicity by counteracting endoplasmic reticulum stress and autophagy impairment, as demonstrated by the reduced accumulation of the autophagyrelated proteins p62/SQSTM1 and LC3-II ($p<0.05$). At the same time, the early pharmacological activation of TPC2, obtained by using some already-approved antidepressants and anti-psychotic drugs, was able to increase neuronal viability and restore autophagy in ALS motor neurons.
Conclusions: Collectively, we identified new lysosomal channel modulators rescuing motor neurons from L-BMAA-induced toxicity through the restoration of the autophagic flux.

Keywords: amyotrophic lateral sclerosis; lysosomal calcium channels; L-BMAA; autophagy; drug repurposing

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Testing reliability of in vitro neuronal cultures in pharmacological treatment of a genetic form of migraine

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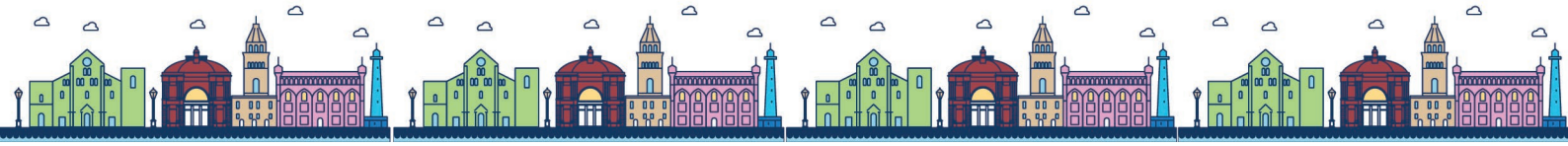
Aim: Familial hemiplegic migraine type 3 (FHM3) is a genetic form of migraine with aura caused by gain-of-function mutations in the SCN1A gene encoding the Nav1.1 channel. Specific pharmacological treatment has not been developed for this disease yet. In this work we evaluated the effect of the sodium current blocker GS967, already considered as antiarrhythmic and antiepileptic drug.
Methods: We employed two KI mouse models, each one bearing an FHM3-related mutation, Scn1aL1649Q or Scn1aL1670W, respectively, in the C56BL/6N background. Brain cortices were dissected from E17embryos and dissociated neurons were cultured in defined medium for up to 25 days. Cultures were studied by patch clamp recording on single neurons, while population excitability was monitored using microelectrode arrays (MEAs). RT-qPCR was used to detect possible dysregulation caused by FHM3 disease.
Results: Evaluation of kinetic parameters of Na+ currents by patch clamp pointed out sizeable differences between genotypes confirming an overall gain-of function-effect of FHM3 Nav1.1 mutants. Analysis of burst properties of spontaneous activity recorded with MEA indicated that cortical network from homo and heterozygous FHM3 mice is less sensitive to GS967 than wild type.
Conclusions: The results suggest that the in vitro model can recapitulate several features observed in FHM3. It is worth emphasizing that, despite its limitation, the use of dissociated cultures may be a valid alternative method to reduce the number of animals used in experimental studies.

Keywords: Nav1.1 channel; Na current; Na current blocker; MEA; Patch clamp

Sessione 1

Poster



CHARACTERIZATION OF THE EFFECTS OF JMV2894, A SYNTHETIC GROWTH HORMONE SECRETAGOGUE, IN A CELLULAR MODEL OF DUCHENNE MUSCULAR DYSTROPHY

Elena Bresciani[1], Laura Rizzi[1], Ramona Meanti[1], Ornella Cappellari[2], Paola Mantuano[2], Elena Conte[2], Francesca Sanarica[2], Brigida Boccanegra[2], Alessandro Giovanni Cerchiara[2], Antonella Liantonio[2], Sonia Cantel[3], Séverine Denoyelle[3], Jean-Alain Fehrentz[3], Vittorio Locatelli[1], Annamaria De Luca[2] and Antonio Torsello[1]

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Aim: Chronic treatment with JMV2894 has demonstrated beneficial effects in preserving muscle function in the mdx mouse, a model of DMD. JMV2894 decreased inflammatory status and fibrotic tissue deposition, and induced positive modifications in muscle metabolism. However, JMV2894 mechanism of action on muscle cells is largely unknown.
Methods: We investigated JMV2894 effects on muscle cell precursors involved in the DMD muscle regeneration process. H2K-SF1 are muscle cells characterized by the absence of dystrophin. Short-term, 48 and 96 hours (h), or long-term incubation (9 days), with 1 µM JMV2894 alone or in combination with 0.2 µM methyl-prednisolone (m-pdn), commonly used in DMD patients, were performed to assess the expression levels of specific myogenic markers, the cell fusion index and the mRNA levels of RYR1.
Results: Short-term incubations with JMV2894 significantly altered Myf5 and myogenin mRNA expression and enhanced the cell fusion index, compared to control; long-term tretaments significantly increased slow myosin mRNA levels. Similar effects were induced by m-pdn, althought m-pdn stimulated slow myosin mRNA expression more effectively than JMV2894. The long-term co- administration of m-pdn with JMV2894 significantly increased the slow myosin mRNA levels compared to the JMV2894 and m-pdn groups. Interestingly, 9-day treatment with JMV2894 as well as m-pdn stimulated RYR1 mRNA levels, a receptor involved in Ca2+ homeostasis regulation.
Conclusions: Our results suggest that in vivo JMV2894 beneficial effects may likely depend on its capability to impact in the muscle cell regeneration efficiency and differentiation process, supporting that JMV2894 could be developed as a possible modulator of the pathology.

Keywords: Duchenne muscular dystrophy (DMD); Growth Hormone Secretagogues (GHSs); dystrophic H2K-SF1 cells; myogenesis; Ca2+ homeostasis

SRT2104, a new SIRT1 activator, is an effective metabolic enhancer that promotes muscle recovery in DMD

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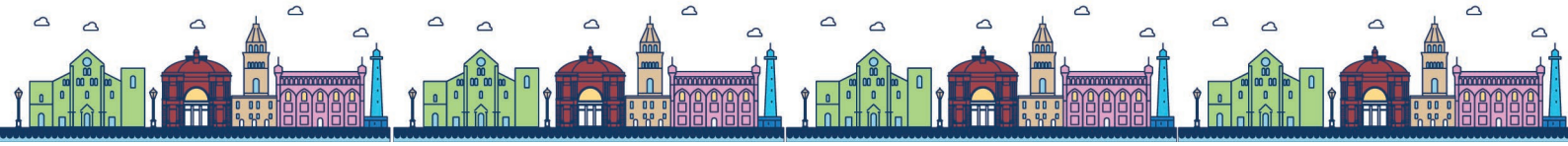
Duchenne Muscular Dystrophy (DMD) is a degenerative disorder caused by mutations in the DMD gene encoding dystrophin. Despite remarkable progress has been made in genetic approaches to restore dystrophin or its function, targeting secondary pathological mechanisms remains an important issue. Sirtuin 1 (SIRT1) is a NAD+-dependent class III histone deacetylase that controls several key cellular processes. Different attempts have been done to increase SIRT1 expression or activation in mdx mice, and to date, the most promising one seems to be resveratrol. However, more potent and selective activators exist, and among these SRT2104 is the most promising one. We performed molecular dynamics simulations on SIRT1 available structures, proving that a conformational selection mechanism was responsible for the activity of SRT2104, i.e., the open inactive conformation of SIRT1 explored a more compact intermediate state that is stabilized by the drug, then converted into its active form.
Even more potent and specific than resveratrol, it has never been tested in DMD therefore we challenged SRT2104 effects in mdx mice.
We orally administered SRT2104 for 12 weeks in 8-week-old mdx/PhAM mice [generated crossing C57BL/10ScSnDmdmdx/J with PhAM mice (C57BL6/129SV)] obtaining promising results (n5 each group, analyzed by unpaired two-tailed t-test). SRT2104 promoted muscle OxPhos capacity and improved muscle performances and phenotype in mdx mice. The proteomic profile of SRT2104 treated muscle revealed the specific enrichment of fatty acid oxidation and mechanotransduction signals, both contributing to muscle recovery.
In conclusion, SRT2104 can be considered a good metabolic enhancer and an interesting treatment for DMD.

Keywords: metabolism, sirtuin 1, mitochondria



SRT2104 effects on human muscle cell model of Duchenne Muscular Dystrophy, as a SIRT1 highly selective activator

De Santis C[1], Quarta R[1], Cristiano E[1], Cerchiara AG[1], Zecchini S[3], Barile SN[2], Lasorsa FM[2], Mouly V[4], De Palma C[3], Clementi E[3], De Luca A[1], Giovarelli M[3], Cappellari O[1]

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Aim: In Duchenne Muscular Dystrophy (DMD) lack of dystrophin leads to sarcolemma weakening, regenerative failure, inflammation and mitochondrial dysfunctions. SRT2104, a highly selective activator of SIRT1 (NAD+ dependent deacetylase Sirtuin 1), raised interest for its potential to foster muscular functionality by reducing inflammation and boosting mitochondrial metabolism. In preliminary study in mdx mouse model of DMD, SIRT1 overexpression tends to improve exercise performances, heart pathology and fibrosis. The aim of this project is to determine SRT2104 cytotoxic profile and to verify its mechanism of action, in terms of mitochondrial biogenesis and function, in a human DMD cell model, to reinforce the translatability of these data.
Methods: Two dystrophic patient-derived immortalized muscle cell lines have been used: HDMD1 (with exon 51 mutation) and HDMD2 (with a stop-codon mutation). SRT2104 (3, 5 and 10 μM) has been tested at different time point of differentiation: 24/48/96 hours and 17 days. Cell viability has been evaluated with CCK-8 solution. Mitochondrial respiration has been assessed with measurements of oxygen consumption rates (OCR) through Seahorse platform (Agilent). High-content immunofluorescence studies with Operetta are ongoing.
Results: Preliminary data show no toxicity at the tested concentrations. Test of long-term exposure and of higher concentrations have been hindered by compound solubility issues and experiments for obtaining more suitable formulation are ongoing. Early OCR data analysis support an impaired mitochondrial function in dystrophic cells.
Conclusions: Given its metabolic and anti-inflammatory capability, the characterization of SRT2104-mediated Sirt1 activation effects on human cell model of DMD could be pivotal to further understand its role in DMD settings (Grant PRIN 2020 + Trampoline 24077).

Keywords: Duchenne Muscular Dystrophy, SIRT1, Cytotoxicity, Mitochondrial Respiration

LKB1 as a novel diagnostic and therapeutic player in Duchenne Muscular Dystrophy: new insights from cellular and murine dystrophic models

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The liver kinase B1 (LKB1), is a highly conserved protein with emerging roles in skeletal muscle homeostasis. LKB1 activation occurs with the formation of a heterotrimeric complex with two accessory proteins, the pseudokinase STE20-related adaptor (STRAD) and the scaffolding mouse protein 25 (MO25). When activated, LKB1 shuttles from nucleus to cytoplasm and phosphorylates the adenosine monophosphate-activated protein kinase, a ubiquitous master regulator of mitochondrial biogenesis and metabolic processes, dysregulated in Duchenne muscular dystrophy. In this frame, we questioned whether LKB1 could be an important upstream player in the aberrant mechanic-metabolic coupling characterizing dystrophic myofibers.
Hence, to determine if the impairment of LKB1 expression happens in early and/or in chronic pathology phase, we analysed gene expression levels of LKB1 and its accessory proteins in gastrocnemius muscle (GC), of mdx and D2mdx mice, a new murine model with a more severe dystrophic phenotype, throughout lifespan (4,8,28,52 weeks of age). Furthermore, we performed immunofluorescence (IF) analyses in mdx GC muscles and in dystrophic SF1 cells to identify the exact localization of LKB1.
Preliminary results showed that LKB1-STRADA-MO25 genes were severely downregulated in both dystrophic mouse models, at all ages, with an overall reduction of more than 50% vs. background- related WT mice. IF analysis in SF1 cells as well as in GC of adult mdx mice (6-months-of-age) displayed a nuclear localization for LKB1 whilst the protein was detected at sarcolemmal level in WT controls.
Our study provides new insights into the role of LKB1 as a possible player in the defective metabolic signalling observed in dystrophic conditions. Further qRT-PCR analyses in DIA and heart as in patients-derived cells are currently ongoing. (supported by: PRIN 2020)

Erucin, a natural hydrogen sulfide (H2S) donor, improves DMD-induced SKM dysfunction

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Aim: Duchenne muscular dystrophy (DMD) is a rare X chromosome-linked disease that leads to progressive degeneration of skeletal muscle (SKM) tissues. An impairment of hydrogen sulfide (H2S) pathway in the SKM of dystrophic mice has been recently demonstrated. This study aims to evaluate the beneficial effect of Erucin, a natural slow H2S donor, in a murine model of DMD.
Methods: *In vivo* studies were performed on mdx dystrophic mice, and their littermates (WT). Mice (n=10) were treated with Erucin (3mg/kg) for 2 weeks and locomotory activity was performed. At 7 weeks of age, mice were sacrificed, and quadriceps were collected for evaluation of oxidative stress markers (H2O2, and GSH/GSSG ratio). Statistical analysis was assessed using one-way ANOVA.
Results: *mdx* mice displayed a significantly reduced SKM performance compared to WT (**p<0.01). Increased oxidative stress was detected in the quadriceps of *mdx* mice compared to WT (H2O2: 0.32 ± 0.06 vs. 0.11 ± 0.04 μM/mg of protein; **p<0.01; GSH/GSSG: 2.0 ± 0.25 vs. 3.06 ± 0.43; *p<0.05). Erucin treatment fully recovered the impaired SKM performance observed in mdx mice in both locomotory tests (rotarod: 293 ± 4.7 vs 219 ± 25 sec; weight test: 14.3 ± 0.26 vs. 12 ± 0.49 sec; °°p<0.01). This effect was associated to a reduction of oxidative stress (H2O2: 0.10 ± 0.03 vs 0.32 ± 0.06 μM/mg of protein; °°p<0.01; GSH/GSSG: 3.1 ± 0.17 vs. 2.0 ± 0.25 °p<0.05).
Conclusions: our results suggest that Erucin exerts a protective effect in DMD, improving SKM performance by reducing oxidative stress via H2S release

Dasatinib as a booster of mutation-specific molecular therapies in Duchenne muscular dystrophy: first assessment of safety in murine and human cell models

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Background: Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene, leading to dystrophin protein absence in myofibers; in DMD Src Tyrosine Kinase(TK) is over-activated and promotes the degradation of -dystroglycan(-DG). Previous preclinical studies showed that dasatinib, a competitive inhibitor of Src-TK, increased non-phopshorylated -DG protein levels in mdx mice1,2 muscles, without improving diseases-related indices. Accordingly, it could be potentially useful to combine dasatinib with dystrophin-restoring therapeutic approaches, such as ataluren, a small molecule that acts with a stop-codon read-through mechanism. Our aim is to first assess any potential cytotoxicity of dasatinib and ataluren on myogenic cells.
Methods: We used murine (H2K-2B4 and H2K-SF1) and human (HDMD1 and HDMD2) myoblasts and myotubes. Cells were treated individually with dasatinib and ataluren at increasing concentrations (0.1μM-250μM), in proliferation and differentiation conditions and then, in association on murine myoblasts. Cell viability was assessed with CCk-8 solution.
Results: Preliminary results confirm, on murine cells, a cytotoxic effect of dasatinib on myoblasts at high concentrations while, disclosing a remarkable cytotoxicity at lower concentrations on myotubes after 4 days of differentiation. However, on human cells, the drug appears to be toxic already at low concentrations in both proliferation (after 5 days of drug exposure) and differentiation conditions (after 11 days of differentiation) irrespective to the underlying mutation.
Conclusions: Further experiments will define the concentration range of dasatinib that could be used safely in association with ataluren and qPCR/immunofluorescence experiments will allow to characterize the effects of the drugs association on the myogenic program, dystrophin expression and DGC restoration. (PRIN 2020, ELYA32_002).

Keywords: Duchenne muscular dystrophy, dasatinib, dystrophin restoration, cytotoxicity

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Class I selective HDAC inhibitors as new potential treatment for DMD: *in vivo* and *ex vivo* readouts in D2-mdx mouse model

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Background and aim: Duchenne Muscular Dystrophy (DMD) is a rare, incurable and degenerative disease caused by the absence of structural protein dystrophin. This leads to a complex pathogenetic cascade that ends in gradual loss of muscle function, oxidative stress, collagen deposition and aberrant epigenetic control. In fact, in dystrophic myofibers, the overexpression and overactivation of epigenetic silencers Histone Deacetylases (HDACs) deregulates the transcription of genes involved in pathways that are pivotal to preserve muscle function and homeostasis. A New Drug Application for the US Food and Drug Administration approval in DMD has been recently completed for the pan histone deacetylase inhibitor (HDACi) givinostat, underlying the interest towards HDAC inhibitors in DMD. In this frame, the aim of this new study is to evaluate the potential benefit and antifibrotic effects of class I selective HDACi in D2-*mdx* mouse, an emergent murine model for the study of DMD with a severe pro-fibrotic phenotype.
Methods: We administered intraperitoneally to 5-week-old D2-*mdx* mice, for 8 weeks, 5 times a week, rodin (4mg/kg), an inhibitor of HDAC1 and 2, and vorinostat (5mg/kg), a pan HDACi, as positive control. In addition, groups of age-matched D2-WT and D2-*mdx* mice were used as control and treated with 5% DMSO (vehicle). The outcomes were evaluated by using a multidisciplinary approach, including *in vivo* indices of muscle function and ex vivo functional, biochemical and histological readouts.
Results: None of the treatments improved forelimb force, whereas treadmill results show a stabilization of muscle performance only in D2-*mdx* treated with vorinostat, underlined by loss of significant difference vs D2-WT, both in distance run and in time to exhaustion (RS:27,3%; RS:25%).
Conclusions: Histological and biochemical analysis are currently ongoing to evaluate treatments effects on collagen deposition and genes expression.

Gut microbiota-endocannabinoid interplay in rare skeletal muscle myopathies: an intricate relationship that must be taken into consideration

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Background and aim: Little is known about the potential implications of gut microbiota and the endocannabinoid system (ECS) in rare skeletal muscle disorders. We analysed the composition of faecal microbiota along with circulating levels of short-chain fatty acids (SCFAs) and activity of the endocannabinoid system (ECS) in the mdx mouse model of Duchenne muscular dystrophy (DMD), which is the most frequent and severe form of hereditary myopathy for which there is no cure. Additionally, we explored the role of the ECS in preclinical models of statin-induced myopathy, a rare condition that occurs in about 1 in 10,000 patients worldwide who use this class of drugs to lower cholesterol and risk of cardiovascular disease.
Methods: In our studies, we used a multi-disciplinary approach including advanced techniques. The main ones included 16S rRNA gene sequencing analysis for faecal bacterial taxonomic analysis and liquid Chromatography-Mass Spectrometry (LC-MS) analysis to measure endocannabinoid levels in murine C2C12 cells, primary human myoblasts, blood, and skeletal muscles of mice. We used bioinformatics tools complemented by cellular fluorescence-activated cell sorting (FACS) molecular biology techniques (miRNA screening, qPCR, and western blot analyses) as well as pharmacological tools to explore the pharmacological role of the ECS in in vivo, in vitro, and ex vivo models of disease. The animals used in our study were: a) 5-week-old male control (C57BL/10ScSnJ; n=6); b) dystrophic (C57BL/10ScSn-DMDmdx/J; n=6) and c) 5-week-old male C57BL/6 mice treated with vehicle or simvastatin 20 mg Kg-1 (n=6).
Results: We provide evidence that the microbiota composition along with circulating levels of short-chain fatty acids (SCFAs) and related metabolites are altered in the mdx mouse model of DMD compared to healthy controls. Supplementation with sodium butyrate (NaB) in mdx mice rescued muscle strength and autophagy, and prevented inflammation associated with excessive endocannabinoid signaling at CB1 receptors to the same extent as deflazacort (DFZ), the standard palliative care for DMD. In C2C12 myoblasts, we found that the effects of NaB depend on the activation of GPR109A and PPAR receptors. In mice exposed to simvastatin, we found dysregulated expression of the endocannabinoid CB1 receptor as well as impairment of its downstream signaling pathways. Similar alterations were found in murine C2C12 and primary human myoblasts.
Conclusions: In summary, we report here a novel mechanism by which gut dysbiosis associated with ECS dysregulation contributes to the severity of DMD. This discovery may lead to a novel disease-modifying approach that could also benefit other muscular dystrophies. We also discovered that ECS dysfunction is a pathological mechanism contributing to statin-induced myopathy.

Keywords: Gut microbiota; Endocannabinoid system (ECS); Duchenne muscular dystrophy (DMD); simvastatin

Targeting unfolded protein response reverts ER stress and ER Ca2+ homeostasis in cardiomyocytes expressing the pathogenic variant of Lamin A/C R321X

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Aim: The lmna nonsense mutation R321X, identified in several members of an Italian family, produces a truncated LMNA variant co-segregating with a severe form of dilated cardiomyopathy. R321X mis-localized into the endoplasmic reticulum (ER) of HL-1 cardiomyocytes, inducing ER stress response and apoptosis. The aim of this work was to analyze whether targeting the UPR can be used to revert the ER dysfunction associated with LMNA R321X expression in HL-1 cardiomyocytes.
Methods: HL-1 cardiomyocytes stably expressing LMNA R321X were used to assess the ability of 3 different drugs targeting the UPR for 48h, salubrinal (200 μM), guanabenz (20 μM) and empagliflozin (10 μM) to rescue ER stress. In these cells, the state of activation of both the UPR and the pro-apoptotic pathway were analyzed monitoring the expression levels of phospho-PERK, phospho-eIF2, ATF4, CHOP and PARP-CL. Moreover, we measured ER-dependent intracellular Ca2+ dynamics as indicator of proper ER functionality.
Results: We found that salubrinal and guanabenz increased the expression levels of phospho-eIF2 and empagliflozin inhibited PERK phosphorylation in LMNA R321X-cardiomyocytes. Furthermore, all drugs tested downregulated the apoptosis markers CHOP and PARP-CL and restored ER ability to handle Ca2+ in these cardiomyocytes.
Conclusions: We provided evidence that the different drugs, although interfering with different steps of the UPR, were able to counteract pro-apoptotic processes and to preserve the ER homeostasis in R321X LMNA-cardiomyocytes. Of note, two of the tested drugs, guanabenz and empagliflozin, are already used in the clinical practice, thus providing preclinical evidence for ready-to-use therapies in patients affected this mutation.

Keywords: Lamin A/C; Cardiolaminopathies; Salubrinal; Guanabenz; Empagliflozin.

Potential application of Growth Hormone Secretagogues (GHS) for Amyotrophic Lateral Sclerosis (ALS) treatment: mechanisms of action and neuroprotective effects in human SH-SY5Y SOD1G93A cells

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Aim: ALS is a rare motor neuron disease for which all interventions are currently only symptomatic and palliative. The strong need to characterize more effective drugs led us to propose hexarelin and JMV2894, two synthetic compounds belonging to GHS family, as potential drugs for the treatment of ALS.
The interest in these two compounds is because: i) Hexarelin exerts neuroprotective effects and in cardiac and skeletal muscles has protective effects at the mitochondrial level; and ii) JMV2894 stimulates calcium mobilization in vitro and GH release in vivo, and modulates mitochondria activity and ROS production.
Methods: Human neuroblastoma cells overexpressing $SOD1^{G93A}$ mutated protein (SH-SY5Y $SOD1^{G93A}$ cells) were treated with H_2O_2 (150μM) and GHS (1μM) for 24h. Photomicrographs of stained cells were quantified by skeleton and fractal analysis. The mRNA expression levels of apoptotic and inflammatory markers were quantified by real-time PCR, while protein levels were measured by WB. Data were analysed by one-way ANOVA, followed by Tukey's t-test.
Results: Morphometric evaluation, mRNA levels and effector proteins quantifications showed that H_2O_2 treatment induced apoptotic activation ($p<0.001$). Both GHS significantly blunted H_2O_2 effects decreasing Bax/Bcl-2 ratio ($p<0.001$), inhibiting the activation of caspases ($p<0.05$) and modulating MAPKs and PI3K/Akt phosphorylation ($p<0.01$). These effects were probably mediated by epigenetic mechanisms as shown by the significantly decreased percentage of H2AX-positive cells in the GHS-treated group compared to the H_2O_2 group ($p<0.001$).
Conclusions: GHS are capable of protecting cells from cytotoxicity induced by oxidative stress, suggesting that they could be developed as novel drugs with improved potential in ALS.

Keywords: ALS, SOD1, GHS, neuroprotection, drug validation

Effects of Irisin Treatment on the Expression of Genes Associated with Myogenesis, Inflammation, Mitochondrial Metabolism, and Neuroprotection in an Amyotrophic Lateral Sclerosis "In Vitro" Model

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Aim: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons and compromises skeletal muscle function. The only drug in therapy is riluzole. Previous studies highlighted that the skeletal muscles of mice which overexpress SOD1G93A mutation shown a reduction of irisin mRNA together with impaired function (Camerino et al 2019). Irisin is a myokine that favors the muscles homeostasis and the cross-talk between muscles and neurons and has been found to be beneficial in different neurodegenerative diseases.
Our objective is to investigate the therapeutic potential of irisin in ALS.
Methods: We evaluated the impact of irisin application on the expression of genes related to myogenesis, inflammation, mitochondrial metabolism, and neuroprotection through qPCR in an immortalized murine myoblast cell line (C2C12) transfected with the $SOD1^{G93A}$ or $SOD1^{WT}$ gene.
Results: The expression of PAX7 and PAX3, the transcription factors involved in cell proliferation, was unchanged in the C2C12-$SOD1^{G93A}$ cells compared to C2C12-$SOD1^{WT}$, but was downregulated upon irisin application. However, irisin treatment effectively restored the expression of differentiation genes (MyoG and MyoD), which were downregulated in C2C12-$SOD1^{G93A}$ cells compared to C2C12- $SOD1^{WT}$. In addiction, irisin treatment reduced the expression of inflammation genes (IL6 and NFKB) in C2C12-$SOD1^{G93A}$ cells and promoted the upregulation of mitochondrial Citrate Synthase, PGC1 α and BDNF expression in both cell lines.
Conclusions: These findings suggest that irisin may have a positive impact on ALS by promoting muscle fiber differentiation, counteracting neurodegenerative and neuroinflammatory processes, and enhancing mitochondrial function. Therefore, irisin is a potential therapeutic agent for ALS

Keywords: Amyotrophic lateral sclerosis Irisin gene expression

References:

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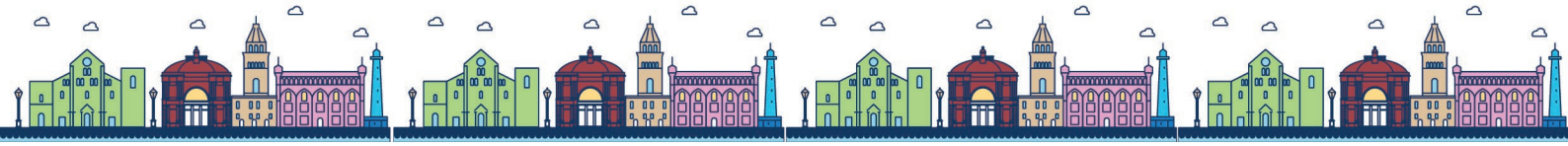

Preclinical study showing a gender specific protective properties of conjugated linoleic acid (CLA) for amyotrophic lateral sclerosis

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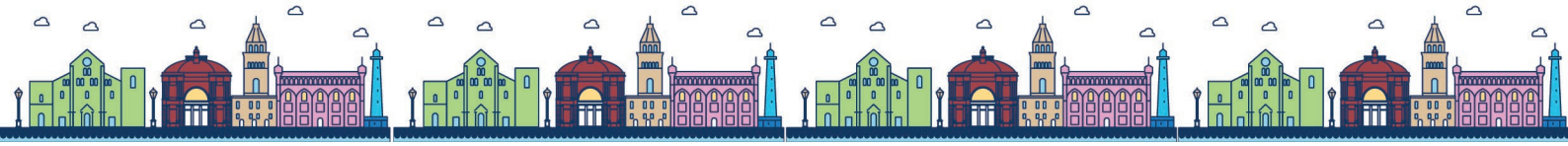
Aim: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with no effective therapies, characterized by degeneration of motor neurons and muscle atrophy. Mitochondrial dysfunction, oxidative stress, and inflammatory response are pathognomonic signs of ALS. In this scenario, a crucial role is played by the nuclear erythroid-related factor 2 (Nrf2). Nrf2 has been indicated as a therapeutic target for ALS, but the efficacy Nrf2 activators has been limitedly investigated. Recent in-vivo pre-clinical studies showed that dietary supplementation with the conjugated linoleic acid (CLA) activates Nrf2 in the liver of healthy animals and shows antioxidant effects towards age-dependent pro-inflammatory status.
Methods: We evaluated the effects of the dietary supplementation of CLA (600 mg/kg/day) starting from the pre-symptomatic stage of the disease, in $SOD1^{G93A}$ mice, by in-vivo behavioral studies and ex-vivo biochemical analysis (n=30 and n=15 animals for in-vivo and ex-vivo experiments respectively).
Results: CLA treatment was able to significantly delay the disease onset and improve the functional scores in male $SOD1^{G93A}$ mice, while no effects were observed in females. Biochemical analysis on blood and tissues dissected from the treated and non-treated mice revealed that the treatment led to an amelioration of different oxidative stress parameters and improved mitochondrial functions.
Conclusions: The sex specific effect of CLA unveiled an unexpected aspect that need to be investigated. However, our pre-clinical results overall suggest that CLA could be exploited as a promising dietary supplement to improve the antioxidant response in ALS.



In-vitro and in-vivo pre-clinical evidence unveiling the mGlu5 receptor as a promising pharmacological target for ALS clinical treatment

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Aim: Amyotrophic lateral sclerosis (ALS) is fatal neurodegenerative disease characterized by a progressive degeneration of motor neurons (MNs). The etiology is partially obscure, and several mechanisms have been proposed, including glutamate-mediated excitotoxicity. In this context, group I metabotropic glutamate receptors (mGluR1/5) play an active role, since their expression and functions are altered in ALS. Behavioral, histological, and functional experiments have been performed to characterize the effects of mgluR5 modulation in both in-vivo and in-vitro ALS models.
Methods: We investigated the effect of mGluR5 genetic ablation exploiting the $SOD1^{G93A}$ ALS mouse model. We tested in-vivo the pharmacological blockade of mGluR5 by the negative allosteric modulator CTEP (2 mg/kg/48h or 4 mg/kg/24h). Moreover, we studied in-vitro the effects of mGluR5 genetic or pharmacological modulation, on spinal cord astrocytes and microglia primary cell cultures (n=105 and n= 120 animals for in-vivo and in-vitro experiments respectively).
Results: The in-vivo genetic ablation of mGluR5 as well as the pharmacological treatment with CTEP translated into a delayed disease onset, decreased glial activation, increased MNs preservation and prolonged survival probability in $SOD1^{G93A}$ ALS mice. In-vitro experiments with primary spinal cord astrocytes and microglia cells genetically lacking mGluR5 showed a modulation of the reactive phenotype, diminished release of glutamate and other neuroinflammatory factors and reduced neurotoxic effects.
Conclusions: The constitutive genetic downregulation, or the pharmacological blockade of mGluR5 have a positive outcome in ALS mice, paving the way for potential application of mGluR5 modulators as favorable pharmacological tools that can be tested in clinical trials for ALS treatment.

Keywords: amyotrophic lateral sclerosis; metabotropic glutamate receptors; in-vivo preclinical trial; glutamate excitotoxicity

New therapeutic intervention for amyotrophic lateral sclerosis: analysis of the effects of acetazolamide on the biophysical properties of skeletal muscle in a transgenic mouse model

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Aim: Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of motor neurons and muscle atrophy. Transgenic mice carrying mutations on SOD-1 gene (SOD1-G93A) develop ALS-like symptoms and the muscle-specific animal model (MLC/SOD1-G93A mice) demonstrated a severe skeletal muscle involvement. In this model we found a reduction of expression and activity of the ClC-1 chloride channel. This channel is important for skeletal muscle function since it controls the electrical stability of sarcolemma. At the aim to find effective therapies we evaluated the effects of acetazolamide. Its mechanism of action may include the activation of the skeletal muscle ClC-1 or of the Ca^{2+}-activated-K^+ channels.
Methods: We performed an in vivo treatment with acetazolamide (5mg/kg, i.p. for 2 weeks) in a new generated animal model of ALS, the SOD1-G93A Knock-In (KI) in which the endogenous SOD1 has been substituted with the mutated one. The component conductances and muscle excitability have been measured electrophisiologically in current clamp by two microelectrodes.
Results: We found a significant reduction of resting chloride conductance (gCl) (27%) in the SOD1-G93A KI model with respect to WT. The excitability parameters were modified accordingly, since the threshold current needed to obtain the first action potential was decreased and the maximum number of spikes was increased. Acetazolamide in vivo treatment was able to completely restore the gCl toward the WT value.
Conclusions: These results show that ClC-1 channel can be a pharmacological target. At this aim, acetazolamide and analogues deserves to be better investigated as promising, prompt-to-use drugs in ALS.

Gene therapy for the treatment of childhood SMA1: Onasemnogene abeparvovec. Observations of results of early Zolgensma administration thanks to newborn screening

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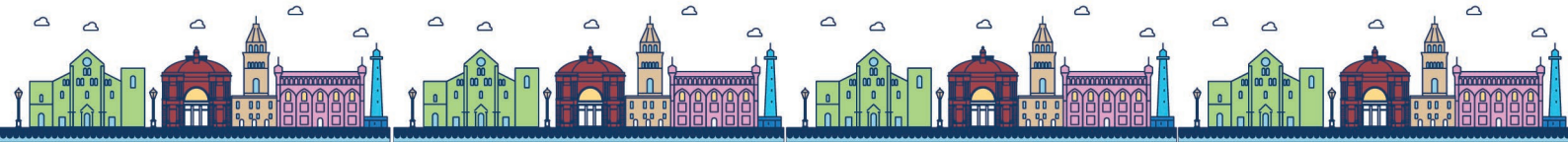
Aim: Onasemnogene abeparvovec (Zolgensma) is a prescription gene therapy used to treat children less than 2 years old with spinal muscular atrophy (SMA). Zolgensma is given as a one-time infusion into a vein. In Italy reimbursement is provided in patients weighing up to 13.5 kg with clinical diagnosis of SMA1 with 1 or 2 copies of the SMN2 backup gene.
In Apulia, SMA is early detected through newborn screening, which is provided by Local Healthcare Service since 2021.
This analysis highlights the improvement of motor function through 1-3-6-12 mounths checks -assesed by CHOP INTEND- of four newborns screened in 2022/23 who were administrated with Zolgesma in first days of life.
Methods: CHOP test was created to measure the motor development of children with SMA1. The healthcare provider measures 16 types of muscle movements, including head control, arm and leg mobility and hand grip. Each of the 16 motor skills is given a score from zero to 4. The highest possible score is 64.
Results: Patient 1, female; birth score=31. She received the drug dose on 23rd day of life. T1=39; T3=45; T6=61; T12=64.
Patient 2, male, T0 score=38. He recived the dose on 20rd; T1=48; T3=T6=T12=62. Patient 3, female, T0 score=45. She recived the dose on 16th; T1= 24; T3=35; T6=38. Patient 4, male; T0=62. He received the dose on 13th. T1=63.
Conclusions: Patients achieved rapid improvements in motor function thanks to Zolgensma treatment, and most have already achieved motor milestones not observed in the natural history of SMA1.



KIF5A, a protein involved in axonal transport, represents a new druggable target in a mouse model of spinal muscular atrophyl

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Aim: Spinal muscular atrophy(SMA) is a neurodegenerative disease characterized by spinal cord and brainstem motor neuron (MN) loss. Axonal transport defects are a common hallmark of MN degeneration and KIF5A, a major component of the axonal transport machinery, has been associated with MN diseases, but its role in SMA has not been elucidated yet. Our aim was to investigate a possible correlation between KIF5A and SMA pathogenesis.
Methods: Western Blot, qRT-PCR and immunohistochemistry analysis were performed to investigate KIF5A and its adaptor protein complex Miro1/Milton expression in the spinal cord of a mouse model of SMA, at different stages of the disease. Computational analysis was used to identify putative consensus sequences for microRNAs able to modulate KIF5A expression.
Results and conclusions: Our results showed a down-regulation of KIF5A and Miro1 in the spinal cord of SMA mice at P5 and P12, mirrored by an upregulation of mir-140-3p levels in this region. To assess the hypothesis that miR-140-3p could be a possible regulator of KIF5A expression, luciferase assay was performed and mice were intracerebroventricularly injected with antimiR140, a molecule able to specific block miRNA-140. Behavioral tests showed an increase in the lifespan of SMA pups and an improvement of their behavioral performance after antimiRNA administration. Therefore, our study suggests an implication of KIF5A in the anterograde transport defects observed in SMA mice and identifies miR-140-3p as a new possible therapeutic target for the treatment of the disease.

Sessione 2

Poster

Biallelic inheritance of two novel SCN1A variants results in loss of Nav1.1 channel function and developmental and epileptic encephalopathy

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Aim: Loss- (LoF) and gain-of-function (GoF) variants in the *SCN1A* gene (Nav1.1 voltage-gated sodium channel) have been associated with a spectrum of neurologic and neurodevelopmental disorders (e.g., epilepsy) with different severity and drug-responsiveness. Most *SCN1A* variants are heterozygous changes occurring de novo or dominantly inherited. However, recessive inheritance has been reported in a few cases. We identified two siblings carrying two novel *SCN1A* variants, N935Y (paternal origin) and H1393Q (maternal origin), presenting developmental and epileptic encephalopathy, and born to heterozygous asymptomatic parents. The aim of this study is to provide a detailed clinical description of the family and assess *SCN1A* variants pathogenicity.
Methods: For this purpose, HEK293 cells were transfected with different combinations of WT and mutant cDNAs and resulting sodium currents were recorded through whole-cell patch-clamp.
Results: H1393Q channels showed current amplitudes comparable with those of WT, whereas N935Y currents were 50% increased compared with WT. In co-expression studies, WT+H1393Q and WT+N935Y channels showed current levels, respectively, similar and 20% higher than WT+WT. Interestingly, N935Y+H1393Q channels showed 30% reduced current amplitude compared with WT+WT.
Conclusions: Our results show that H1393Q and N935Y variants cause subtle changes in Nav1.1 that are not sufficient to cause the disease when in the heterozygous state, as in the parents. Conversely, biallelic inheritance of the two variants causes loss of Nav1.1 function that may decrease the seizure threshold and possibly explain the disease in the carriers.

Keywords: Nav1.1, epilepsy, patch clamp, biallelic inheritance

SCN2A A1659V loss-of-function variant causes early infantile onset encephalopathy

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Aim: Pathogenic variants in *SCN2A* coding the voltage-gated sodium channel Nav1.2 are known to cause a phenotypic spectrum including epileptic encephalopathy and intellectual disability or autism without epilepsy. Classically, early infantile onset encephalopathies are well respondent to sodium channels blockers, assuming gain-of-function (GOF) mutation of the channel. We evaluated the expression and functional effect of a *SCN2A*: c.4976C>T (p.A1659V) pathogenic variant found at a mosaic state in two infants non-respondent to carbamazepine.
Methods: The *SCN2A* pathogenic variant was identified through NGS technique and the mutation was inserted by site-directed mutagenesis in the pIR-CMV-IRES-mScarlet plasmid encoding for Nav1.2. In HEK293 cells transfected with the WT or mutated channel, we evaluated the expression of the protein using western blot and immunohistochemical assays and membrane currents by whole-cell patch-clamp technique to functional characterization.
Results: *SCN2A* A1659V mutation does not impact on channel expression as shown by western blot and immunofluorescence assays. Electrophysiological analysis showed that A1659V induced a smaller current respect to WT channel. The quantitative analysis of A1659V activation properties show a shift of V1/2 about 10 mV towards more negative potentials and a time constant slower than the WT channel.
Conclusions: SCN2A loss-of-function mutations may cause a severe phenotype like GOF mutation but with no response to sodium channel blockers. Functional characterization may direct clinical interventions and expand funotype-phenotype correlations.

Keywords: Sodium-channel, encephalopathy, epilepsy, drug resistance, personalized medicine

Automated Patch Clamp for assessing the effects of Mexiletine and its pyrroline derivative on Nav1.4 and Nav1.5: towards anti-myotonic drugs with improved safety profile

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Aim: LMyotonia is an inherited rare skeletal muscle disorder characterized by sarcolemma hyperexcitability caused by ion channels mutations. Mexiletine is a class I antiarrhythmic drug approved in myotonia, exerting a use-dependent block of skeletal muscle sodium channel(Nav1.4). Discriminating drug action between Nav1.4 and cardiac Nav1.5 is critical for reducing cardiac toxicity risk. Thus, robust pre-clinical tests are pivotal also to find novel anti-myotonic agents with better safety/efficacy profile.
Methods: We assessed the effects of mexiletine (Mex) and its pyrroline analogue (VM11) on Nav1.4 and Nav1.5 by automated patch-clamp Patchliner(Nanion) using tissue-specific pathophysiologic stimulation frequencies. Tonic-(0.3 Hz;TB), voltage-(-70/-140 mV at 0.3 Hz;VDB) and use- dependent blocks(3 Hz for Nav1.5 and 10 Hz for Nav1.4;UDB) were evaluated using TE671 cells endogenously expressing Nav1.4 and CHO cells heterologously expressing Nav1.5.
Results: Mex blocked Nav1.5 more than Nav1.4 in TB/VDB conditions (IC50: 24±3 vs 87±5μM for TB; 2±0.3 vs 15±1μM for VDB -70mV; 159±8 vs 226±16μM for VDB -140mV). VM11 produced similar TB/VDB(-140mV) of Nav1.4 and Nav1.5(7±0.6 vs 9±0.5μM for TB; 48±3 vs 54±5μM for VDB) but exerted greater VDB at -70mV on Nav1.5 than Nav1.4(3±0.2 vs 7±0.4μM). At 3Hz, VM11 blocked Nav1.5 more than Mex (5±0.3 vs 35±1μM), while at 10Hz, Mex and VM11 potently blocked Nav1.4 but still at concentrations(24±2 and 3±0.2μM) in the range of blocking Nav1.5.
Conclusions: Patchliner allowed to easily assess use-dependent behavior of Mex-like compounds, disclosing a still narrow safety range even at myotonic-like frequencies. This biophysical approach can help the development of novel Nav1.4 blockers with improved safety profile for muscle disorders(MNESYS_PE0000006).

Keywords: sodium channels; mexiletine; cardiac safety; automated patch clamp

Combined *in silico* and in vitro approaches to repurpose drugs towards Kv1.1 and Kv1.2 potassium channels for epileptic encephalopathy and ataxia pharmacological treatment

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Aim: . Kv1.1 (*KCNA1*) and Kv1.2 (*KCNA2*) are voltage-gated potassium channels expressed in the central and peripheral nervous systems responsible for dampening excitability.[1] Loss- and gain-of-function variants in *KCNA1* and *KCNA2* have been associated with episodic ataxia type 1 (EA1) and with developmental and epileptic encephalopathy (DEE).[1] Current treatments with antiseizure medications are still far from being really effective; moreover, drug-resistance very often occurs. Given these concerns, Kv1.1 and Kv1.2 selective blockers as well as activators are necessary to provide patients with new therapeutic options having higher benefit-risk profiles. To date, some poor Kv1 activators and blockers are known, such as 4-AP, niflumic acid and some plant extracts.[2,3] In this study, we mapped the putative Kv1.2 binding pockets and carried out a molecular docking virtual screening to repurpose marketed drugs to speed up the discovery of new agents effective to tackle DEE and EA1 diseases.
Methods: Using a homology model of Kv1.2 protein, binding site mapping has been performed by employing BioGPS software[4]. A multiple-grid docking-based virtual screening has been run by using an *in house* curated collection of 1730 marketed drugs.
Results: A pool of repurposed drugs has been prioritised as candidate regulators of Kv1.2 activity to be confirmed and validated by experimental studies.
Conclusions: The herein proposed drug repurposing strategy has suggested potential drugs which may act as regulators of Kv1.2 activity. While experimental validations are in progress, *in silico* screening of novel drug-like compounds towards Kv1.1 is undertaken to improve DEE and EA treatments.

Keywords: Episodic Ataxia Type 1, Voltage-gated potassium channels, Drug Repurposing, Virtual Screening

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Automated Patch Clamp for assessing the effects of Mexiletine and its pyrroline derivative on Nav1.4
Structure-based identification and characterization of novel inhibitors of K_{Na}1.1 potassium channels

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K_{Na}1.1 potassium (K+) channels are sodium-activated K+ channels widely expressed in neurons, where they regulate neuronal excitability. Pathogenic variants in K_{Na}1.1 cause a range of epileptic syndromes associated to intellectual disabilities and drug-resistant seizures. Functional studies revealed that the largest majority of pathogenic KCNT1 variants increase K_{Na}1.1 currents; therefore, quinidine, a well-known antiarrhythmic drug also acting as a K_{Na}1.1 blocker, has been used as a personalized treatment in these patients. However, quinidine is only partially effective in controlling seizures, and its use is limited due to its non- selective activity on others target including cardiac channels, such as hERG. In this work we combined in silico and in vitro screening techniques to identify novel KNa1.1 channel blockers. Virtual screening of an in-house library of 945 compounds using a human homology model of the chicken K_{Na}1.1 channel was performed and 21 molecules were selected. All these 21 compounds were characterized in vitro using a Thallium (Tl+)-based fluorescent assay (FluxOR™) in CHO cells stably expressing K_{Na}1.1 subunits and five molecules (CP-K 4, 13, 16, 19 and 21) were more effective and potent than quinidine in reducing the fluorescent signal, showing IC50s at least 20-times lower (around 5-10 μM). Patch-clamp electrophysiology experiments confirmed the higher K_{Na}1.1 channel-blocking efficacy of these five compounds when compared to quinidine and two of these compounds (CP-K 16 and 21) showed little effect on the cardiac hERG channels. These compounds may provide starting points for the development of novel pharmacophores for K_{Na}1.1 inhibition, with the aim to treat K_{Na}1.1-assocuated epilepsy.

Keywords: K_{Na}1.1, antiseizure medications, epilepsy, electrophysiology, virtual screening

De novo variants IN *KCNA3* cause developmental and epileptic encephalopathy

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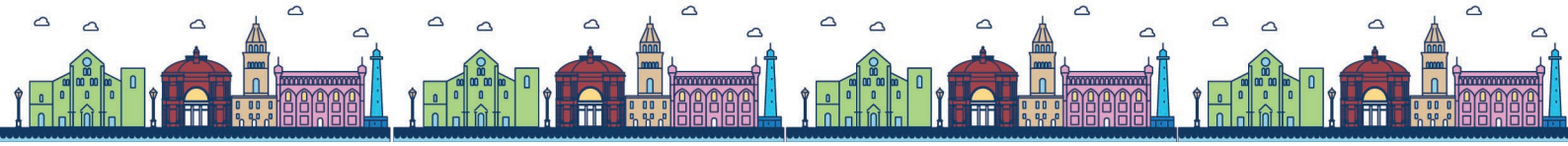
Aim: To investigate the functional alterations prompted on Kv1.3 channels by mutations identified in patients affected by Developmental and Epileptic Encephalopathy (DEE), and a pharmacological approach to counteract these possible functional changes.
Methods: In this study, ten de novo missense mutations in *KCNA3* were identified by whole- exome sequencing. To functionally characterize these variants, Kv1.3 subunits carrying each variant were transiently expressed in CHO cells and their currents recorded using whole-cell patch-clamp. Statistical significances were evaluated by using Student's t-test or ANOVA followed by Student-Newman-Keus post-hoc test, with the threshold set at $p < 0.05$.
Results: Except for A361T, electrophysiological recordings revealed significant functional changes for all variants, supporting their role in disease pathogenesis. These ranged from faster inactivation kinetics (P11R), to reduced currents with (T443I, G468F, and P477H) or without (I431N) dominant-negative effects indicative of "pure" loss-of-function (LoF), to mixed loss- and gain-of-function (GoF) effects (A357V, I455V, V460M, and V478M). Kv1.3 currents in lymphoblasts from the proband carrying the V478M variant displayed functional changes qualitatively similar to those observed in CHO cells. The antidepressant drug fluoxetine blocked Kv1.3 and Kv1.3 V478M channels, suggesting a personalized treatment approach for individuals carrying KCNA3 variants with GoF effects.
Conclusions: *KCNA3* is a novel DEE-causing gene and Fluoxetine could be a possible therapeutic approach for patients harboring GoF variants.

Keywords: *KCNA3*; Whole-Cell Patch-clamp; Drug-repurposing; Fluoxetine



Potassium channels and TRPV1 modulators on SU-DIPG-36 and SU-DIPG-50 cells: in vitro effects on cell proliferation and channel currents characterization

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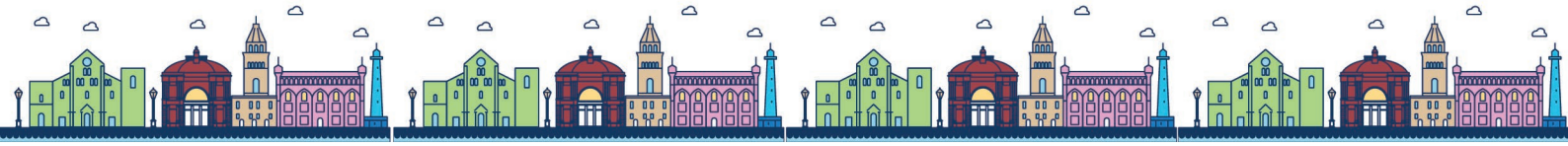
Diffuse intrinsic pontine glioma (DIPG) is a rare fatal brainstem tumour in children. We studied targetable K+ and TRPV1 ion channels in this disease. In vitro cell viability experiments with crystal violet and CCK-8 assays were performed on DIPG-36 and DIPG-50 cells using K+ and TRPV1 channels modulators; the most effective were repaglinide and glibenclamide (50nM-200μM) after 6- 72 hours of incubation time. Repaglinide (100μM) was also effective on SU-DIPG-36 cells in clonogenic assay. The agonist of TRPV1 channels, capsaicin (1-100μM), showed cytotoxicity on both cells. Patch-clamp experiments showed that DIPG-36 cells were sensitive to BaCl2-TEA (5 mM). On DIPG-36 cells (N=7 cells), the application of glibenclamide (10μM) reduced control currents at +80 mV (Vm) (834.525+176.20 pA) and at -80 mV (Vm) (-201.198+46.23 pA) by - 27.57% and -9.057%, respectively. On 4 cells, capsazepine (1μM), reduced control currents at +80 mV (Vm) (904.541+241.291 pA) of -16.43% and at +180 mV (Vm) (4069.52+1049.74 pA) of -
31.389%. Instead, DIPG-50 were less sensitive to BaCl2-TEA (5mM). On DIPG50 (N=5 cells), repaglinide (100μM) at +60 mV (Vm) reduced control currents (178.50+96.62 pA), with a percentage of -27.63%; at -80 mV (Vm), reduction of the control current (-60.263+15.23 pA) was of -15.0374%. The acute application of diazoxide (250 μM) did not further activate the currents. Repaglinide and glibenclamide were therefore the most potent antiproliferative drugs on both cells, so KATP channels are investigated as a new target for DIPG therapy; also, TRPV1 channels modulators have been shown to have antiproliferative effects.

Keywords: DIPG, KATP, TRPV1, REPAGLINIDE, CAPSAICIN



ANTIPROLIFERATIVE EFFECTS OF TYROSINE KINASES (TK) INHIBITORS STAUROSPORIN/MIDOSTAURIN ON SU-DIPG CELLS AND ON CATION CURRENTS: ROLE OF K+ CHANNELS AND TRPV1 CHANNELS AS DRUG TARGETS

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Emerging data proposed tyrosine kinases as dis-regulated pathways in Diffuse Intrinsic Pontine Glioma (DIPG) which is a rare, paediatric high-grade glioma. Investigations are carried out on DIPG patient-derived H3.1K27M (SU-DIPG-36) and H3.3K27M (SU-DIPG50) cells using staurosporin and its structural analogue midostaurin, multitarget tyrosine kinases inhibitors. Previous data showed that staurosporin also modulates ion channels. These compounds (0,1M-100μM), were potent antiproliferative drugs in both cells in crystal violet and CCK-8 cytotoxic assays at all incubation times (6-72 hrs) reducing cell volume and diameters. These data were confirmed by clonogenic assay. Patch-clamp investigations showed that the whole-cell inward and outward cation currents of DIPG36 cells were inhibited by -74%±56 at +20mV (Vm), -50%±43.78 at +40mV (Vm) and -46%±30.17 at
+60mV (Vm) by the acute application of staurosporin 2.14μM vs controls. These currents were fully inhibited by TEA-BaCl2 (5mM). Staurosporin also blocks the capsazepine and glibenclamide- sensitive currents in the same cells. On DIPG50 cells, midostaurin at t=0 failed to inhibit the currents at negative membrane potentials while after 20 min of incubation time reduced currents by -28% at - 80mV (Vm) and -22% at -60mV (Vm) vs controls. At positive potentials, at t=0, midostaurin reduced currents by -64%±57,5 at +60mV (Vm) with an increasing inhibitory effect of -97% after 20 min of incubation time. These data suggest that staurosporin/midostaurin inhibits both classes of K+ and TRPV1 channels being more effective on outward currents at positive membrane potentials not allowing potassium and calcium exchange with cell apoptosis and suggesting an additional cytotoxicity mechanism.

Keywords: DIPG – Midostaurin – Ion Channels

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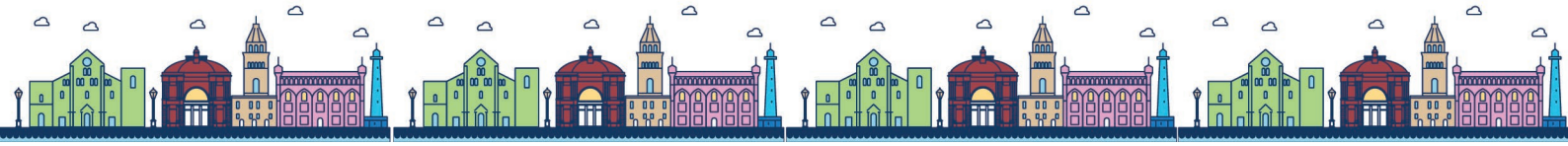

Kir6.1- and SUR2-dependent KATP overactivity caused intestinal tight junction protein alterations in the intestinal epithelium in murine models of Cantú syndrome

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Cantu syndrome (CS) is a rare genetic disorder [1] caused by gain-of-function (GOF) mutations in pore-forming (Kir6.1, KCNJ8) and accessory (SUR2, ABCC9) ATP-sensitive potassium (KATP) channel subunit genes, characterized by a variety of features, including distinctive facial features [2], cardiac abnormalities, hypertrichosis (excessive hair growth), and skeletal abnormalities [3]. One of the lesser-known but significant aspects of CS is its association with gastrointestinal (GI) dysmotility. In intestinal motility, KATP channels can influence smooth muscle activity and thereby impact the movement of contents through the GI tract [4]. Dysfunction of KATP channels has been implicated in various GI disorders, including some forms of irritable bowel syndrome (IBS) and motility disorders like gastroparesis [5].
In the CS conditions, the overactivity of KATP channels might disrupt the normal patterns of smooth muscle contractions, leading to alterations in intestinal motility. Furthermore, KATP channels are present in neurons and regulate neurotransmitter release and neuronal excitability. Overactivity of KATP channels in enteric neurons or nerves innervating the intestines could dysregulate neural motility control. While the precise mechanisms underlying the potential interaction between KATP channels and tight junctions (TJ) protein structures intercellular that play a role critical for building the epithelial barrier and maintaining epithelial polarity remain to be fully elucidated, but emerging evidence suggests that alterations in cellular signaling, energy metabolism, and ion homeostasis associated with KATP channel dysfunction could contribute to disruptions in TJ structure and function [6].
The in vivo and ex vivo characterization of skeletal muscle in mouse models of CS syndrome, CS mice with A478V in SUR2 and V65M in Kir6.1, showed reduced forelimb strength and amplitude of diaphragm movement, whereas RT-PCR evaluation on intestinal smooth muscle detected the expression of both Kir6 subunits. x and SurA/Surxb specifically in the muscle layer of all gastrointestinal tract segments. Furthermore, WB investigation of intestinal biopsies showed significant alterations of intestinal TJ proteins, including Zonula occludens (ZO-1), Occludin and claudins 1 and 2 in CS mice vs. control mice.
In summary, KATP overactivity can disrupt the normal regulation of intestinal motility by affecting smooth muscle function, neural regulation, and contributing to GI disorders. Understanding the role of KATP channels in intestinal motility may lead to developing new therapeutic approaches for managing motility disorders and related GI conditions.

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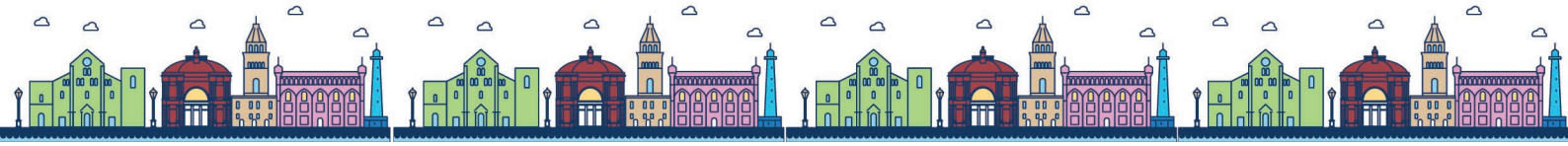

New insights into the involvement of rare genetic variants in *CLCN6* and *CLCN7* associated with neurological diseases: a functional in vitro study

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Aim: Rare genetic mutations in *CLCN6* and *CLCN7* genes encoding the ClC-6 and ClC-7 Cl^-/H^+ antiporters respectively, have been found to be associated with several neurodevelopmental and neurodegenerative diseases (1–3). Predominantly expressed in neurons, ClC-6 and ClC-7 are localized in late endosomes and lysosomes respectively (4). In particular, the GoF variant, ClC-6^{Y553C} causing severe neurodegenerative disorder shows a strong transport activation, being activated at less positive voltages compared to WT ClC-6 when heterologously expressed (2,5). Organomegaly and delayed development are associated with the ClC-7^{Y715C} GoF mutant displays a similar electrophysiological behavior as seen in the ClC-6^{Y553C} variant (3,6). We aimed to explore the GoF roles of disease-associated mutants.
Methods: Here, we measured Cl^-- and pH-dependences of ClC-6^{Y553C} and ClC-7^{Y715C} and their corresponding ClC-7^{Y577C} and ClC-6^{Y781C} constructs comparing them to the respective WT constructs, over-expressed in HEK293 cells and using whole cell patch clamp. It has to be kept in mind that the extracellular solution corresponds to the intraluminal endo-lysosomal compartment.
Results: Lowering extracellular Cl- reduced ClC-6 transport activity, whereas it increased activity of ClC-7. In ClC-7, the activation mediated by low Cl- persisted under acidic pH_{ext}. Interestingly, ClC-7Y577C recapitulated the GoF effect seen in the corresponding ClC-6^{Y553C}. Additionally, the ClC-6^{Y781C} showed similar faster kinetics to those observed in ClC-7^{Y715C}. Of note, all mutants overall reflected WT behaviors.
Conclusions: These findings provide new insights into the non-overlapping ClC-6 and ClC-7 physiological roles in their respective compartments contributing to a better understanding in the molecular mechanism underlying their involvement in human diseases.

Standard abbreviations: gain of function (**GoF**), wilde-type (**WT**), extracellular pH (**pH_{ext}**)

Keywords: ClC-6, ClC-7, CLC antiporters, neurological diseases, rare genetic mutations

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Preclinical evaluation of safinamide as an antimyotonic drug in myotonic ADR mouse model

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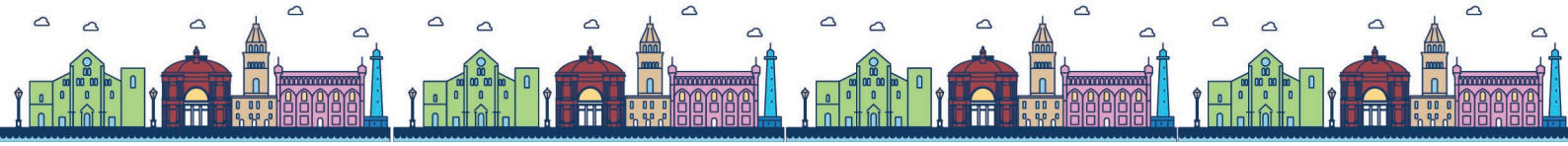
Aim: Nondystrophic myotonias are caused by mutations in Nav1.4 sodium and ClC-1 chloride channels expressed in skeletal muscle and are characterized by muscle weakness. Mexiletine is the drug of first choice in the treatment of myotonia, but a subgroup of patients experience little benefit. Safinamide has been identified as a potential antimyotonic drug. It is indicated for Parkinson's disease by reducing excessive glutamate release and by blocking sodium channels.
Methods: In vitro studies were performed using the electrophysiological technique of the two intracellular microelectrodes in current-clamp. The number of spikes and after discharges were evaluated in skeletal muscle fibers isolated from adr/adr myotonic mice before and after drugs application. We evaluated the effects of Safinamide 1 μM, 3 μM and 10 μM in comparison to Mexiletine 10 μM and 30 μM.
Results: The number of spikes (Nspikes) is higher in the muscle fibers of ADR mice than in those of healthy mice. The application of 10 μM safinamide reduced this value moving it toward that found in wild-type muscles. Whereas the application of 10 μM mexiletine was significant but less effective with respect to safinamide. Indeed, the effect of 30 μM mexiletine was comparable to those of 10 μM safinamide. Also at the dose of 3 μM safinamide showed a significant reduction of Nspikes.
Conclusions: These results demonstrate that safinamide exerts a more potent antimyotonic effect with respect to mexiletine. Thus, safinamide may represent a valid alternative to mexiletine based on its efficacy and specificity.

Keywords: myotonia congenita, mexiletine, safinamide, adr mice



Functional and pharmacological characterization of sodium and chloride channel mutations in Italian families affected by non-dystrophic myotonias

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Aim: Non-dystrophic myotonias (NDMs) are rare diseases characterized by delayed muscle relaxation leading to muscle stiffness. We report the functional and pharmacological characterization of novel Nav1.4 sodium channel (p.T592I, p.N1180I, p.K1302R) and ClC-1 chloride channel (p.H838P) mutations identified in Italian families affected by NDMs.
Methods: Mutations were introduced in Nav1.4 and ClC-1 channel cDNAs and expressed in HEK cells. Sodium and chloride currents were recorded with patch-clamp technique.
Results: Both T592I and K1302R induced a hyperpolarizing shift of activation, increasing open probability at negative potential that may explain muscle stiffness. K1302R Nav1.4 and H838P ClC- 1 mutation were found co-expressed in patients with an atypical and mixed phenotype. H838P induced a reduction in chloride current and a small positive shift of voltage dependence. Thus, concomitant expression of K1302R and H883P mutations may explain the complex clinical phenotype. Pharmacological studies revealed that K1302R was sensitive to mexiletine and lamotrigine, suggesting that both drugs might be useful to the K1302R carriers.
N1180I was identified in two Italian families showing a peculiar phenotype characterized by myopathy and myotonia. Functional study evidenced the slowdown of fast inactivation and the larger window current area, suggesting a gain-of-function possibly responsible for myotonia. Meanwhile, the reduction in expression efficiency and maximal current amplitude, together with the positive shift of activation, suggested a loss-of-function more compatible with myopathy.
Conclusions: Functional studies represent valuable tools for genotype/phenotype correlation in patients with unusual clinical manifestation and allows addressing therapy in individual patients. (Supported by University of Bari "Horizon Europe Seeds"-project Medineuropa).

Keywords: non-dystrophic myotonia, sodium channel, chloride channel, ion channel pharmacology

Sessione 3

Poster

Fingerprinting cardiolipin in leukocytes by MALDI-TOF mass spectrometry as a screening tool for Barth Syndrome

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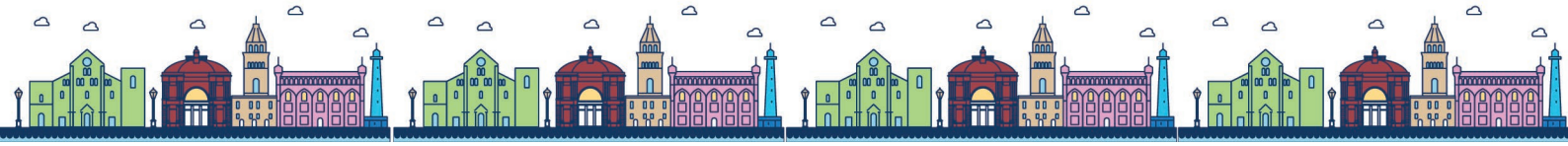
Barth syndrome (BTHS) is a rare and often misdiagnosed genetic disease characterized by early-onset cardiomyopathy, skeletal muscle myopathy, growth delay, neutropenia, and variable mitochondrial dysfunction. It results from mutations of the Tafazzin (TAFAZZIN) gene localized to chromosome Xq28.12 causing deficient remodeling of cardiolipin (CL).
CL is the phospholipid marker of mitochondria, wherein it plays a crucial role in the structure and functioning of the inner membrane. Its metabolite monolysocardiolipin (MLCL) is physiologically nearly absent in the lipid extract of human cells and its appearance is the hallmark of BTHS.
Upon TAFAZZIN loss-of-function, CL remodeling fails and specific phospholipid abnormalities arise in mitochondria of BTHS patients: mature CL content (CLm) is decreased, while increased levels of monolysocardiolipin (MLCL) and altered CL acyl composition (i.e., immature CL species, CLi) occur.
Our method here described generates a "cardiolipin fingerprint" and allows a simple assay of the relative levels of CL and MLCL species in cellular lipid profiles. In the case of leukocytes, only 1 mL of blood is required to measure the MLCL/CL ratio via matrix-assisted laser desorption ionization - time-of-flight/mass spectrometry (MALDI-TOF/MS), just within 2 h from blood withdrawal.
The assessment of elevated MLCL/CL ratio in isolated leukocytes discriminates BTHS patients from healthy subjects and other heart failure patients with 100% sensitivity and specificity.

Keywords: Cardiomyopathy, lipid analysis, mitochondria, white cells



The Keap1/Nrf2/ARE pathway as a potential therapeutic target for the treatment of Huntington's disease

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Aim: Huntington's disease (HD) is a rare neurodegenerative disorder caused by an expansion of cytosine-adenine-guanine repeats that triggers the formation of a mutant huntingtin protein leading to neuronal death, predominantly in the striatum. Due to the impact of mitochondrial dysfunction and oxidative stress in the early stages of HD, targeting the antioxidant Keap1/Nrf2/ARE pathway presents a promising therapy for this neurodegenerative disease. Here we have assessed the potential neuroprotective effects of sulforaphane (SFN), a potent Nrf2 inducer, through canonical and non-canonical activation of the Keap1/Nrf2 pathway.
Methods: We used inducible PC12 HD- Q23 and HD-Q74 cells expressing normal and mutant huntingtin protein (mHtt), respectively, to model early and advanced stages of HD.
Results: We found that before the full aggregation of mHtt, PC12 HD-Q74 cells exhibited an early activation of endogenous antioxidant defence mediated by Keap1/Nrf2 pathway, which was insufficient to counteract ongoing oxidative stress, mitochondrial dynamics impairment and neuronal death. Reinforcing Nrf2 nuclear translocation and its antioxidant transcriptional activity through SFN prevented neuronal death. SFN also decreased phosphorylation of Erk and GSK-3 kinases, but not AKT kinase, which are upstream regulators of Nrf2 activation. This suggests a canonical mechanism of Nrf2 release after disruption of Keap1- Nrf2 complex mediated by SFN. Interestingly, SFN metabolites such as SFN-glutathione, SFN- cysteine, and SFN-N-acetylcysteine exhibited similar neuroprotective effects as SFN.
Conclusions: These results demonstrate that SFN and its metabolites may prevent the neurotoxicity induced by mHtt through activation of Keap1/Nrf2/ARE pathway.

Keywords: Huntington's disease, oxidative stress, Nrf2, neuroprotection, sulforaphane

A clinical case of corpus callosum agenesia: a neuroscience multidisciplinary evaluation

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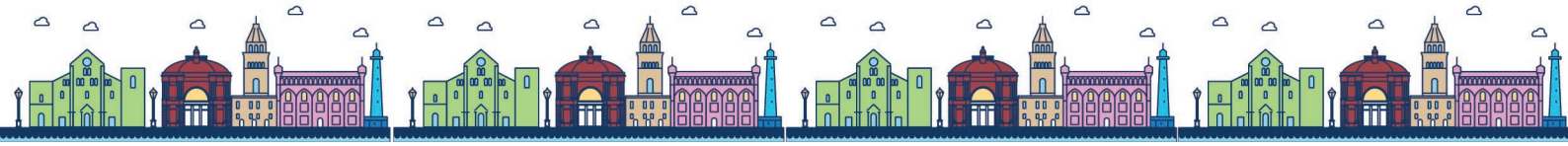
The CC is the most voluminous interhemispheric commissural structure. CC, cytologically is composed of glial cells such as oligodendrocytes, astrocytes and few neurons. The CC is topographically subdivided into 7 regions, in which the functional distribution of the fibers is still little known. Moreover, the CC fibers are mainly composed of myelinated fibers and a smaller number of unmyelinated fibers, both originate from neocortical neurons of the layers III, V, VI and have a heterogeneous neurochemical composition. Studies have shown in the CC regions a different expression profile of proteins involved in regulatory calcium signals and in oxidative stress. A rare malformative disorder is the agenesia of the corpus callosum (AgCC) characterized by an absence or residual presence of CC fibers. Nonetheless, AgCCs are often neglected or undiagnosed. Several studies demonstrated the coexistence of AgCC to other brain anomalies, however, the reciprocal influences of AgCC with other brain malformations and the correlations between neurological and psychiatric symptoms are scantly. The aim of this translational study was to analyze morphofunctional and neuropsychological functions, clinical symptoms, and the reciprocal influence of AgCC with other brain anomalies. The multidisciplinary analysis highlighted the coexistence of other brain abnormalities, the presence of psychiatric and neurological symptoms, and the presence of neurocognitive deterioration, all closely related to the AgCC. The multidisciplinary analysis of this clinical case of AgCC suggests that this type of approach can highlight the presence of undiagnosed AgCC or CC abnormalities. Furthermore, it may provide the basis for new neuropsychological and pharmacological therapies.

Keywords: rare brain disease; corpus callosum agenesia; neuropsychology; neuroscience



A clinical case report of cerebellar vermis hypoplasia related to deletion 15q21.3-22.31

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Cerebellar vermis hypoplasia (Vm-Hyp) is a rare malformative condition related to a wide variety of causes characterized by an elective reduction of cerebellar vermis, while the cerebellum mainly conserved its overall shape. Currently, considerable studies highlight a role of the cerebellum in sensory-motor functions and also in non-motor functions (e.g. language, emotion, cognition). However, with high difficulty, non-motor deficits are electively related to cerebellar malformations. In fact, Vm-Hyp is a rare and heterogeneous malformative condition often neglected. In fact, much more often Vm-Hyp is related to malformations of other brain regions. Here, we report the clinical case of a young 29 year old woman presenting an elective Vm-Hyp and a specific 15q21.3-22.31 deletion of about 7Mb. The neuroradiological analyses demonstrated mainly the presence of a Vm-Hyp. However, minor malformations in other brain regions, such as slight hypoplasia of the brainstem and corpus callosum, and a broad dilation of the ventricular system has been also detected. Moreover, in other imaging analyses the absence of malformations in other body regions have been evidenced. The neuropsychological evaluation highlighted a serious impairment of practical-concrete skills, aimlessness, reduced social adaptation, medium-severe cognitive impairment. Although, the correlation between the 15q21.3-22.31 deletion and Vm-Hyp is quite evident, further investigations are necessary to understand the malformative mechanisms, which neuropsychological functions and cerebellar circuits are mainly compromised. In addition, further studies are also necessary for the application of innovative pharmacological and neuropsychological therapies to improve the clinical conditions of patients suffering from rare genetic cerebellar malformations.

Keywords: Human cerebellum, Cerebellar malformations; Genetic deletions; Neuropsychology; Neuroscience

Calretinin in the human brain: a light microscopy immunohistochemical study

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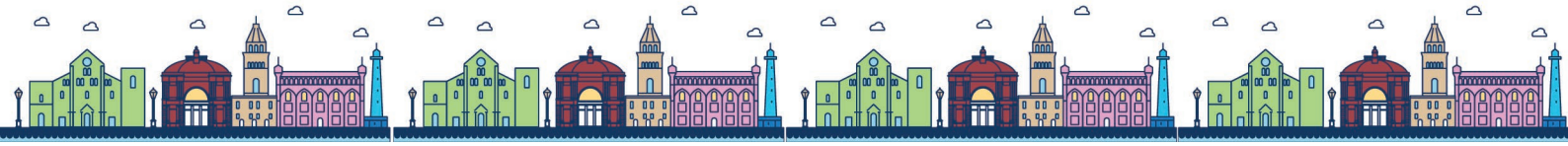
Calretinin (CR) is a highly conserved 29 kDa intracellular calcium binding protein of the EF-hand family, firstly described in the chick retina. CR is composed by 261–271 amino acids. CR and Calbindin D28k present 58% of identical amino acids residues. CR is widely expressed in the neuronal cell bodies and processes and form asymmetrical and symmetrical synaptic specializations on spines, dendrites and somata. Although, studies suggest a role of CR in neurologic, psychiatric and neuropsychiatric disorders and in some rare brain disorders (e.g. Huntington's disease, spinocerebellar ataxias). Currently, non-exist human brain mapping of CR. Therefore, the aim of this study is to carry out, using an immunohistochemical approach, a detailed distribution of CR in the adult human brain and in particular, in the human brain areas affected by rare diseases (e.g. basal ganglia, cerebellum). The study was carried out on postmortem fragments of human brains fixed in neutral buffered formalin, embedded in paraffin, cut into 4 µm sections and subjected to light microscopic immunohistochemistry with mouse polyclonal antibody for CR. For positive controls were used fragments of human mesothelioma subjected to the same experimental procedure. CR-immunoreactivity was observed in the gray matter in bodies and processes of neurons and astrocytes, in the white matter in bodies and processes of oligodendrocytes of different regions of the adult human brain (e.g. midbrain, cerebellum and neocortex gyri). Therefore, these results indicate a role of CR in calcium homeostasis and in neurotransmission and gliotransmission mechanisms. Furthermore, CR mechanisms could be damaged in brain diseases.



Genetic variants and inborn errors of immunity in bone marrow failure: novel potential drug targets for precision medicine

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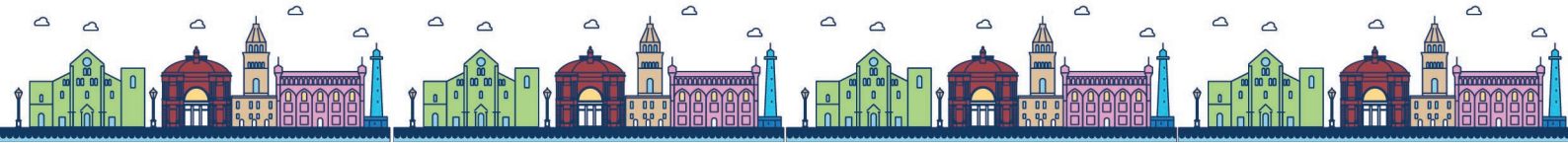
Aim: Recently, we identified a rare Gain of Function (GOF) STAT1 gene heterozygous variant (p.Cys174Arg) in a 32-year-old female patient with symptomatic severe aplastic anemia (AA), suggesting that genetic factors might be involved in bone marrow (BM) failure.
Methods: To investigate the association between potential genetic variants and idiopathic AA, we examined six patients with severe AA. BM biopsy and immunohistochemistry were performed to confirm the diagnosis and whole exome sequencing carried on uncovering inborn errors of immunity.
Results: In all enrolled patients, we found enhanced phospho-STAT1 levels (by BM immunostaining), suggesting that dysregulated JAK-STAT signaling with associated abnormal function of CD8+ T cells might contribute to the pathogenesis of BM failure. In addition to the STAT1 variant, two other underlying genetic events were identified: one patient had a TNFRSF13B (TACI) gene variant involved in B-cell development and function. Two patients had mosaicism of trisomy 8, a chromosomal abnormality associated with BM failure and other blood disorders. Interestingly, treatment with the JAK inhibitor ruxolitinib reduced STAT1 phosphorylation and downregulated the expression of cytotoxicity-related genes in CD8+ T cells from AA patients. These findings suggest the potential of targeting the JAK-STAT pathway as a therapeutic approach for AA and related disorders characterized by dysregulated STAT1 signaling, TNFRSF13B (TACI) variants or mosaicism of trisomy 8.
Conclusions: Our results emphasize the importance of identifying driver genetic events such as the STAT1 variant, TNFRSF13B (TACI) variant, and mosaicism of trisomy 8, as crucial step toward developing more effective diagnostic and treatment strategies.

Keywords: inborn errors; rare genetic variants; autoimmunity diseases; aplastic anemia; JAK/STAT inhibitors



Pharmacological block of prokineticin system and microglia inhibition counteract pain in a murine model of fabry-anderson disease

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Fabry-Anderson disease (FD) is an X-linked lysosomal storage disorder. A deficient alpha-galactosidase A (Gal-A) leads to Gb3 accumulation in different tissues, including the nervous system. FD patients suffer from lifetime-lasting neuropathic pain that develops in childhood and is resistant to specific treatments. Glial and infiltrating immune cells are involved in neuropathic pain pathogenesis. The prokineticin system (PKS) is a family of chemokines important in inflammation and pain, composed of PK2 and its receptors, that may represent a new therapeutic target. PKS antagonists counteract chronic inflammatory/neuropathic pain, contrasting neuroinflammation. This study wants to validate if novel pharmacological strategies (PKS antagonism with PC1 and glial inhibition with Minocycline), contrasting neuroinflammation, may relieve FD neuropathic pain.
FD male mice, Gal-A deficient, were treated with minocycline (10 mg/kg, intraperitoneal, once daily) and PC1 (150 µg/kg, subcutaneous, twice daily) for fourteen consecutive days. Hypersensitivity was constantly monitored. Levels of PKS, cytokines and (neuro)inflammatory markers were evaluated in the sciatic nerve, dorsal root ganglia, spinal cord and gut as mRNA and protein.
FD mice suffer from thermal hyperalgesia, mechanical allodynia, abdominal pain and are slightly hyposensitive to cold stimuli. Moreover, these alterations are associated with marked neuroinflammation. In these animals, there is PKS up- regulation, pro-inflammatory cytokines increase, and glia/immune cells' markers overexpression in all the analyzed tissue. Both PC1 and minocycline contrast all the painful symptoms and reduce neuroinflammation. Our data demonstrate that pharmacological inhibition of the PK system (by PC1) or the glial cells (by minocycline) may be a promising approach to control FD pain.

Exploring the link between GALC and GPR65: implications for Krabbe Disease

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Krabbe disease (KD) is a lysosomal storage disorder caused by the deficiency of galactosylceramidase (GALC), causing psychosine (PSY) accumulation, demyelination and neurodegeneration. Current cure is mostly supportive, and correcting GALC-deficiency may not fully rescue the KD phenotype, hinting at unidentified contributors to pathogenesis.
We identified evidence supporting the G protein-coupled receptor 65 (GPR65) as a key mediator in the PSY-independent mechanism of the disease.
Notably, GALC and GPR65 genes are closely linked since the regulatory non-coding enhancer region (GH14J087949), which activates the transcription of both GALC and GPR65 genes, is annotated in an intron of the GALC gene. Recently, genome-wide association studies reported how variants in the non-coding regions, especially in enhancers, contribute to the pathogenesis of several diseases. Interestingly, one of the most common mutations leading to KD, the 30Kb- deletion, is in the region of the GH14J087949 enhancer, suggesting a correlation between GALC genetic alterations and a decrease in GPR65 expression. GPR65 is highly expressed and exerts its activity in immune system cells, which are some of the cell populations affected in KD. The GH14J087949 enhancer is associated with an active epigenetic state specifically in immune cells. GPR65 induces anti-inflammatory response, and KD is characterized by profound neuro-inflammation.
Preliminary studies on GPR65 expression in KD. Our data clearly show that GPR65 is highly downregulated in the lymphocytes of patients with KD compared to healthy controls, and also in thyme of mice with KD, suggesting a route for the identification of a new PSY- independent mechanism of the disease.

Keywords: Krabbe disease, leukodystrophy, GALC, GPR65, inflammation

Phosphodiesterase 5 Inhibitors as a new treatment for Maternally Inherited Leigh Syndrome

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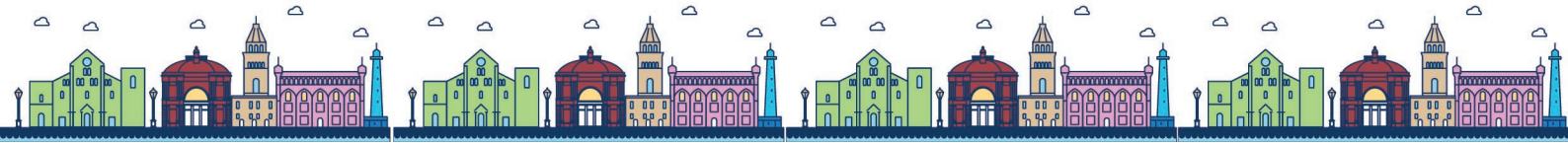
Aim: This project aims to: i) establish a novel preclinical model for Maternally Inherited Leigh Syndrome (MILS) to be exploited for a large (> 5500 compounds) high-throughput screening of repurposable drugs; ii) validate the phosphodiesterase 5 inhibitor (PDE5i) Sildenafil as an effective therapeutic option for MILS.
MILS is a severe, incurable, pediatric neurological disorder caused by mutations in the mitochondrial DNA (mtDNA), most frequently affecting the MT-ATP6 gene encoding for the "subunit a" of the mitochondrial ATP Synthase (or Complex V). Recently, PDE5 inhibitors emerged as candidate compounds for the therapy of MILS.
Methods: through a reprogramming-based strategy, we generated human MILS-neural precursor cells (NPCs) carrying homoplasmic mutations 8993T>G, 8993T>C, m.9185T>C and m.9176T>G and matched controls, and we characterised their metabolism, mitochondrial membrane potential (MMP), Complex V activity and assembly. We performed overnight treatment with Sildenafil 10 μM, checked its pharmacological effects on MILS-NPCs pathological hallmarks, and investigated its mechanism of action. At least n=3 biological replicates were performed for each assay, and data were analysed through 1-way ANOVA or paired t-test when appropriate.
Results: MILS-NPCs displayed consistent pathological hallmarks, including mitochondrial hyperpolarization (fold increase: 1.23-1,32 $p < 0.05$ for all cell lines), reduced complex V stability and activity (residual activity: 20%-29%; $p < 0.005$ for all cell lines), and metabolic defects. MMP was selected as the readout for high-throughput drug screening. Sildenafil restored physiological MMP ($p < 0.005$ for all cell lines) through a cGMP- dependent mechanism.
Conclusions: MILS-NPCs are an effective tool for drug screening and validation.

Keywords: mitochondrial disease; MILS; PDE5i; NPCs, drug screening



Theβ3-AR agonist BRL37344 ameliorates the main symptoms of X-Linked nephrogenic diabetes insipidus in the mouse model of the disease

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X-linked Nephrogenic Diabetes Insipidus (X-NDI) is a rare congenital disease caused by inactivating mutations of the vasopressin type-2 receptor (AVPR2), characterized by impaired renal concentrating ability, dramatic polyuria, polydipsia, and risk of dehydration. The disease, which still lacks a cure, could benefit from the pharmacologic stimulation of other GPCRs, activating the cAMP-intracellular pathway in the kidney cells expressing the AVPR2. On the basis of our previous studies, we here hypothesized that the β3-adrenergic receptor could be such an ideal candidate.
In the mouse model of X-NDI we evaluated the effect of continuous 24h stimulation of the β3-AR with the agonist BRL37344 and assessed the effects on urine output, urine osmolarity, water intake and the abundance and activation of the key renal water and electrolyte transporters.
We demonstrate that the β3-AR agonism exhibits a potent antidiuretic effect. The strong improvement in symptoms of X- NDI produced by a single i.p. injection of BRL37344 (1mg/Kg) was limited to 3h, but repeated administrations in the 24h, mimicking the effect of a slow release preparation, promoted a sustained antidiuretic effect, reducing the 24h urine output by 27%, increasing urine osmolarity by 25%, and reducing the water intake by 20%. At the molecular level, we show that BRL37344 acted by increasing the phosphorylation state of NKCC2, NCC, and AQP2 in the renal cell membrane, thereby increasing electrolytes and water reabsorption in the kidney tubule of X-NDI mice.
Taken together, these data suggest that human β3-AR agonists might represent an effective possible treatment strategy for X-NDI.

Dual Targeting of the G protein-coupled receptors CaSR and V2R for treating autosomal dominant polycystic kidney disease (ADPKD)

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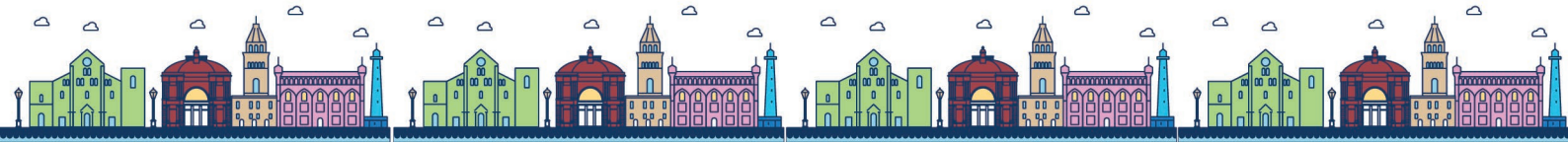
ADPKD, caused by mutations in PKD1 or PKD2 genes, is characterized by development and growth of cysts causing progressive kidney enlargement. Reduced resting cytosolic calcium and increased cAMP levels, associated with the tonic action of vasopressin, are two central biochemical defects in ADPKD. Currently, the vasopressin V2 receptor (V2R) antagonist tolvaptan is the only drug approved to delay the progression of ADPKD, however it causes serious hepatocellular toxicity. Simulations on a multiscale computational model of drug-induced liver injury indicate that the novel V2R antagonist lixivaptan has a safer liver profile.
Here, we show that co-targeting two GPCRs, the Calcium Sensing Receptor (CaSR) and the V2R, using the calcimimetic R-568 in combination with lixivaptan, reduced cyst progression in two animal models of human PKD.
PCK rat and Pkd1RC/RC mouse littermates were fed ground rodent chow without or with lixivaptan and R-568, alone or in combination.
In PCK rats, the combined treatment strongly decreased kidney weight, cyst and fibrosis volumes by 20%, 49% and 73%, respectively, compared to animals fed with standard diet. In Pkd1RC/RC mice the same parameters were reduced by 20%, 56%, and 69%, respectively. Interestingly, recent data in human conditionally immortalized Proximal Tubular Epithelial cells, with stably down-regulated PKD2, demonstrated the possibility that CaSR is involved in PC2 functional regulation.
These data suggest an intriguing new application for two known drugs. The potential for synergy between these two compounds and the functional coupling of CaSR and PC2 warrant further investigation in clinical settings.

Keywords: GPCRs, ADPKD, vaptans, calcimimetic



What is hidden in patients with unknown nephropathy? The genetic screening could represent the missing link in the diagnosis and management in kidney transplantation

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Background: 15-20% of patients with end stage renal disease (ESRD) do not know the cause of the primary kidney disease and can develop complications after kidney transplantation. We performed a genetic screening in 300 patients with kidney transplantation, or un-diagnosed primary renal disease, in order to identify the primary disease cause and discriminate among overlapping phenotypes.
Methods: We used a custom-made panel for Next Genera-tion Sequencing (Agilent technology), including genes associated to Fabry disease, podocytopaties, complement-mediated nephropathies and Alport syndrome related diseases.
Results: We detected candidate diagnostic variants in genes associated to nephrotic syndrome and FSGS in 29 out of 300 patients, solving about 10% of the probands. We also identified the same genetic cause of the disease (PAX2: c.1266dupC) in 3 family members with different clinical diagnosis. Interestingly we also found one female patient carrying a novel missense variant, c.1259C>A (p.Thr420Lys) in the GLA gene not previously associated to Fabry disease, and in silico defined as Likely pathogenic and destabilizing, associated to a mild alteration in GLA enzymatic activity.
Conclusions: The identification of the specific genetic background may give the opportunity to evaluate the risk of recurrence of the primary disease especially for patients candidate to living donor kidney transplant.

Characterization of lipid and lipoprotein profile in Alagille syndrome

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Aim: Alagille syndrome (ALGS) is a rare disease (1/70.000) variably characterized by chronic cholestasis due to paucity of intrahepatic bile ducts, peripheral pulmonary artery stenosis, vertebrae segmentation anomalies, characteristic facies, pigmentary retinopathy, and dysplastic kidneys. Most patients experience intractable pruritis and the presence of xanthomas, localized cholesterol and fats deposits under the skin caused by this liver dysfunction. Hypercholesterolemia is a well-known cardiovascular risk factor associated with atherosclerosis and arterial stiffening in liver disease. However, the role of lipid in ALGS-associated cardiovascular risk is unclear and lipids and lipoproteins are poorly characterized. Aim of this work was to characterize lipid and lipoprotein alterations in ALGS to identify potential pharmacological targets.
Methods: Six pediatric patients with ALGS were enrolled and lipid and lipoprotein profile was characterized.
Results: Three patients show high LDL-C, the presence of LpX and reduced HDL-C associated to alteration in subclasses distribution. Moreover, an impaired activity of LCAT enzyme is detected and can explain the increased free to total cholesterol ratio in these patients. Interestingly, the in vitro incubation with a synthetic LCAT activator is able to ameliorate lipid profile. However, these alterations are not observed in all patients.
Conclusions: In our study, patients showing lipid abnormalities have more severe symptoms compared to patients with normal lipid profile. The correlation between lipids and the disease severity needs further studies to identify possible targets for therapeutic approaches aimed at preventing the onset and progression of cardiovascular and renal complications in ALGS.

Riboflavin Transporter Deficiency Type 2 (RTD2, OMIM #614707): a focus on the Endoplasmic Reticulum responses

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Background and aim: Riboflavin (Rf) in its active forms, FMN and FAD, provides important functions in bioenergetics, epigenetics and oxidative protein folding [1]. Rf Transporter Deficiency Type 2 (RTD2, OMIM #614707) is a rare neurodegenerative disorder (prevalence: 1 in 1 million births) associated with biallelic pathogenetic SLC52A2 variations. The current treatment involves supplementation with high doses of Rf together with antioxidant and other supplements [2].
We are involved in studying the molecular and cellular consequences of SLC52A2 variations and defining the molecular mechanisms underlying RTD2, on the way to search for molecules of pharmacological interest.
Methods: Patients' cells were grown as in [3, 4]. Cytosolic Ca^{2+} dynamics and mitochondrial morphology were assessed by FURA-2 AM and MitoTracker® Green, respectively. WB and qPCR were used to evaluate the levels of sensors of unfolded protein responses (UPRER) [3]. Rf transport assays were performed with recombinant WT and mutated RFVT2s reconstituted in proteoliposomes [5].
Results: In fibroblasts and iPSC-derived motor neurons from RTD2 patients, we observed an altered mitochondrial morphology together with altered Ca^{2+}-mediated responses. Interestingly, in both cell models, UPRER sensor levels increased, presumably due to altered protein folding/maturation. If this is the case, a chaperone-based therapy might be suggested.
In parallel, we set up a RFVT2/proteoliposome model to perform both kinetic and molecular characterization of the protein mutants, on the way to test the effect of Rf and compounds of pharmacological interest [4].
Conclusions: The results of our basic research on RTD2 introduced UPRER as a novel putative pharmacological target and a new molecular proteoliposome model for the screening of potential drugs.

Keywords: RTD2, SLC52A2, Ca^{2+} signalling, mitochondria derangement, UPR^{ER}

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Off-label use of Liraglutide counteracts the immune dysregulation associated with Wolfram syndrome

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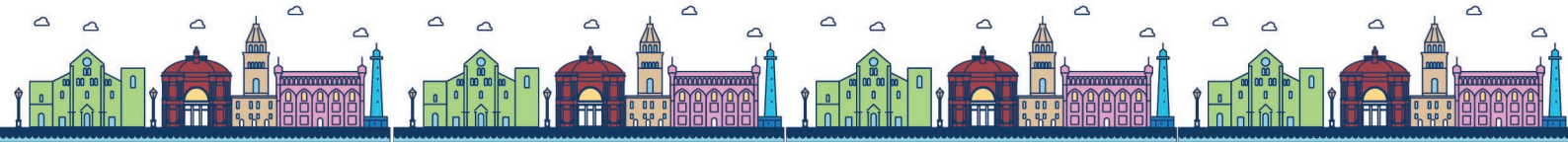
Aim: Mutations in the WFS1 gene, encoding wolframin (WFS1), causes Wolfram Syndrome (WS). WS is clinically characterized by childhood-onset diabetes mellitus, optic atrophy, deafness, diabetes insipidus, neurological signs. We recently described the case of a WS patient bearing two novel WFS1 mutations with immune dysregulation and high levels of proinflammatory cytokines produced by peripheral blood mononuclear cells (PBMCs).
This study aimed to expand the cohort of patients to investigate the possible impact of WFS1 mutations on the immune system. Furthermore, since some patients undergo an off-label use of the GLP1-receptor agonist liraglutide, we investigated if this treatment could affect the immune cells.
Methods: Flow cytometry multi-colored staining analysis of PBMCs from peripheral blood samples (from San Raffaele Hospital in Milan (Italy)) from 10 WS patients (4 males, 6 females) bearing different types of WFS1 mutations and with different clinical manifestations. Among these, 6 patients were receiving liraglutide. Statistical analysis was performed using ANOVA followed by post-hoc Bonferroni's test.
Results: The comparison between WS patients and healthy donors highlighted the activation of the immune system, as there is a significant ($p<0.05$) increase in HLA-DR+ cells, B cells, CD8+ effector memory cells, Th1 CD4+ T cells and plasmacytoid DCs (pDCs). On the contrary, CD8+ naïve and CD8+ effector cells were significantly decreased ($p<0.05$). The off-label use of liraglutide was capable of counteracting the dysregulation.
Conclusions: Overall, the immunophenotyping of PBMCs highlighted in WS patients an important alteration of several immune subsets, and revealed that liraglutide counteracts this immune dysregulation.

Keywords: Wolfram Syndrome, wolframin (WFS1), liraglutide, immune dysregulation



Anti-yo mediated paraneoplastic cerebellar degeneration: a case report

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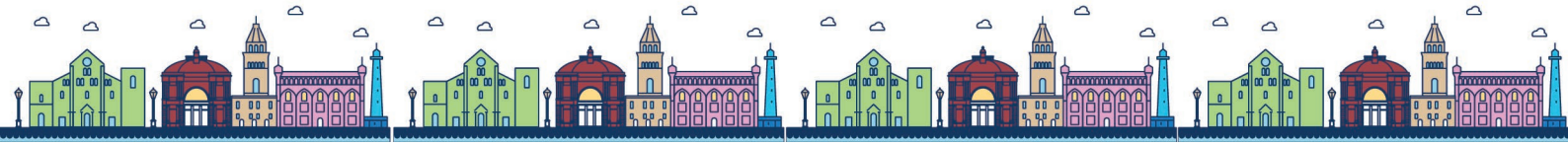
Paraneoplastic cerebellar degeneration (PCD) mediated by anti-Yo autoantibodies is a rare cerebellar disease that occurs during specific tumors (e.g. breast cancer, female pelvic cancer). Among PCDs, that caused by anti-Yo autoantibodies has a female predominance. Anti-Yo are specific autoantibodies directed against a cytoplasmic antigen of Purkinje neurons. We report a translational evaluation of a clinical case of PCD due to anti-Yo in a 58-year-old woman with breast cancer, estrogen positive, progesterone negative, Ki67 positive, Her-2 positive and highly positive for anti-Yo autoantibodies. The patient was not affected by previous neurological, psychiatric and autoimmune diseases. The multidisciplinary evaluation showed the co-presence of cerebellar symptoms and signs, i.e. bilateral deficits characterized by trunk and limb ataxia, nystagmus, diplopia, dizziness and ataxic dysarthria. Depression and anxiety were noted in the psychiatric evaluation. MRI analysis of the brain revealed atrophy of the cerebellar vermis and a dilation of all the cerebellar sulci. Neuropsychological evaluation revealed a probable cerebellar cognitive affective syndrome, characterized by deficits in executive functions, spatial cognition and visuospatial memory, inappropriate behavior and depression. Furthermore, the patient underwent pharmacological therapies (corticosteroids and plasmapheresis) and neuromotor rehabilitation treatments, achieving only modest improvements. In conclusion, the translational evaluation highlighted the coexistence of motor and non-motor (e.g. cognitive-affective) cerebellar deficits. In conclusion, further specific studies are necessary to identify early diagnostic criteria and effective pharmacological therapies aimed at improving the evolution of this rare autoimmune disease of the cerebellum.

Keywords: Human cerebellum; Paraneoplastic cerebellar degeneration; anti-Yo autoantibodies; Breast cancer; Neuroscience



CD90-TGF β1 co-expression promotes chemoresistance in intrahepatic cholangiocarcinoma

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Aim: Intrahepatic cholangiocarcinoma (iCCA), a biliary tract cancer, is the second most common malignant tumour developing in liver. As reported, stemness induces chemoresistance in several tumors. In iCCA, CD90 stem cell marker, is associated with a poor prognosis and TGFβ pathway causes tumor progression. Based on these key points, our aim is to investigate CD90-TGFβ1 correlation and the functional mechanisms that explain this relationship.
Methods: RNA sequencing expression data on iCCA patients' tissues and matched surrounding normal liver tissues from two databases was analyzed by bioinformatic approach. Protein expression of TGF β1 pathway was evaluated in transfected HuCCT1 cell line to overexpress CD90 by western blot. Viability 2D and 3D assay was performed on HuCCT1 empty vector (CD90-) or CD90+. Cell lines were treated with increasing concentrations of gemcitabine in monotherapy or in combination with galunisertib, a TGFβ1RI inhibitor, for 72 hours.
Results: In this study, by two GEO database cohorts of iCCA samples, we demostrate that TGF β1 gene expression correlates with CD90 mRNA. In vitro, we confirm that HuCCT1 CD90+ express high levels of pSMAD compared to HuCCT1 CD90- cell lines. HuCCT1 CD90+ treated with gemcitabine alone at increasing doses result resistant compared to CD90- cells that instead respond in a dose-dependent manner. Furthermore, the gemcitabine-galunisertib combination has a synergic effect only on HuCCT1 CD90+ proliferation unlike CD90, in which the combination shows no change.
Conclusions: These data prompted us to consider that CD90-TGFB co-expression is a novel dualism to promote chemoresistance and gemcitabine-galunisertib combination could be a potential terapheutic option for iCCA patients.

Keywords: iCCA, CD90, TGF β1, Gemcitabine, Galunisertib

Sessione 4

Poster

Human cellular preclinical models to understand congenital myopathies: from pathogenic mechanism to new therapeutical targets in TAM and SEPN1-RM diseases

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Background: Congenital myopathies are a group of genetic muscle diseases classified based on distinctive morphological abnormalities in skeletal muscle. We focus our interest on Tubular Aggregate Myopathy (TAM) and Selenoprotein1(SEPN1)-related myopathy (SEPN1-RM), which are part of this group of diseases. TAM is a rare muscle disease caused by Gain-of-Function mutations in STIM1/ORAI1, the major protein involved in a mechanism critical for maintaining Ca^{2+}homeostasis, called SOCE, and characterized by the presence of tubular aggregates in muscle biopsies. SEPN1-RM belong to a group of rare congenital myopathies in which SEPN1 mutations alter the selenoprotein1 expression, an endoplasmic reticulum protein involved in redox-based Ca^{2+}homeostasis and cell protection against oxidative stress. In both cases patients show muscle weakness, fatigue, myalgia, and, for SEPN1-RM, life-threatening respiratory distress. Therapeutic options remain limited and, although the availability of animal models get a hopefully chance in this context (1-3), they partially replicate human disease.
Methods: In our laboratory, we have created a reliable cellular model useful for TAM preclinical studies, consisting of myoblasts and myotubes deriving from TAM patients' biopsy carrying Leu96ValSTIM1 mutation. With the same methodological approach, we began to generate and validate a new mutation causing SEPN1-RM.
Results: We characterized for the first time the molecular mechanism underlying TAM disease associated to Leu96ValSTIM1 mutation, showing the presence of Ca^{2+}overload and a defect of myogenesis at the late differentiation phase. The characterization of the patient biopsy- derived myoblasts and myotubes associated to a SEPN1mutation are ongoing.
Conclusions: Our study will allow to identify new therapeutical targets, with a reliable translation in the clinical management of SEPN1-RM and other congenital myopathies.

Keywords: patient-derived cellular model; SEPN1-RM TAM; Calcium homeostasis myogenesis

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In vitro 3D-model of mitochondrial myopathy human skeletal muscle

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Background: Mitochondrial myopathy is one of the most common neuromuscular disorders for which no cure is currently available. Performing mechanistic study and screening compound libraries on myoblast cultures is not time and cost effective, since myoblasts are structurally and metabolically distinct from mature muscle fibres.
Aim: The aim of this study is to develop functional *in vitro* 3D-models of mitochondrial myopathy human SKM that could be applied for the search of a suitable compound to treat mitochondrial myopathy patients.
Methods: We used primary myoblasts obtained from mitochondrial myopathy patients carrying nuclear variants in *TWNK*, *RRM2*B and *SURF1* genes. After differentiation into myotubes, the characterization of the *in vitro* 3D-models of mitochondrial myopathy included the assessment of SKM structure by immunofluorescence and contraction and Ca^{2+} dynamics induced by electric pulse stimulation.
Results: The generated *in vitro* 3D-models present different SKM structure. *RRM2B* myoblasts are not able to differentiate, therefore the SKM specific marker SAA is not expressed in the 3D-model, oppositely to *TWNK* and *SURF1* 3D-models, which both present good SKM directionality (d=0.76 and d=0.93, respectively) and express high SAA level. Moreover, *TWNK* and *SURF1* 3D-models functionally contract when electrically stimulated, although displaying different SKM Ca^{2+} dynamics compared to healthy control 3D-muscles.
Conclusions: We generated functional and contractile *in vitro* 3D-models of mitochondrial myopathy human SKM using primary cells from patients carrying nuclear variants. In the future, primary cells from patients carrying mtDNA variants will be used. Overall, the application of such a *in vitro* 3D- model will accelerate the search of a suitable cure for mitochondrial myopathy patients.

Abbreviations: Ca^{2+} (calcium), mtDNA (mitochondrial DNA), SAA (sarcomeric alpha-actinin), SKM (skeletal muscle).

Keywords: mitochondrial myopathy, human skeletal muscle, *in vitro* 3D models, electric pulse stimulation, Ca^{2+} dynamics

Optogenetic skeletal muscle-on-chip as a drug screening and disease modelling platform for rare neuromuscular disorders

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Duchenne Muscular Dystrophy (DMD) is a X-linked recessive, muscle-wasting disorder that leads to progressive tissue wasting, loss of function1 and premature death2. The disease is caused by mutations in the gene that encodes the dystrophin protein (DMD). Thus far, there is no resolutive cure for the DMD. Preclinical research of neuromuscular diseases mostly relies on in vitro and animal models of the pathology, but such models fail to fully translate the human disease setting. In this study, we aim to build a 3D optogenetically engineered muscle-on-chip to serve as a platform for disease modelling and drug testing. To develop a muscle-on-chip, we used a patient-derived immortalized dystrophic cell line, (granted from the biobank MyoLine held by Prof. Mouly) from a 11-year-old patient with mutation in exon 59. Bundles were formed with a hydrogel solution (Matrigel/Geltrex and fibrin) by using microchips (provided by BIOND Solutions). Chips were treated using plasma technology to enhance cell adhesion and differentiation. In parallel, cells were transfected with channelrhodopsin-2 using lipid nanoparticles (LNPs) as transfected reagent to induce differentiation by light stimuli.
We obtained hydrogel-based bundles on plasma-treated chips by using human dystrophic myoblasts. Bundles at day 5 of differentiation were immunostained to evaluate their level of maturation. Parallel 2D experiments on plasma-coated plates revealed that plasma treatment enhanced cell differentiation significantly. Transfection experiments with LNPs formulation revealed that 96% of our cells was successfully transfected with ChR2. qPCR and electrophysiological evaluations will be performed to further assess the level of maturation and differentiation of our tissues.

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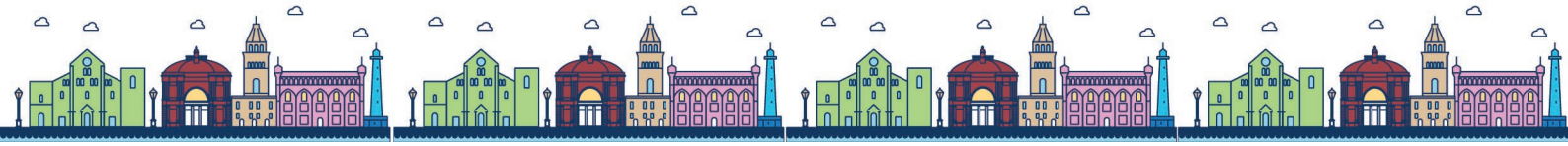

Characterization of Cellular Differentiation in an in vitro model of Neuromuscular Junction using a Co-Culture system: A Preliminary Study of a Drug Testing Platform

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Aim: Neuromuscular junction (NMJ) alteration is involved in a variety of diseases, among them, the Amyotrophic Lateral Sclerosis. In vitro models of the NMJ have proven to be invaluable platforms for testing new drugs. In this preliminary study, our goal was to establish a simple and adaptable in vitro platform for drug investigations. We focused on the cellular differentiation preceding the formation of the NMJ using a co-culture system derived from immortalized muscle (C2C12) and neuronal (NCS-34) cells, as previously described (Taro Inoue et al. 2021).
Methods: We assessed the expression of genes associated with neuronal (Hb9 and Map2) and muscular (Mhc1, MyoD, MyoG, and AChRE) differentiation in the co-culture at 0, 7, and 9 days of differentiation (0D, 7D, 9D) by RT-PCR. Additionally, differentiation was examined at 9D, through immunofluorescence analysis using alpha-bungarotoxin and antibodies targeting peripherin and laminin.
Results: The expression of MyoG and Map2 increased from 0D to 9D, while Mhc1 and MyoD expression was only detectable at 9D. However, the expression of mature NMJ markers (Hb9 and AChRE) was not detectable at 9D. Immunofluorescence with laminin revealed the initiation of differentiation in both cell types, including myocyte formation. Peripherin exhibited the development of elongated neurites in NSC-34 cells, while alpha-bungarotoxin was not detectable.
Conclusions: After 9 days of co-culture, both C2C12 and NSC-34 cells began their differentiation process, but the synaptic junction still remained immature. The study of cell differentiation before the NMJ has formed can help in understanding mechanisms underlying the impairment in neuromuscular diseases.

Keywords: neuromuscular junction, in vitro co-culture system, muscle and neuronal differentiation

Urine-derived stem cells and derived skeletal muscle cells as a functional model to study calcium homeostasis perturbation in neuromuscular diseases

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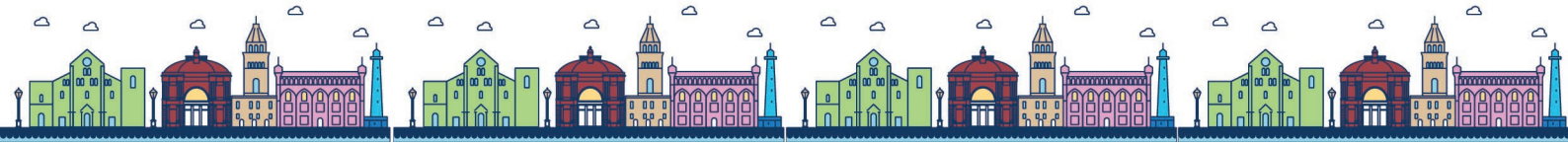
Aim: Rare muscular diseases are characterized by a wide genetic diversity with the Ca^{2+}-signalling machinery often perturbed. An appropriate personalized cellular model is required to understand their pathophysiology, and which may aid in developing novel therapies. The present study aimed to establish a functional cellular model of skeletal muscle cells (USC-SkMCs) differentiated from urine-derived stem cells (USCs).
Methods: We isolated USCs and differentiated into USC-SkMCs by MyoD LV-transduction. We assessed myogenic markers and calcium homeostasis players expression. Then, we evaluated the variation in Ca2+-transient under different stimuli in live imaging microscopy and the contraction capability by collagen assay.
Results: USC-SkMCs expressed high level of myogenic markers. USCs and differentiated cells differed in the expression of key proteins involved in Ca^{2+}-homeostasis and displayed different Ca2+-responses to external stimuli, confirming that during differentiation there was a transition from a non-excitable to an excitable phenotype. In USCs, the main mechanism of calcium entry was IP3 dependent, while in USC-SkMCs both store- and receptor-operated calcium entry were active. Furthermore, a ryanodine receptors and the voltage-operated calcium channels are operative in USC-SkMCs, unlike in USCs. USC-SkMCs rapidly contracted under acetylcholine stimulus.
Conclusions: This study opens an avenue to establish patient-derived disease models where perturbations of Ca^{2+} homeostasis in muscular disorders can be evaluated. This model, therefore, provides a tool to evaluate the single pathological defects of each patient, without requiring experimental manipulations to reproduce the mutation, thereby providing the opportunity to carry out personalized translational characterizations, avoiding invasive or ethically unacceptable methods for sample sampling.



Patient-specific neuronal stem cells as an in vitro model for screening drug safety and efficacy for Aicardi-Goutières pediatric patients

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Background and aim: Aicardi-Goutières Syndrome (AGS, ORPHA:51) are rare genetic neuro- inflammatory diseases, classified among the type I interferonopathies. Dysregulation of the cGAS-STING pathway is pathogenetic for AGS [1-5]; in clinics, treatments are only partially effective and prognosis is poor. Aim of the study is to generate patient-specific neural stem cells (NSC) and neurons to screen in vitro effect and safety of conventional and potential innovative therapies for AGS (e.g. immunosuppressant dexamethasone and cGAS inhibitor mepacrine, respectively, among others).
Methods: IPSCs from three AGS patients with different causative gene mutations (AGS1, AGS2, AGS7) and healthy donor (BJ) were previously generated [6-8], and differentiated into NSCs. Cytotoxic effects of a panel of drugs were tested by MTT assay; results were analyzed using a nonlinear regression. Expression of stemness genes and/or key genes involved in drug pathways were analyzed by real-time PCR. Comparison between NSCs and immortalized cells used as control were performed by ANOVA and Bonferroni's post-test. Statistical significance was set at $p<0.05$.
Results: Characterization of NSCs confirmed their neural stemness profile. NSCs were resistant to dexamethasone in contrast to control NALM6 (IC50: 7.7 ± 0.8 nM; $p<0.0001$); all cell lines were sensitive to mepacrine (mean NSC-IC50: 1.63 ± 0.7μM; NALM6 IC50: 0.25 ± 1.5μM; $p<0.0001$). A lower expression of *NR3C1* gene encoding the glucocorticoid receptor and MB21D1 gene encoding cGAS was observed in NSC vs NALM6 ($p<0.05$, $p<0.0001$, respectively).
Conclusions: Dexamethasone (but not mepacrine) seems safe for NSC; the effects of these drugs will be further investigated in patients-derived neurons, once established.

Keywords: Aicardi-Goutières syndrome, Neural stem cells; therapy personalization; dexamethasone, mepacrine

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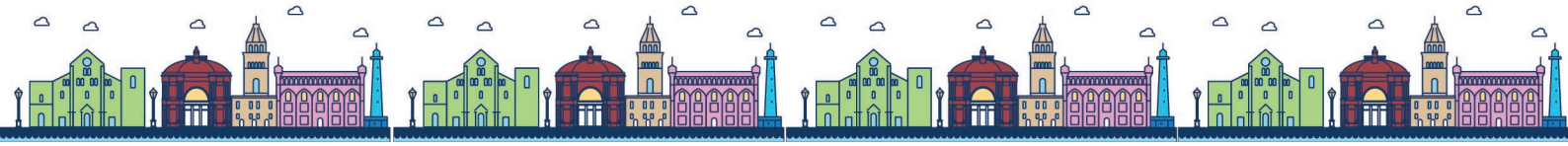

Impaired bioenergetic profile in neuron progenitor cells from iPSCs of patients affected by AGC1 deficiency

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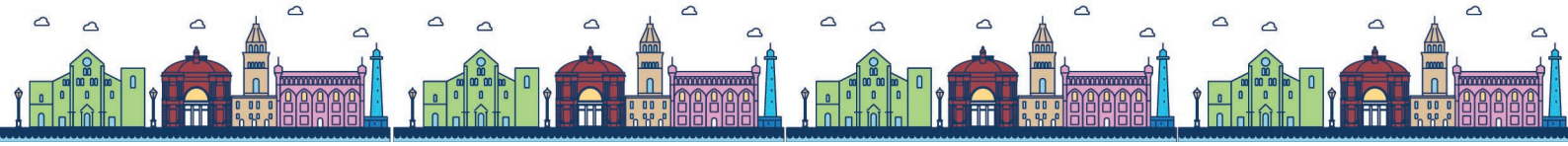
AGC1 deficiency is a rare encephalopathy (DEE39, OMIM# 612949) that manifests in infants with neuromuscular delay, hypotonia, epilepsy, and hypomyelination associated with reduction of brain N-acetyl- aspartate, the precursor of the myelin lipids in CNS. AGC1 deficiency is caused by mutations of SLC25A12 gene, encoding the isoform 1 of the mitochondrial aspartate/glutamate carrier (AGC1). AGC1 catalyses a Ca^{2+}- stimulated entry of glutamate into mitochondria in exchange for aspartate and is essential for the correct oxidation of glucose in neurons and the import of the glycolysis-derived reducing equivalents in the mitochondrial matrix since it is a component of the malate-aspartate NADH shuttle [1].
To study the underlying pathogenetic mechanisms of AGC1 deficiency, fibroblasts from two patients carrying different mutations in *SLC25A12* were reprogrammed into induced Pluripotent Stem Cells (iPSCs) for the subsequent differentiation in neuronal progenitor cells (NPCs). NPCs from one patient expressed the R353Q AGC1 mutant, which retained about 15% of the normal AGC1 transport activity [2], while NPCs from a newly identified patient carried compound heterozygous mutations (c.225del; p(Glu76Serfs*17) and c.1747C>A; p.(=)) that completely prevented carrier expression due to altered splicing.
Patient NPCs showed reduced cell size and higher tendency to aggregate, as compared with control NPCs from healthy individuals. In addition, patient NPCs revealed a proliferation deficit, particularly when deprived of glutamine, with increased cell death and elevated expression of apoptotic markers, as compared to controls. Along with higher lactate production, NPCs from both patients revealed a higher glycolytic activity and a significant increase in total ATP production, although associated with a dramatic reduction of mitochondrial oxygen consumption rates (OCR), when measured in the presence of glucose alone or in combination with other respiratory substrates, such as pyruvate or lactate. Metabolomic analyses revealed that the observed OXPHOS impairment in patient NPCs was correlated with a significant reduction in the intracellular content of key Krebs Cycle intermediates, as well as a decrease in the NAD+/NADH ratio. These findings suggested a compromised mitochondrial pyruvate oxidation and an altered redox balance in AGC1-deficient NPCs. Since the administration of the ketogenic diet improves myelination and the clinical outcomes in the patients with AGC1 deficiency [3], we evaluated the effect of ketone bodies acetoacetate and beta-OH- butyrate on NPCs mitochondrial respiration. OCR measurements revealed that ketone bodies significantly enhanced mitochondrial respiration in NPCs with impaired AGC1, but only in the absence of glucose and particularly in combination with glutamine. Overall, our data suggest that the administration of alternative metabolites, which generate acetyl-CoA in the mitochondria or TCA cycle intermediates, may benefit NPCs of AGC1 deficiency patients by bypassing the limited oxidation of pyruvate derived from glycolysis.



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A novel renal collecting duct model to study secondary Nephrogenic Diabetes Insipidus associated to Cystinosis

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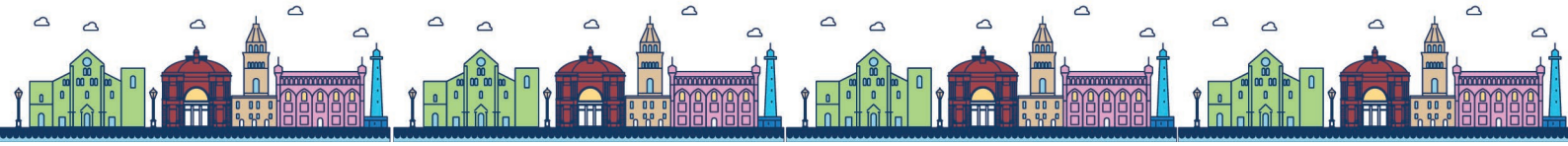
Cystinosis, a lysosomal disease, is caused by mutations in CTNS gene, encoding for cystinosin. It manifests as renal Fanconi syndrome, leading to end stage renal failure. Secondary Nephrogenic Diabetes Insipidus (NDI) occurs as complication of cystinosis, due to the resistance to vasopressin, a key player of the physiological mechanism of water reabsorption in the collecting duct, as well as the vasopressin-sensitive water channel Aquaporin-2 (AQP2). To date, no collecting duct in vitro models have been established for cystinosis, nor the involvement of the Vasopressin-AQP2 pathway has been investigated. Here we established and characterized a collecting duct in vitro model for cystinosis and we investigate the molecular mechanisms causing vasopressin-resistance. CRISPR/Cas9 CTNS KO model derived from MCD4 cells, a mouse collecting duct cell line, stably expressing human AQP2 and vasopressin receptor 2 (V2R), was established and validated by Sanger sequencing, qPCR and mass spectrometry (MS). Osmotic permeability measurements in presence or absence of Desmopressin (DDAVP), a synthetic vasopressin analog, were performed to investigate AQP2 function. Sanger sequencing demonstrated that CTNS was efficiently CRISPRed. This was further confirmed by significant reduction of CTNS transcript levels in KO cells compared to wt (p=0,0006), and significant cystine accumulation by MS in KO cells compared to wt (p=0,070). Osmotic water permeability measurements indicated that, compared to control, KO cells do not significantly increase water permeability in response to DDAVP, consistent with an impairment of vasopressin-AQP2 pathway. We provide here the first cystinotic collecting duct model for the study of secondary NDI in cystinosis.

Keywords: AQP2; NDI; CRISPR/Cas9; Vasopressin



A molecular docking-based virtual screening study to tackle the cure for Lafora Disease

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Aim: Lafora disease (LD) is a rare, recessively inherited condition causing progressive myoclonic epilepsy and declines in cognition and motor function.[1] This neurodegenerative disease is caused by the mutation of two genes, *EPM2A* and *EPM2B*, encoding two proteins involved in the regulation of glycogen synthesis, laforin and malin, respectively. Their dysfunctions result in brain insoluble glycogen inclusions called Lafora bodies, which accelerate neurodegeneration[2]. At the date, no treatment can arrest the disease outcomes and restrict glycogen storage. Only symptomatic approaches are available based on antiseizure medications (ASMs), ketogenic diet or metformin3,4.
Methods: Starting from the hypothesis that modulating glucose access into neurons and astrocytes or inhibiting glycogen synthesis could reduce glycogen accumulation, human glycogen synthase (hGYS1) and glucose transporters (hGLUT1/3) have been investigated by employing molecular docking based virtual screening by using an *in house* curated database of 65 ASMs.
Results: Satisfactorily, preliminary *in vitro* assays performed on a set of candidate compounds caused the reduction of the intercellular glucose concentration confirming and validating the computational studies.
Conclusions: To guarantee that patients receive prompt and efficient treatment, novel pharmacological targets and medications must be found. In parallel *in silico* and experimental studies focusing on drugs acting on glycogen metabolism are ongoing.

Keywords: Lafora Disease, Virtual screening, Molecular docking

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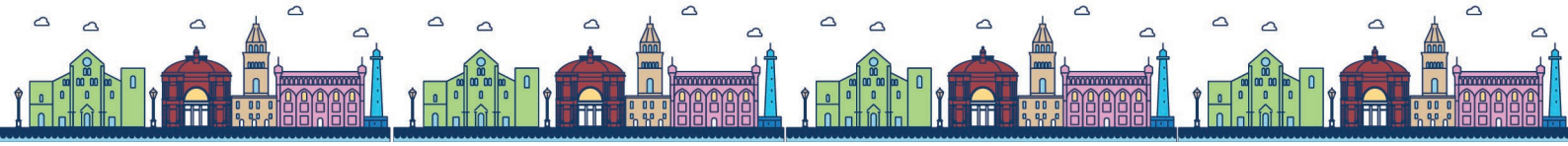

Computational Screening for Lafora Disease: Repurposing of approved Drugs as a Novel Treatment Strategy

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Aim: Lafora disease (LD) is a rare, recessively hereditary disorder that results in progressive myoclonic epilepsy as well as cognitive and motor function losses.[1] The major causes of LD are mutations in two genes, EPM2A and EPM2B, which encode two proteins involved in the regulation of glycogen production, laforin and malin, respectively. These dysfunctions, in fact, generate insoluble glycogen inclusions in the brain known as Lafora bodies, which hasten neurodegeneration. There is currently no medication that can slow the disease's progression and limit glycogen accumulation. [2,3] In this respect, the purpose of this project is to accelerate the discovery of novel active compounds by utilizing computational approaches validated by *in vitro* research.
Methods: human glycogen synthase (hGYS1) and glucose transporters (GLUT1/3) have been investigated to identify putative binders among commercial drugs using the virtual screening workflow (belonging to the Schrödinger suite[4,5]), which implements three different and increasingly refined molecular docking steps. A database composed by the approved drugs retrieved within the latest version of ChEMBL database was thus used for the computational analyses towards hGYS1 and GLUT1/3 3D structures.
Results: Satisfactorily, initial in vitro assays performed on a set of candidate compounds returned from the virtual screening campaign resulted in a decrease of intercellular glucose concentration, verifying and validating the computational investigations.
Conclusions: Novel pharmacological targets and drugs must be discovered to ensure that patients receive fast and efficient therapy. In this scenario, parallel *in silico* and experimental investigations on compounds that affect glycogen metabolism are ongoing.

Keywords: *In silico* analysis, Virtual Screening, Molecular Docking

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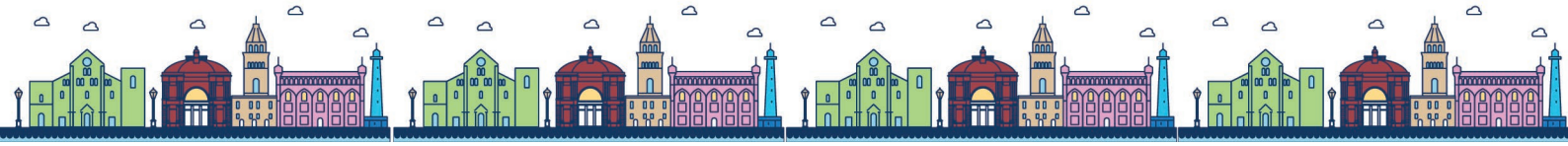

Innovative research methodologies in the EU regulatory framework: an analysis of EMA Qualification procedures in the perspective of rare diseases

Silvia Torretta[1], Viviana Giannuzzi[2], Arianna Bertolani[1,3], Giorgio Reggiardo[3], Eleonora Toich[3], Donato Bonifazi[1,3], Fedele Bonifazi[1,2], Adriana Ceci[1,2] on behalf of the European Paediatric Translational Research Infrastructure (EPTRI)

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Aim: The integration of novel methodologies into medicines research & development is pivotal for addressing challenges in rare diseases research, characterized by the scarcity of data and validated endpoints. We aimed to analyse the applicability of the innovative methodologies qualified by the European Medicines Agency (EMA) into the rare diseases field.
Methods: We identified the EMA's positive qualification opinions (QOs) for novel methodologies (spanning from 2008 to 2023), and examined the duration of the procedure, the methodology type, specific disease or disease area addressed, the type of applicant. We performed multi-level analyses to investigate the interest and applicability of the qualified methodology for/to rare diseases.
Results: Out of the 27 QOs issued by the EMA, only 6 specifically addressed rare diseases, and 9 were potentially of interest for rare diseases.
Biomarkers, endpoints, and registries were the most represented type of innovative methodologies among those of interest for rare diseases (27%, 20% and 20%, respectively), while neurology and infectious and immune system emerged as predominant disease areas (20% and 13%, respectively).
Procedures with interest in rare diseases had a longer adoption time (median time 7.0 months vs. 5.5 months; p-value = 0.363).
Conclusions: Our analysis underscores the critical need for advancing and regulatorily qualifying innovative methodologies in rare diseases. Providing regulatory support and strategic advice to develop methodologies in medicine development tailored to rare diseases is crucial in this research field.

Keywords: Innnovative methodologies, rare diseases, regulatory, European Medicines Agency, Qualification Opinions

Target Therapy for High Grade Neuroblastoma treatment: integration of regulatory and scientific tools is needed

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Aim: High risk Neuroblastoma (NBL) is the commonest paediatric extracranial solid tumour in need for new therapy. Several Active Substances (ASs) targeting NBL molecular targets have been identified and studied but not yet approved for use. This analysis compares the development and the approval status of these ASs to identify gaps limiting access for patients.
Methods: Preclinical studies and clinical trials (CTs), marketing authorisations, paediatric Investigation Plans (PIPs) and waivers were searched from literature, ClinicalTrials.gov and European Medicines Agency website.
Results: Out of 188 ASs targeting NBL, 133 were considered 'under development' as included in preclinical studies (115) and/or CTs (70, of which 5 in phase 3). 37 ASs were granted with a PIP, 41 with a waiver, and 18 with both PIPs and waivers. For the majority of the ASs with PIPs (19) a mechanism of action criteria was adopted, and the PIP paediatric indication was different from the original adult indication. However, for only 2 products of this group (Vitrakvi® and Rozlytrek®) a new paediatric indication, including NBL, was approved.
Conclusions: Many target therapies are under development, but only few of them were approved for use in NBL. With the agreement of several PIPs covering specific paediatric tumours, including NBL, EMA Paediatric Committee provided a relevant contribution, even if with limited impact. To cover the gap, stricter integration between developers and regulators is necessary. A multi- stakeholder platform called Accelerating Clinical Trials in the EU (ACT EU), in which the TEDDY network is involved, could help fill this gap.

Academic development of a paediatric formulation of Budesonide for the treatment of Eosinophilic Esophagitis to cover a high unmet medical need

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Aim: Eosinophilic Esophagitis (EoE) is an antigen-mediated oesophageal inflammatory rare disease. Jorveza® (budesonide - BUD) and Dupixent® (dupilumab) are approved treatments for EoE by the European Medicines Agency (EMA) in adults but not in children. For this population, we propose the development of an extemporaneous mucoadhesive oral BUD solution., We describe the experimental and regulatory steps necessary to this aim.
Methods: The developmental needs were identified: lack of an appropriate paediatric formulation and paediatric clinical data supporting its use in paediatric EoE. Moreover, the orphan designation has been considered as incentive to reach the market.
Results:
Oral formulation
A liquid vehicle, containing carboxymethylcellulose sodium as mucoadhesive agent and hydroxypropyl--cyclodextrin as solubilizing agent, was developed to solubilize BUD. We demonstrated that BUD solutions at concentrations up to 0.7 mg/mL were stable for 120 days at both 4°C and 25°C, easy to dose and administer, and prolonging its residence time on the oesophageal mucosa.
New paediatric data
The protocol of a pilot phase 2 open label, mono-centre paediatric trial and related documents like informed consent, case report form, and centre-specific documents were finalised to be submitted to the competent authority and ethics committee.
Orphan Designation application
An orphan designation application was prepared and submitted to EMA. A literature review was conducted to demonstrate the therapeutic need of BUD in paediatrics for EoE, calculate the disease prevalence in the EU, and the medical plausibility was justified.
Conclusions: A new extemporaneous BUD formulation has been developed, which can be easily prepared by hospital pharmacists. Moreover, after completing the regulatory process, BUD may be repurposed on the market.

Keywords: Budesonide, Eosinophilic esophagitis, Paediatric treatment, Extemporaneous formulation, Mucoadhesive solution

Distinction of drug flows for rare disease anad monitoring of expenditure through optimization of IT systems used in study area

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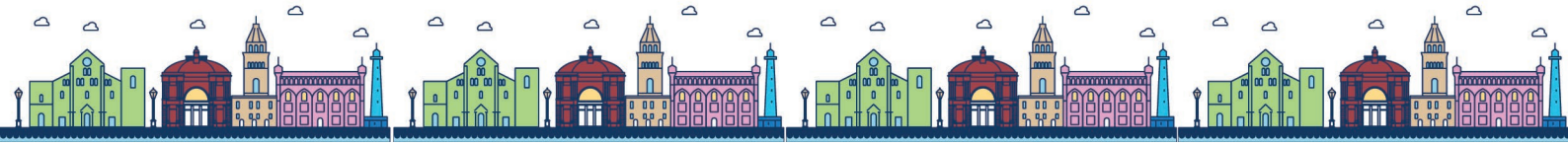
Aim: The Rare Diseases Information System (SIMaRRP) has been operating in the Apulia since 2012 and is currently shared with other regions. The system feeds the National Register. It also allows the information transfer between Hospital Centers for rare deseases and Social Health Districts: diagnostic certificates and patient treatment plans ready for Social Health District to draft exemption scheme for rare deseases. The aim of this work is to identify opportunities for integrating SIMaRRP with the management programs of regional health care companies to identify exactly the type and cost of care for rare disease patients compared to non rare.
Methods: The observations of the present study are based on the comparison between the number of diagnoses available and the corresponding personalized treatment plans reported by doctors in the Apulian SIMaRRP, as well as on the difficulty of matching them with the flow of drugs delivered
Results: SIMaRRP can be configured to meet the needs of distinguish drugs prescribed for rare from non-rare diseases integrating it with pre-existing IT systems for drugs delivered. This will allow to connect contribution to health service costs to single nosological entity.
Conclusions: Connection among SIMaRRP and the others IT systems for drugs management in Apulia could immediatly provide a clear panorama of this specific pharmaceutical expenditure.
This would include several class of drugs. This distinction could also be used to collect data on single nosological entity deseases with therapeutic adherence and provide information on drug utilization.



Axicabtagene ciloleucel (axi-cel) for relapsed/refractory (R/R) large B cell lymphoma beside efficacy: data analysis of EudraVigilance database

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Diffuse large B-cell lymphoma is the most common subtype of non-Hodgkin lymphoma in adults characterized by a median age of presentation in the sixth decade of life with the initial presentation being single or multiple rapidly growing masses in nodal or extranodal sites and that can be accompanied by symptoms of fever, night sweats and weight loss. DLBCL has an aggressive disease course, with the elderly having a poorer prognosis than younger patients, and with relapses being common.
Axi-cel is a second-generation CAR-T-cell therapy directed against CD19, which dramatically improved the prognosis of intractable B-cell lymphomas; however, data about its tolerability are still scant. Therefore, additional information on this issue is urgently needed. In the present paper, EudraVigilance database has been analyzed focusing on all reports of adverse events with axi-cel. About 80% of the reported adverse events were serious, and about 20% of them did not fully resolve or caused death. The adverse events most-frequently reported were Nervous system disorders (25.6%) and, among them, immune-effector-cell-associated neurotoxicity syndrome, followed by Immune system disorders (23.1%), General disorders and administration site conditions (12.0%), Blood and lymphatic system disorders (7.2%), and Infections and infestations (5.8%). Disproportionality analysis showed that the frequency of reported adverse events related to the nervous system was higher with axi-cel than with the other approved CAR-T-cells. In conclusion, real-world pharmacovigilance data showed that nervous system and immune system disorders are the adverse events most reported in axi-cel-related ICSRs and suggest that axi-cel could be more neurotoxic than other CAR-T-cells.

Keywords: EudraVigilance; axi-cel; cytokine release syndrome; immune-effector-cell-associated neurotoxicity syndrome; pharmacovigilance.