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Shaping the future of pediatric health:

Innovative research approaches for rare and metabolic diseases in children

Libro degli Abstract

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Indice *(Ordine alfabetico presenter)*

Comunicazioni Orali

Pharmacovigilance And Real Word Evidence in Paediatrics and Rare Diseases

Comparison of biologic use in pediatric patients for the treatment of immune-mediated diseases in pivotal clinical trials vs the real-world setting using the large-scale Italian VALORE project

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Aim: To compare the characteristics of pediatric patients with immune-mediated inflammatory diseases (IMIDs) who have been enrolled into pivotal trials of immunomodulators biologics vs those treated in Italian real-world (RW) setting.

Methods: We performed a retrospective, population-based cohort study using the VALORE distributed database network, which includes fully anonymized data from 14 Italian Regions. The RW cohort was identified as follows: 1) first ever biologic dispensing (i.e., incident treatments); 2) age <18 years at the time of dispensing; and 3) a gastrointestinal, dermatological or rheumatological IMID as indication for use using a validated algorithm. Pediatric pivotal phase III RCTs of biologic drugs, approved for pediatric IMID indication, were identified. Eligibility criteria of those pivotal RCTs were extracted and RW criteria analogues were developed in the VALORE distributed database network.

Results: Twenty pediatric RCTs of biologics for IMIDs were identified, enrolling a total of 1,211 patients in thetreatment arm (mean age: 12.7 years, F/M ratio=1.7). For the RW cohort, a total of 8,834 incident biologic treatments for IMID (mean age: 11.9 years, F/M ratio=1.5, 55% with juvenile idiopathic arthritis) were identified. Of these, 3,680 (41.7%) pediatric biologic users did not meet eligibility criteria for respective pivotal RCT. Among ineligible patients, adalimumab was the most frequent incident treatment (46.0%) and Crohn's disease was the main indication for use (39.0%).

Conclusions: Nearly half of pediatric patients from the RW settings would have been ineligible for inclusion in the respective indication-specific pivotal RCTs, suggesting that post-marketing surveillance of biologics should be prioritized for those patients.

Keywords Biologic, immune-mediated inflammatory diseases, claims databases, RCT

Pharmacovigilance of orphan drugs: a descriptive analysis of EudraVigilance spontaneous reports

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Aim: Orphan drugs are authorized for the treatment of rare diseases, but their safety profile is often based on limited pre-authorization clinical trials. For rare diseases, post-marketing evidence is particularly important to support regulatory and clinical decisions. The aim of this study was to obtain real- world data on the safety profile of cerliponase alfa and risdiplam by analyzing spontaneous reports of suspected adverse reactions (SARs).

Methods: A descriptive analysis of SARs related to cerliponase alfa and risdiplam was performed using data retrieved from the EudraVigilance database, managed by the European Medicines Agency. Reports were analyzed according to patient age, sex, indication for use, and type of adverse reaction. Only descriptive statistics were applied.

Results: SARs related to cerliponase alfa and risdiplam were more frequently reported in female patients (60.5% and 51.8%, respectively). According to age, SARs to cerliponase alfa were more common in children aged 3–11 years, while those to risdiplam were more frequent in adults aged 18–64 years. The most frequently reported adverse reactions to cerliponase alfa were pyrexia, device-related infections, vomiting, seizures/convulsions, and respiratory disorders. For risdiplam, the most common adverse reactions involved gastrointestinal disorders, infections, general disorders, respiratory disorders, and nervous system disorders.

Conclusions: This descriptive analysis of real-world spontaneous reports appears to confirm the known safety profile of cerliponase alfa and risdiplam. However, an asymmetrical sex distribution of reported adverse reactions was observed, suggesting the need for further post-marketing investigations in patients treated with orphan drugs.

Keywords: Orphan drugs; Pharmacovigilance; EudraVigilance; Cerliponase alfa; Risdiplam

Safety Monitoring of GLP-1 RA liraglutide in paediatric patients by Using EudraVigilance Pharmacovigilance Database

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Aim: The treatment of type 2 diabetes mellitus (T2DM) has evolved with the introduction of glucagon-like peptide-1 receptor agonists (GLP-1 RAs), which improve glycemic control, promote weight loss, and reduce appetite. Nowadays, GLP-1 RA, such as liraglutide, has received recent extensions of indication in paediatric patients affected by weight disorders. The aim of this study is to evaluate the safety profile of liraglutide in paediatric and adolescent patients with type 2 diabetes mellitus and weight-related disorders using pharmacovigilance data from EudraVigilance (EV).

Methods: We conducted a descriptive analysis of all ICSRs in terms of patient characteristics, adverse events encoded according to the Medical Dictionary for Regulatory Activities (MedDRA), outcome and seriousness of the case, therapeutic indication, primary source qualification, primary source country for regulatory purposes.

Results: A total of 124 Individual Case Safety Reports from EV database reporting liraglutide as suspected drug. Many patients experiencing GLP-1–related adverse events (AEs) were female (56.5%) and belonged to the 12–17-year age group, followed by those aged 3–11 years. The most frequently reported outcome was recovered/resolved; however, for most AEs, the outcome was reported as unknown. Most AEs were classified as serious, with the most common seriousness criteria being “caused or prolonged hospitalization” (n = 84; 25.5%), followed by “other medically important condition” (n = 69; 21.8%).

Conclusions: Considering the adverse events of liraglutide-induced, close monitoring of patients receiving this drug is recommended. In this context, further pharmacovigilance studies evaluating the safety profile of liraglutide are needed.

Keywords: GLP-1 RA, safety, paediatric, Diabetes mellitus, Adverse event

Selumetinib for Symptomatic Plexiform Neurofibromas in NF1: A Monocentric Real-World Experience in Pediatric and Adult Patients

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Aim: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder in which up to 60% of affected individuals develop plexiform neurofibromas (PN), frequently symptomatic and not amenable to surgery. Selumetinib is approved for pediatric patients with symptomatic, inoperable NF1-PN and has shown efficacy in adults. We report a monocentric real-world experience in pediatric and adult NF1 patients.

Methods: Retrospective observational study of 29 NF1 patients treated with selumetinib 25 mg/m^2 twice daily: 16 pediatric patients (mean age 13.1 ± 3.7 years; one off-label for optic pathway glioma, OPG) and 13 adults (mean age 38.2 ± 14.9 years one with a hybrid schwannoma/neurofibroma lesion). Clinical symptoms, volumetric MRI changes (REiNS), adverse events (AEs), and treatment modifications were collected.

Results: Pain significantly improved at first follow-up (p < 0.001). PN volume reduction was observed in 54% at 6 months and 65% at 12 months. Overall response rate (ORR) per REiNS occurred in both age groups, with earlier responses in children. Rash occurred in 87.5% of children and in most adults. Gastrointestinal symptoms occurred in 45%. Onychomycosis was more frequent in adults; facial edema occurred in 2/13 adults (15.4%). Treatment discontinuation occurred in two patients due to hypertension and thrombosis, respectively, while another patient temporarily interrupted for lymphedema with erysipelas and successfully resumed treatment after PN regrowth. The OPG case showed initial stability.

Conclusions: Selumetinib provided early symptomatic relief and sustained volumetric tumor reduction with manageable toxicity; pediatric patients showed slightly greater volumetric responses, while adults experienced a higher AE burden.

Keywords: Neurofibromatosis type 1; plexiform neurofibroma; selumetinib; MEK inhibitors; real-world evidence

Mitochondrial Stress Biomarkers in Neonatal Encephalopathy: From Experimental Models to Clinical Application

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Aim. Mitochondrial dysfunction is a key feature of ischemia/reperfusion brain injury, and preservation of mitochondrial dynamics is crucial for neuronal survival. Our preclinical studies demonstrated that ischemia significantly disrupts mitochondrial network integrity, respiratory chain function, and the balance between fusion and fission, leading to neuronal injury. Melatonin, which exhibits significant neuroprotective properties against ischemic brain damage, mitigated these effects in both in vitro and in vivo models by preserving mitochondrial integrity, reducing oxidative damage, and supporting fusion–fission balance. Melatonin also enhanced mitochondrial transfer through tunnelling nanotubes and reduced the release of mitochondrial DNA (mtDNA), thereby limiting inflammatory activation. These findings prompted us to investigate mitochondrial stress signals as potential biomarkers for encephalopathy in neonate (NE).
Methods. Circulating cell-free mtDNA (ccf mtDNA), nuclear DNA (ccf nDNA), and mitokines (FGF-21, GDF-15, and Humanin) were evaluated in an observational cohort study of 48 neonates with NE treated with therapeutic hypothermia. Participants were stratified according to MRI findings into moderate/severe injury (Group I) and no/mild injury (Group II). Biomarkers were quantified in serum, urine, and saliva/buccal swabs and correlated with clinical variables and neurodevelopmental outcomes.

Results. Serum ccf mtDNA and ccf nDNA levels correlated with adverse neurodevelopmental outcomes, including lower Bayley Cognitive, Language, and Motor scores. Ccf mtDNA levels inversely correlated with final Sarnat scores but not with MRI-detected injury, suggesting sensitivity to functional impairment rather than structural damage. Among mitokines, only Humanin was associated with MRI-detected white matter and cerebellar injury, supporting a possible neuroprotective role. Non-invasive samples did not mirror serum trends, likely due to the limited cohort size.

Conclusions. Ccf mtDNA and ccf nDNA emerge as promising early biomarkers for risk stratification in NE, complementing MRI by capturing dynamic metabolic and inflammatory processes. Their dissociation from structural injury highlights their potential to predict functional outcomes and support future personalized interventions. Larger cohorts are needed to confirm their clinical applicability.

Keywords. Neonatal Encephalopathy; Mitochondrial DNA; Mitokines; Neurodevelopmental Outcomes; Non-Invasive Biomarkers.

Sex differences in vancomycin pharmacokinetics in pediatric patients: real-world data from a therapeutic drug monitoring registry

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Introduction: Drug pharmacokinetics often differ between sexes and women are often disadvantaged due to underrepresentation in clinical studies. This is relevant for drugs requiring rapid therapeutic levels, such as vancomycin. This study assessed sex-related differences in vancomycin pharmacokinetics and target attainment in pediatric patients, with age-stratified analysis between groups with reduced or developed sex differences.

Methods: Real-world data from the therapeutic drug monitoring counselling service at the Clinical Pharmacology of IRCCS Burlo Garofolo (Trieste) was used. Pediatric (<18 years) patients with at least one vancomycin concentration measured between September 2017 and December 2025, undergoing continuous infusion, were considered. Young children were those <8 years at sampling. Vancomycin levels were measured using an immunochromatographic assay. Therapeutic range was defined as AUC 400–600 mg/L·h. Associations were assessed using LMM and GLMM, accounting for repeated measurements per patient.

Results: We analysed 227 measurements (130 males; median age 5.25 years; 144 young children). Levels and dose- normalised levels were lower in females, although not statistically significant. Females more frequently had sub-therapeutic levels (57.4% vs 39.2%; p=0.03). In the 8–18-year cohort, females showed lower vancomycin levels (median 18.1 vs 20.4 mg/L; p=0.08), lower dose-normalised levels (median 0.31 vs 0.57;

p=0.03) and were significantly more likely to be below the therapeutic range (51.6% vs 21.6%; p=0.01). No significant differences were observed in the 0–8-year group.

Conclusion

Vancomycin levels and achievement of therapeutic levels were lower in pediatric females, except in sexually undeveloped young children. If confirmed, clinicians should consider higher vancomycin doses in females < 8 years.

Keywords: Vancomycin, TDM, sex, sex differences, pediatric

Lysosomal acid lipase activity as a novel biomarker of histological progression in MASLD: a single- center prospective study

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Aims: The prevalence of MASLD is increasing also in the pediatric population and the identification of patients at a higher risk of progression is an unmet need. Genetic lysosomal acid lipase deficiency (LAL-D) is characterized by liver steatosis and hypercholesterolemia, indicating a key role for LAL in cellular and systemic lipid metabolism. Since patients with MASLD can develop an acquired LAL-D, aim of the study was to investigate whether low LAL activity could predict MASLD progression.

Methods: Liver phenotype of 144 adult MASLD patients was reassessed after ≥5 years from enrollment and LAL measurement, by elastography (Controlled Attenuation Parameter, CAP and Liver Stiffness Measurement, LSM) and biopsy, when indicated.

Results: Mean follow-up was 8.2 years, and 94/144 patients had paired biopsies. Changes in hepatic features over time evaluated by elastography were not associated with baseline LAL/platelet ratio. Interestingly, in patients with paired biopsies a lower baseline LAL/platelets ratio was significantly associated with histological deterioration evaluated as worsening of NALFD activity score (NAS), of fibrosis or as having a composite histological endpoint (i.e. development of MASH and/or worsening of NAS or fibrosis). In multivariate analysis, the composite endpoint was associated with baseline LAL/platelet ratio (OR=0.51) and with HOMA index (OR=1.32), after adjustment for age, gender, CAP and/or LSM.

Conclusion: LAL activity could help in identifying patients at higher risk of MASLD progression towards MASH and fibrosis. In particular, lower LAL could be associated with the development of inflammation, a feature that is not assessed by available non-invasive techniques. Supported by RF-2021-12374481.

Keywords: Lysosomal acid lipase; metabolic dysfunction-associated steatotic liver disease; inflammation; prospective study.

Individualised Treatments for Rare and Ultra-Rare diseases: Ethics and Regulatory Insights from a First ERDERA multi-stakeholders Consensus Meeting

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Aim: While individualised Antisense Oligonucleotides (also known as n-of-1 or n-of-few ASOs) offer treatment promise for patients affected by ultrarare diseases, clinicians and scientists face biological, clinical, regulatory and ethical challenges that fall outside traditional drug development pathways (Synofzik et al. 2022, doi:10.1089/nat.2021.0039). Through participating in a multi-stakeholder consensus meeting held as part of the ERDERA (European Rare Diseases Research Alliance) project, we aimed to identify the key ethics and regulatory issues surrounding individualised ASO treatments.

Methods: Discussions focused on patient eligibility, treatment initiation, benefit/risk assessment, communication and consent, and regulatory requirements. A consensus document was drafted outlining state-of-the-art and preliminary recommendations.

Results: The following ethics and regulatory insights emerged: 1) communication and consent require new approaches in personalised treatments, given significant uncertainties regarding safety, efficacy, feasibility and timelines; these processes should be iterative and maintained throughout the treatment, rather than limited to a single pre-trial interaction; 2) clinical trial design, regulatory compliance and treatment access are significantly challenged, because individualized ASOs are developed for single patients. Notably, “named-patient basis” treatment, which is carried out on an individual basis under the direct responsibility of the doctor, is currently the only feasible access route, although this largely depends on national laws.

Conclusions: Communication between researchers and patients should be effective and informed consent process continuous and adaptive. Given the emerging regulatory uncertainties on individualised treatments, mapping national regulatory frameworks would be essential to facilitate access to these innovative therapies, which do offer cutting-edge therapeutic opportunities, and in which ERDERA is pioneering.

Keywords: Individualised Treatments; Antisense Oligonucleotides; Ultrarare Diseases; Ethics; Regulatory Frameworks.

Mechanism and Models for Drug Development in Pediatrics and Rare Diseases

Genotype-phenotype-drug response correlation of newly identified SCN1A variants from the Italian Registry of Dravet Syndrome Residras

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Aim. Variants in the SCN1A gene, encoding the Nav1.1 sodium channel, are associated with a spectrum of neurological disorders, ranging from mild genetic epilepsy with febrile seizures plus (GEFS+) to severe Dravet syndrome (DS). These variants may cause loss-of-function (LoF), gain-offunction (GoF), or mixed LoF/GoF defects, leading to significant phenotypic variability and diverse responses to available treatments (Guerrini et al., Physiol. Rev., 2023). Functional studies are therefore essential to establish genotype-phenotype correlations and guide therapeutic decisions, particularly to avoid contraindicated drugs. From the Italian Registry of Dravet Syndrome, Residras, we selected nine novel SCN1A variants that were predicted as pathogenic/neutral and to cause LoF or GoF effects using a machine learning model (Heyne et al., Sci Transl Med, 2020; Balestrini et al., Epilepsia Open 2023). The variants R1634Q, F984L and A1772T are associated with DS and predicted as GoF. Four variants are associated with GEFS+ and predicted as GoF (V1339A and R1646H) or LoF (A1940S and F773L); Q3H and N1327H are associated with generalized epilepsy and focal epilepsy, and predicted as LoF and GoF, respectively. The aim of this study was to functionally characterize Nav1.1 mutant channels in order to clarify their pathogenic mechanisms and support therapeutic decisions. We also compared in silico predictions with functional in vitro data.

Methods. Nav1.1 wild-type (WT) and mutant channels were expressed in HEK293 cells, and sodium currents were recorded via whole-cell patch-clamp.

Results. The mutant channels displayed current amplitude and voltage-dependent activation similar to Nav1.1 WT. The variants R1634Q, F984L, and V1339A caused a negative shift in the voltagedependence of fast inactivation by +16, +10, and +7 mV, respectively, compared with WT. R1634Q also slowed the recovery from fast inactivation, albeit not significantly. The variant F773L slightly accelerated the kinetics and recovery of fast inactivation. Q3H, A1940S and N1327H showed no significant biophysical change in our experimental conditions. For the A1772T and R1646H variants experiments are ongoing.

Conclusions. R1634Q and F984L show clear LoF defect consistent with DS phenotype and data from literature, but not with in silico prediction. Accordingly, sodium channel blockers are avoided in these carriers. The mild LoF of V1339A correlates with a mild biophysical phenotype, typical of GEFS+ but again not in accordance with in silico prediction. F773L showed mixed GoF/LoF behavior unusual for GEFS+ but part of the spectrum of defects associated with SCN1A-related disorders (Gallagher et al., Epilepsia 2024). The comparison of in vitro and in silico data for the seven variants suggested that the functional defect predicted in silico is not always confirmed by in vitro functional study (Knox et al., Ann Clin Transl Neurol 2025). Indeed, de novo SCN1A variants with early onset show more severe LoF biophysical phenotype than that of inherited variants. DS variants are more severe than GEFS+. Inherited variants causing focal or generalized epilepsy and involving not conserved residues are often similar to WT. These data highlight the crucial role of functional studies in understanding molecular mechanisms and in personalizing treatment for SCN1A-related disorders. (PNRR: M6/C2_CALL 2022 Full Proposal, Project Code: PNRR-MR1-2022-12376642)

Keywords: Dravet Syndrome, SCN1A, epilepsy

Aquaporin-9 has a role and relevance as drug target in Wolman disease

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Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive disease due to mutations in the lysosomal acid lipase gene (Lipa). Reduced LAL activity leads to a progressive accumulation of cholesterol esters and triglycerides in hepatocytes, adrenal glands, intestine, and macrophagemonocyte system cells throughout the body. LAL-D induces two clinical severity spectra, an infantile form called Wolman disease (WD) and a less aggressive form known as cholesterol ester storage disease (CESD). To date, there is no effective therapy against LAL-D. Here, a transgenic mouse model of CESD (Lipa-/- mice) was used to further characterize the LAL-D phenotype and to check the relevance of AQP9, an aquaporin channel highly expressed in liver and leukocytes with pivotal roles in energy balance and inflammation. Compared to healthy control mice, CESD mice revealed early onset of hepatic steatosis already from the 9th day of postnatal life with rapid progression to severe hepatosplenomegaly and microvesicular liver steatosis at day 90 associated with a strong infiltration of neutrophils and Kupffer cells in liver parenchyma. Interestingly, this phenotype was significantly improved after genetic ablation of AQP9 by the generation of Lipa-/-/Aqp9-/- double knockout mice indicating pathophysiological relevance of AQP9 in the disease progression. Taken together, these results suggest AQP9 as a suitable target in the treatment of WD/CSD. Preclinical investigation with cell and animal models is in progress in our laboratory for this purpose using potent and AQP9-selective small heterocyclic compounds.

Keywords: Lysosomal acid lipase (LAL), Aquaporins, hepatic steatosis, inflammation, drug targets, heterocyclic compounds, macrophages.

Functional and pharmacological characterization of the p.L689F Nav1.4 sodium channel variant associated with Myotonia Permanens

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Aim. Mutations in the hNav1.4 sodium channels cause sodium channel myotonia or paramyotonia congenita, two allelic diseases characterized by muscle stiffness. We report the case of an adolescent affected by generalized muscle stiffness since infancy carrying the p.L689F mutation.

Methods: The mutation was introduced in hNav1.4 cDNA and expressed in HEK cells for patchclamp studies.

Results: All the symptoms converge on the diagnosis of myotonia permanens. Myotonia was severe and significantly impacted motor skills. Genetic analysis revealed a de novo c.2065C>T variant in the SCN4A gene, which resulted in the substitution of leucine by phenylalanine at codon 689 (p.L689F) in hNaV1.4. Patch-clamp studies of heterologously expressed L689F channels showed a negative shift of voltage dependence of activation, fast inactivation, and slow inactivation, compared to wild-type channels. The shift was more pronounced on activation, leading to a huge increase of channel open probability at negative voltages, which likely accounts for muscle fiber hyperexcitability leading to muscle stiffness. The negative shift of slow inactivation voltage dependence likely reduces the likelihood of muscle weakness occurrence. The patient takes carbamazepine from the age of six with partial stiffness relief, except for a brief, unsuccessful trial of mexiletine. In vitro pharmacological assays suggest that the L689F mutation does not alter sensitivity of the channel to mexiletine, lamotrigine, and flecainide, but slightly increased sensitivity to carbamazepine.

Conclusions: This study discloses the genotype/phenotype relationship of p.L689F variant and supports a pharmacogenetic strategy for applying precision medicine in myotonia. Supported by Medineuropa project granted by University of Bari.

Keywords: sodium channel myotonia, pharmacogenetics in pediatrics, anti-myotonic drugs, electrophysiology, precision medicine.

Molecular basis for ligand-induced switching from activation to inhibition in kv7.2 channels

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Aim: KCNQ-encoded neuronal Kv7 voltage-gated potassium channel subunits (Kv7.2-Kv7.5) control electrical properties of neurons, are involved in common and rare neuropsychiatric disorders, and are targets for novel neurotherapeutics. Retigabine, the first neuronal Kv7 activator clinically approved for epilepsy treatment, was withdrawn due to its chemical liability, off-target effects and lack of selectivity. Here, we describe the structures of human Kv7.2 channels in complex with two retigabine analogues: compound 60 (c60), which acts as a channel opener, and compound 106 (c106), a potent Kv7 inhibitor

Methods: Molecular modelling in silico used molecular docking and molecular dynamics. A truncated human Kv7.2 construct (residues 64–674) co-expressed with calmodulin in HEK293S cells was used for cryo- EM studies. Functional studies were performed by heterologous expression in CHO cells of specific cDNA plasmids; recordings were performed using patch-clamp in whole-cell configuration.

Results: Both c60 and c106 were found to position in the same pocket at the S5-S6 interface in the pore domain between two adjacent subunits. The opposite functional behaviour of c60 and c106 was due to their differential interaction with the L307 residue. In fact, replacing the bulkier leucine with a smaller alanine or valine residue at this position fully prevented the inhibitory effect of c106; instead, Kv7.2 activation by c60 was unaffected by the L307V substitution.

Conclusions: We provide novel mechanistic insights into the molecular mechanisms governing Kv7 channel modulation by exogenous ligands with opposite functional effects. This result may prove useful to target Kv7 channels with more potent and selective modulators.

Keywords Kv7.2; epilepsy; retigabine; ion channel structure

ATM deficiency impairs calcium homeostasis in human neural progenitors: a cellular platform for drug repurposing strategies in pediatric Ataxia-Telangiectasia

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Ataxia-Telangiectasia (A-T) is a rare autosomal recessive disorder marked by progressive cerebellar atrophy, neurodegeneration, and cognitive decline, with no effective disease-modifying therapies. Elucidating the cellular consequences of ATM deficiency is critical for identifying therapeutic vulnerabilities. Here, we reveal a previously unrecognized role of ATM in regulating mitochondrial calcium homeostasis in human neural progenitor cells (hNPCs). ATM-knockout hNPCs were generated from human induced pluripotent stem cells using CRISPR/Cas9. Compartment-specific live-cell imaging revealed widespread dysregulation of cytosolic and mitochondrial calcium dynamics in ATM-deficient cells. Pharmacological inhibition of ATM in wild-type hNPCs recapitulated these defects, confirming a direct mechanistic link between ATM signaling and mitochondrial calcium handling. Loss of ATM altered mitochondrial calcium regulation, disrupted redox balance, and impaired the expression of key calcium transfer proteins. Importantly, pharmacological modulation of mitochondrial calcium uptake partially rescued these defects, suggesting that the mitochondrial calcium uniporter is a critical downstream effector of ATM deficiency. These findings establish ATM as a central regulator of cytosol/mitochondrial calcium in hNPCs and identify a druggable vulnerability in A-T. Candidate clinically approved compounds were prioritized using an in-silico model (PMID: 41285916), and their ability to restore calcium homeostasis, as well as target other known pathological mechanisms, will be evaluated, providing a translational blueprint for drug repurposing. By linking ATM deficiency to calcium dysregulation, this study uncovers a fundamental mechanism of neurodegeneration in A-T and highlights a tractable target for early intervention in paediatric patients.

Keywords: Ataxia-telangiectasia, ATM, mitochondrial calcium, drug repurposing, hNPCs.

Unveiling a novel HDAC inhibitor mechanism to rescue disease-specific LKB1 dysregulation in Duchenne Muscular Dystrophy

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Efficient skeletal muscle contraction relies on tight mechano-metabolic coupling regulated by AMPactivated protein kinase (AMPK). Duchenne muscular dystrophy (DMD) is characterized by aberrant AMPK activation and altered metabolic signalling. We investigated the expression and regulation of the LKB1–STRADα–MO25 heterotrimeric complex, the main upstream activator of AMPK. To assess LKB1STRADα–MO25 dynamics during early and chronic pathology, qRT-PCR analyses were performed in gastrocnemius (GC), diaphragm and heart of two murine dystrophic models (BL10 mdx and D2 mdx at 4,8,28,52 weeks of age). A marked downregulation of the complex was observed at all disease stages (<50% vs. WT counterparts), a defect not detected in tibialis anterior muscles of 15-week-old SOD1G93A mice, a model of amyotrophic lateral sclerosis.

An 8-week treatment with the pan-HDAC inhibitor vorinostat (5 mg/kg, 5 times a week, i.p.) restored LKB1 gene and protein expression in D2 mdx mice (5 weeks of age), with recovery scores (RS) of 197% and 204%, respectively. This effect correlated with significant downregulation of miR-451, miR-195 and miR-17 (one-way ANOVA, $F < 24.27$, $0.0001<p<0.016$), known post-transcriptional repressors of LKB1. In contrast, the selective HDAC1/2 inhibitor Rodin-A (4 mg/kg, i.p.) increased LKB1 mRNA (RS 138%) but did not restore protein levels or modify miRNA expression.

Studies in patient-derived myoblasts (HDMD, deletion 48-50; AB1098) showed impaired LKB1 expression during differentiation (0, 6, 11 day). Moreover, LKB1 fluorescence was widespread in healthy cells but reduced and mainly nuclear in HDMD. Overall, these findings identify the LKB1–STRADαMO25 axis as a regulatory node disrupted in DMD and responsive to epigenetic modulation, highlighting its therapeutic potential.

Keywords: liver kinase B1, Duchenne muscular dystrophy, epigenetic, histone deacetylases inhibitors, animal models

Precision therapy for Aicardi-Goutières paediatric patients using patient-specific in vitro preclinical models based on induced pluripotent stem cells

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Background and Aim: Aicardi–Goutières syndrome type-1 (AGS1) is a rare pediatric autoinflammatory disorder caused by TREX1 mutations, related to chronic type-I interferon (INF-I) release, increased interferon-stimulated genes (ISGs) expression, severe neurological manifestations including encephalopathy and cerebral calcifications. Current treatments provide limited benefit; novel therapeutic approaches are urgently needed. This study aims to generate patient-specific neural stem cells (NSCs) and neurons to evaluate safety and efficacy of conventional and emerging therapies.

Methods: Induced pluripotent stem cells (iPSCs) from one AGS1 patient and one healthy donor (BJ) were differentiated into NSCs and neurons. Cells characterization, under basal condition and after cGAMP stimulation, was performed by quantitative PCR, immunofluorescence and western-blot (WB). Cytotoxicity of lamivudine baricitinib, dexamethasone, mercaptopurine and thioguanine was evaluated by MTT assay on neurons.

Results: Reduced iPSC (OCT4, SOX1) and increased NSC markers (SOX2, PAX6, NESTIN) confirmed NSC differentiation. Neuronal differentiation was validated evaluating mature neurons markers MAP2 and ß- TUBULIN-III. CHAT, GAD and TH levels indicated a predominant population of cholinergic neurons, followed by GABAergic and dopaminergic ones. Tested drugs were safe in BJ and AGS1 neurons. AGS1 cells were more sensitive to mercaptopurine (2-way- ANOVA p<0.0001), lamivudine (p=0.038) and dexamethasone (p=0.048). Preliminary data showed that 24h cGAMP stimulation increased IFN receptors, STING and ISG genes (ISIG15, RSAD, IFI27, SIGLEC, IFI44, IFIT1) in BJ and AGS1 neurons. STING protein was detectable only in neurons, which showed higher p-p65/p65 levels after cGAMP stimulation.

Conclusion: Neurons represent a more suitable disease model compared to NSCs, better recapitulating STING pathway activation and inflammatory responses. Tested drugs resulted safe; further analyses are required to study their pharmacodynamic effects on neurons.

5 keywords: Aicardi–Goutières syndrome type 1, interferonopathies, precision therapy, neural stem cells, neurons

Trehalose partially restores autophagy markers and membrane trafficking in a CLN1 C451T cellular model of Infantile Neuronal Ceroid Lipofuscinosis (INCL)

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Aim: The neuronal ceroid lipofuscinoses (NCLs) are rare, fatal neurodegenerative disorders. Infantile NCL (INCL) is one of the most severe forms and results from mutations in the CLN1 gene, encoding PPT1, a lysosomal depalmitoylating enzyme required for protein turnover and autophagy. PPT1 loss leads to accumulation of palmitoylated proteins, lysosomal dysfunction, and neurodegeneration (Simonati et al., 2009). The C451T (p. Arg151*) nonsense mutation introduces a premature stop codon, abolishing enzymatic activity and defining a severe INCL genotype (Bouchelion et al., 2014). This study investigated whether trehalose can improve autophagic and trafficking defects in a C451T cellular model.

Methods: SH-SY5Y neuroblastoma cells were transiently transfected with PPT1 wild-type or C451T constructs. After 24 h, cells were treated with 100 mM trehalose for 48 h. Gene expression was assessed by RT-qPCR. Protein levels and subcellular distribution were examined by Western blot and surface biotinylation. Data were analyzed by one-way ANOVA with Tukey's post-hoc test.

Results: In untreated conditions, C451T cells showed increased SQSTM1/p62 and MAP1LC3B mRNA, consistent with impaired autophagy, while HSPB8 remained unchanged. Trehalose further induced all these genes, suggesting activation of compensatory pathways. Several palmitoylationdependent ion channels were dysregulated in C451T cells. Kv1.3 mRNA was elevated in the mutant and unaffected by trehalose, while total Kv1.3 protein levels didn't differ between groups. Surface biotinylation revealed no intrinsic trafficking defect; however, trehalose increased membraneassociated Kv1.3 specifically in C451T cells, indicating improved trafficking.

Conclusions: Trehalose enhances autophagy-related responses and partially restores membrane trafficking in CLN1-deficient cells, supporting its potential as a modulator of lysosomal and proteostatic homeostasis in INCL.

Keywords: INCL; PPT1; Autophagy; Trehalose; Ion channels

Hydrogen sulfide restores PGC-1α and promotes slow-twitch muscle fibers in Duchenne Muscular Dystrophy

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Background: Duchenne muscular dystrophy (DMD) is the most common X-linked myopathy, resulting from dystrophin gene mutations causing progressive skeletal muscle (SKM) degeneration [1]. Recent evidence shows that the transsulfuration pathway and its end-product hydrogen sulfide (H_2S) are essential for SKM homeostasis [2-3] but are impaired in DMD muscle [4]. This study aims to evaluate whether erucin, a natural H_2S donor, improves SKM function in mdx mice, a well-known preclinical DMD model.

Methods: male mdx mice received erucin (3mg/kg/die) or vehicle for 2 weeks. SKM performance was assessed by rotarod and weight tests. At 7 weeks, quadriceps (QFA) were collected and analyzed for oxidative stress (evaluated as H_2O_2 levels and glutathione oxidized/glutathione reduced ratio), gene/protein expression, and proteomic profiling. Statistical analyses include the T- test or one-way ANOVA.

Results: Erucin significantly improved SKM performance in mdx mice (n=8; **p<0.01) and reduced oxidative stress in QFA of mdx mice (n=6; p<0.05). Proteomics revealed upregulation of proteins involved in myofiber maintenance and mitochondrial biogenesis. Specifically, QFA from erucin-treated mice displayed increased slow-twitch markers (n=5; *p<0.05) and PGC-1α expression (n=5; *p<0.05), indicating enhanced oxidative metabolism. Erucin upregulated genes promoting muscle regeneration while downregulated fibrotic markers (n=6; ***p<0.001). The mitochondrial complexes I, III, and V of the electron transport chain were also increased in QFA harvested from erucin-treated mice compared to the vehicle.

Conclusions: Erucin enhances SKM function in mdx mice by reducing oxidative stress, promoting a slow-fiber phenotype, and improving mitochondrial bioenergetics via PGC-1α activation. These findings support erucin as a potential adjuvant therapy for DMD.

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Drug repurposing using an integrated in silico/in vitro approach to identify therapies for KCNA1- and KCNA2-related neurological disorders

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Aim Kv1.1 (KCNA1) and Kv1.2 (KCNA2) are voltage-gated potassium channels that regulate neuronal excitability[1]. Variants in KCNA1 and KCNA2, causing loss- or gain-of-function (LoF, GoF) and mixed LoF/GoF defects are linked to neurologic disorders, including episodic ataxia type 1 and severe forms of developmental and epileptic encephalopathy (DEE)[2-4]. There are hardly any effective treatments for these conditions, as no modulators of Kv1.1 and Kv1.2 channels exist. 4aminopyridine, a non-selective Kv1.x blocker, is the only precision medicine approach offered in some patients carrying GoF variants[5,6]. Therefore, development of new molecules acting on Kv1.1 and Kv1.2 is necessary.

Our aim is to identify approved drugs to be repurposed in KCNA1- and KCNA2-related disorders.

Methods Virtual screening has been performed on homology models of Kv1.1 and Kv1.2 channels using the ChEMBL database (~2000 approved drugs). Automated patch-clamp platform, Patchliner (Nanion), was used to validate in silico data on Kv1.1, Kv1.2 and E236K mixed LoF/GoF mutant expressed in HEK293 cells.

Results Different repurposed drugs have been identified as promising modulators of Kv1.1 and Kv1.2, such as paroxetine, fluoxetine and fluvoxamine, selective serotonin reuptake inhibitors (SSRIs), and cannabidiol, recently approved as add on for rare epilepsies. Comparable block was observed for fluvoxamine (IC50: 10.4±2.7 μM for Kv1.1, 11.2±2.7 μM for Kv1.2 and 6.2±1.2 μM for E236K), fluoxetine (10.7±6.4 μM for Kv1.1, 11.4±3.3 μM for Kv1.2 and 11.8±2.4 μM for E236K) and paroxetine (6.4±1.2 μM for Kv1.1, 5.2±1.1 μM for Kv1.2 and 5.2±1.2 for E236K). Cannabidiol blocked Kv1.1, Kv1.2 and E236K, but with lower affinity (41±12.7 μM for Kv1.1, 44.2±13 μM for Kv1.2 and 55.6±11.2 μM for E236K).

Conclusions Our results suggested that SSRIs are potential candidates for repurposing in children harbouring KCNA1 and KCNA2 GoF or mixed variants. These data support previous findings showing fluoxetine effect on other potassium channels variants in DEE[7]. Our data also showed that cannabidiol may modulate Kv1.1 and Kv1.2 activity, albeit with a low affinity. Given the role of these channels in the brain, these drugs may have broader therapeutic applications for other excitability disorders.

Keywords: KCNA1/KCNA2, DEE, in silico screening, automated patch clamp, drug repurposing

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Oxerutin and doxocycline improve quality of life and wound healing in patients with epidermolysis bullosa: a monocentrical pilot study

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Background: Recessive dystrophic epidermolysis bullosa (RDEB) is a rare, severe multisystem genetic disorder caused by COL7A1 mutations, characterized by chronic skin and mucosal fragility, pain, recurrent infections, and progressive extracutaneous involvement. Growing evidence indicates that immune dysregulation, chronic autoinflammation, and endothelial–microcirculatory damage substantially contribute to disease severity and clinical heterogeneity. Targeting these downstream mechanisms may represent a valuable adjunctive pharmacological strategy. Oxerutin is an antioxidant with capillary-stabilizing and anti-edema properties, while doxycycline exerts antimicrobial and anti-inflammatory effects, including inhibition of matrix metalloproteinases.

Methods: We conducted a retrospective case series including six patients with severe RDEB and clinical signs of lower-limb microcirculatory impairment. All patients received oral oxerutin (100 mg/kg/day) for symptomatic venous insufficiency for at least 18 months; three patients also received doxycycline (100 mg twice daily), initially prescribed for Pseudomonas aeruginosa infection and continued due to sustained clinical benefit. Demographic, clinical, and laboratory data were collected. Outcomes included wound burden quantified as body surface area affected by wounds (BSAP), patient- reported pain and pruritus, dressing time, use of wound-care materials, transfusion and infusion requirements, and long- term safety.

Results: All patients showed consistent clinical improvement. Oxerutin alone resulted in an averagereduction of BSAP of approximately 60%, while combined therapy with doxycycline achieved reductions up to 80%. Patients reported a 50–80% decrease in non-traumatic wound formation, reduced blister exudate and cutaneous edema, improved pain and pruritus, and markedly shorter dressing times with decreased use of wound-care materials. A substantial reduction or discontinuation of red blood cell and albumin transfusions was observed, together with decreased need for intravenous iron supplementation. No adverse events were recorded during prolonged treatment.

Conclusions: Long-term administration of oxerutin, alone or in combination with doxycycline, was safe and associated with clinically meaningful improvement in cutaneous and systemic manifestations of severe RDEB. These findings support further prospective controlled studies to confirm efficacy and to better define the role of microcirculation- and inflammation- targeted pharmacological repurposing in pediatric and adult RDEB.

Off-label use of sirolimus in children with autoimmune lymphoproliferative syndrome

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Aim: Autoimmune lymphoproliferative syndrome (ALPS), a rare inherited disorder due to the dysregulation of the FAS apoptotic pathway, is usually manifested in childhood, although symptoms may occur at any age. It is characterized by disturbed lymphocyte homeostasis, leading to chronic lymphadenopathy, splenomegaly, multilineage cytopenias, and increased risk of lymphoma. The treatment includes corticosteroids and steroid-sparing immunosuppressants. However, the relapse rate is high especially during dose reduction, and the only cure for severe cases remain hematopoietic stem cell transplant. Nevertheless, sirolimus demonstrated high efficacy in refractory cases, although not approved from regulatory agencies. The aim of this study was to evaluate the off-label use of sirolimus in patients with ALPS followed at the Pediatric Oncohematology Unit of the University Hospital of Catania.

Methods: A retrospective analysis was conducted on data collected to manage off-label prescriptions at the University Hospital of Catania. Specifically, we performed a descriptive analysis of sirolimus use in patients with ALPS between January 2014 and December 2025.

Results: In the reference period, a total of 10 patients with ALPS (9 males, 1 female, mean age 9.1 years, range 3–18) were treated with sirolimus at a dosage of 2-3 mg/m 2 . The mean treatment duration was 3.4 years, with 4 treatments (40%) still ongoing. Based on available follow-up data, nearly 80% of patients showed an optimal treatment response, in terms of blood count recovery and resolution of associated symptoms, without adverse events.

Conclusions: Our results show that sirolimus represents an effective and well-tolerated treatment option in patients with ALPS.

Keywords: ALPS; sirolimus; off label; rare disease

Targeting the ClC-5 Cl^-/H^+ antiporter in rare diseases: in silico drug repurposing as a first step toward the discovery of pharmacological modulators

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Aim and Methods. The ClC-5 Cl^-/H^+ antiporter plays a crucial role in renal endosomal acidification and protein reabsorption. Loss-of-function mutations in CLCN5 gene are responsible for Dent disease, a rare inherited renal tubulopathy (Jentsch & Pusch, Physiol Rev 2018), whereas recent evidence indicates that ClC-5 overexpression is associated with renal cyst formation in patients affected by tuberous sclerosis complex (TSC) (Barone et al., PNAS 2021). These findings highlight ClC-5 as an attractive therapeutic target, where both activators/potentiators and inhibitors may have high therapeutic potential. In this perspective, in this study we embarked in Structure-Based Virtual Screening (SB-VS) to repurpose existing drugs towards new therapeutic goals (Pinzi& Rastelli Int J Mol Sci, 2019). In this respect, molecular docking was employed to screen an in-house database of ~ 1700 marketed drugs against a set of putative druggable pockets mapped on derived homology model of human CLC-5.

Results. Compounds were ranked based on their predicted affinity and binding modes within selected and functionally relevant regions of the ClC-5 Cl^-/H^+ antiporter. SB-VS revealed several approved drugs as promising ligands candidates for the ClC-5 protein, spanning diverse therapeutic classes, from agents targeting the cardiovascular system to drugs involved in alimentary tract and metabolism. This approach enables the rapid identification of candidate molecules with favorable pharmacokinetic and safety profiles, thereby facilitating their potential translation toward in vitro experimental validation.

Conclusions. Overall, this work represents a first step toward the discovery of pharmacological modulators of ClC-5 and supports drug repurposing strategies as a promising avenue for the treatment of Dent disease and cystic kidney manifestations associated with TSC.

Poster

Pharmacovigilance, Regulatory Science and Real-World Drug Safety

Real-World Safety of Tisagenlecleucel in Paediatric Acute Lymphoblastic Leukaemia: Evidence from the EudraVigilance Database

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Tisagenlecleucel has significantly improved the management of relapsed or refractory B-cell Acute Lymphoblastic Leukaemia (ALL) in paediatric patients; however, its post-marketing safety profile requires further characterization. This study aimed to evaluate the safety of tisagenlecleucel in paediatric patients, and the temporal changes in reporting of related adverse events (AEs) during the COVID-19 pandemic.

From 2017 to 2026, Individual Case Safety Reports (ICSRs) referring to tisagenlecleucel and paediatric patients were retrieved from the EudraVigilance database. Temporal trends in monthly reporting were analysed using Interrupted Time Series (ITS) analyses with quasi-Poisson regression, overall and for predefined safety outcomes.

Overall, 606 out of 3145 ICSRs involved paediatric patients, predominantly males aged 3–11 years. A total of 3099 AEs were reported, of which 85.1% were classified as serious, most frequently as Other Medically Important Conditions. Fatal outcomes accounted for 7.2% of AEs and were mainly associated with malignant neoplasm progression (N = 24; 10.8%), and ALL (N = 16; 7.2%). Cytokine release syndrome (CRS) was the most frequently reported AE (10.7%). Neurotoxicity represented approximately 4.0% of reported events, while about 6.0% were suggestive of therapeutic failure. ITS analyses showed no significant changes in overall paediatric reporting level or trend at the onset of the COVID-19 pandemic or during the vaccination period. Event-specific analyses revealed a significant reduction in the post-COVID reporting trend for CRS, while neurotoxicity remained stable over time.

The study supports preapproval evidence on the safety of tisagenlecleucel in paediatric patients and shows heterogeneous temporal patterns linked to evolving clinical practice.

An easy-to-use flow chart to classify your clinical study

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Fondazione per la Ricerca Farmacologica Gianni Benzi onlus

Aim: Clinical studies should be properly defined before their initiation to ensure compliance with the applicable regulatory framework. This can be challenging from a regulatory perspective, especially for multi-centre multi-national studies involving 'small populations' [1,2]. In the context of the BETTER project, we built an easy-to-use flow chart to support the proper classification of clinical studies from the regulatory point of view within the European framework.

Methods: In the design of the tool, we considered many factors, such as the experimental nature of the study, the processing of personal/sensitive data, the handling of biospecimens and any product(s), including medicines and medical devices. We identified the main applicable European and international regulations and guidelines for classifying a clinical study, as sourced from the regulatory database held by Fondazione Benzi. Key information was then extracted and synthesised to build our flow chart.

Results: The resulting flow-chart was structured using a question-driven framework.

Conclusions: The tool allows the investigator to properly classify the clinical study and to identify the relevant regulatory and ethics framework. It was tested and used within the BETTER project. Further uses will validate its wider applicability, thereby strengthening its role as a practical support tool in study design and classification.

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Keywords: Clinical studies classification, small populations, regulatory, ethics, flow chart.

Desensitization enables resumption of Enzyme Replacement Therapy in two children with Rare Diseases after Hypersensitivity Reactions

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Aim: To assess the clinical efficacy of desensitization protocols for hypersensitivity reactions (HR) to enzyme replacement therapy (ERT) in pediatric patients with lysosomal storage disorders.

Methods: Two pediatric patients with HR to ERT were treated using similar rapid desensitization protocols based on stepwise intravenous administration of increasing drug doses, using a 3-bag, 12-step regimen with premedication including antihistamines and montelukast. One patient with mucopolysaccharidosis type II developed severe generalized urticaria to idursulfase, with positive intradermal skin testing, and additionally underwent an allergen immunotherapy-like (AIT-like) protocol. A second patient, a 6-year-old boy with Infantile-Onset Pompe disease, experienced infusion-related reactions to alglucosidase alfa characterized by flushing, cough, and fever, with negative skin tests, suggesting a non-IgE-mediated mechanism.

Results: Both patients successfully tolerated ERT following desensitization without further HR. The patient with mucopolysaccharidosis resumed idursulfase infusions and, after one year of combined rapid desensitization and AIT-like treatment, showed conversion of skin tests from positive to negative, consistent with immune tolerance. The Pompe disease patient tolerated alglucosidase alfa after desensitization and later switched to avalglucosidase alfa for clinical efficacy reasons. After one year, a similar infusion reaction occurred and was again successfully prevented using the same desensitization protocol, without adverse events.

Conclusions: Desensitization is an effective and safe strategy for managing both IgE- and nonIgE-mediated HR to ERT in pediatric patients, allowing continuation of essential therapies. Keywords: Enzyme replacement therapy; desensitization; hypersensitivity reactions; mucopolysaccharidosis; Pompe disease.

Off-label drug use in children: a ten-year experience of monitoring

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Aim: Off-label use refers to the usage of medications that fall outside the approved indications, dosages,routes of administration, treatment durations. It is particularly widespread in the pediatric population, often excluded from clinical trials, and in critical areas such as onco-hematology and rare diseases.

The aim of this study was to evaluate off-label prescribing among patients followed at the Pediatric Units of the University Hospital of Catania.

Methods: We performed a retrospective analysis of data collected to monitor off-label use in the hospital setting, in accordance with Law 94/1998. Specifically, a descriptive analysis was conducted on off- label drugs prescribed from January 2014 to December 2024 by pediatricians, excluding onco-hematologists.

Results: In the reference period, a total of 197 patients (52.3% males, 47.7% females, mean age 5,1 years, median age 1 year, range 0–52) were treated with 257 off-label drugs (1.3 prescriptions/patient; mean 23.4 prescriptions/year). The most prescribed drug was immunoglobulins (n = 40, 15.6%) to treat ABO hemolytic disease of newborn and several rare and autoimmune disorders, including autoimmune encephalitis and Acute Disseminated Encephalo-Myelitis.

Conclusions: One of the main advantages of off-label prescribing is that it addresses important unmet medical needs in special populations, such as pediatric patients and those affected by rare diseases. Well-performed monitoring of off-label prescriptions in the hospital setting enables detection of unmet medical needs and identification of drugs with a favorable risk/benefit profile. This represents an opportunity for patients, but also a regulatory challenge to guarantee equal access to effective and safe treatment.

Keywords: Off-label; access; pediatric; unmet needs; rare diseases

Post-marketing evaluation of fenfluramine safety and appropriate use in children under two years: analysis of EudraVigilance data

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Aim. Fenfluramine is a serotoninergic drug approved in 2020 in Europe as add-on therapy for the treatment of seizures in Dravet Syndrome (DS) and Lennox-Gastaut syndrome (LGS) in patients aged 2 years and older. Initially developed as an appetite suppressant, it was withdrawn from the market in 1997 due to cardiovascular safety concerns, including valvular heart disease and pulmonary arterial hypertension. DS and LGS are rare, genetic and severe developmental and epileptic encephalopathies (DEE). Patients are particularly vulnerable to the detrimental effects of prolonged seizures on development and, given the early onset of these DEE, timely intervention is critical. Fenfluramine is not only effective and safe for seizure control but may also improve neurodevelopmental trajectories and reduce the risk of sudden death in epilepsy (Boncristiano et al., Epilepsia 2025). In addition, a recently published prospective, independent, open-label study of 5 DS patients suggested its safety in children younger than 2 years (Pietrafusa et al., Epilepsia 2024) and clinical trials are ongoing (NCT06118255; NCT06598449). Therefore, this pharmacovigilance study aimed to assess the safety and appropriate use of fenfluramine in patients younger than 2 years, in whom fenfluramine use remains off-label.

Methods. We retrieved individual case safety reports on fenfluramine suspected adverse drug reactions (ADRs) from the website of suspected ADR (www.adrreports.eu) of the European pharmacovigilance database (Eudravigilance). By using the line listing function, data were collected from the date of marketing authorization of fenfluramine, 2020, to November 2025. A descriptive analysis was performed focusing on the ADRs occurring in children below 2 years.

Results. During the study period, 1.031 ADRs reports were identified, including 39 cases in children aged below 2 years. In 23 cases (59.5%) fenfluramine was used to treat DS and LGS. No cases of cardiac valvulopathy or pulmonary arterial hypertension, the historically most serious safety concerns, were reported. The most frequent ADRs involved nervous system disorders (20 cases of seizures), infections (12 cases, mainly respiratory and gastrointestinal), and mild appetite and weight loss (6 cases), all consistent with package leaflet. A few cases of fever and gastrointestinal discomfort were also noted.

Conclusions. No new or serious safety signals were observed when fenfluramine was used off-label in children under 2 years of age. In most cases, its use was in accordance with disease indications. Consistent with previous findings, these results suggest that fenfluramine use in patients below 2 years is well-tolerated and potentially safe, supporting early therapeutic consideration in DS and LGS.

Keywords: Dravet Syndrome, Lennox Gastaut syndrome, fenfluramine, Eudravigilance

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Defending paediatric medicines in Europe: EPTRI's strategic contribution and actions in shaping the new pharmaceutical legislation

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Aim: The European pharmaceutical system is undergoing major reform through a new Regulation1 and Directive[2] that will redefine the authorisation of medicines. The revision includes the Paediatric Regulation repeal, raising concerns about negative effects on paediatric medicines development and availability. The European Paediatric Translational Research Infrastructure (EPTRI)[3] assessed the potential effects of these changes.

Methods: A qualitative analysis of the proposed legislative documents was conducted, focusing on paediatric-relevant provisions. Based on this assessment, EPTRI suggested specific amendments to improve the forthcoming legislative framework.

Results: Key concerns include the dispersion of the Paediatric Committee's expertise and the weakening of Paediatric Investigation Plan (PIP) obligations. EPTRI elaborated Position Paper[4] and proposed amendments to the European Parliament[5] (EP) in 2023. These focused on establishing a dedicated, permanent Paediatric Working Party at the European Medicines Agency; preserving PIP obligations; strengthening paediatric innovative and repurposed medicines development; improving processes for identifying paediatric unmet needs; ensuring dedicated funding. Some of EPTRI points were included in the revised text voted by the EP in 2024[6]. In June 2025, the proposal of the Council of the European Union[7,8] also incorporated EPTRI-aligned changes, maintaining paediatric expertise and reinforcing research priorities. However, further commitment is required to ensure that paediatric needs remain prioritised in the ongoing negotiations aimed at reaching final agreement. Conclusions: Robust, mandatory paediatric regulatory pathways are essential to safeguard children's health. Despite the setback derived from these legislative changes, EPTRI will continue to advocate for paediatric needs to remain central in the EU pharmaceutical scenario.

Keywords: paediatric research, pharmaceutical legislation, PIP, PDCO.

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Pediatric safety profile of off-label anti-myotonic drugs: real-world evidence from EudraVigilance database

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Aim: Myotonic disorders are rare genetic neuromuscular diseases characterized by delayed muscle relaxation, stiffness, and weakness. Mexiletine is the only EMA-approved treatment for non-dystrophic myotonias. Marketing authorization is restricted to adults, but a clinical trial in children and adolescents is ongoing. Other drugs, including flecainide, propafenone, ranolazine, carbamazepine, lamotrigine, phenytoin, and acetazolamide are used off-label in myotonic individuals including children, despite limited data on safety. This study investigates the pediatric safety profile of these eight drugs using EudraVigilance database.

Methods: Pediatric ADR reports (0-17 years) notified within 2010-2024 for selected drugs were extracted from EudraVigilance. A descriptive analysis assessed demographics and reporting features (age, sex, reporter type, therapeutic indication, outcome, reporting year) and adverse events classified by System Organ Class.

Results: A total of 9034 pediatric ADRs were identified, including lamotrigine (n=3604), carbamazepine (n=3026), phenytoin (n=1200), acetazolamide (n=196), flecainide (n=187), propafenone (n=66), mexiletine (n=41), and ranolazine (n=4). Reported indications reflected approved therapeutic uses, including cardiac and epilepsy-related indications, whereas off-label neuromuscular use was rare (<2%). Among cardiac drugs, mexiletine and propafenone primarily induced cardiac disorders (~55%) and general/systemic reactions, while flecainide showed a slightly lower cardiac risk (~20%). The few reports on ranolazine included nervous system and general disorders. Antiepileptic agents (phenytoin, lamotrigine, and carbamazepine) mainly involved nervous system, skin, and general disorders. Phenytoin showed the highest incidence of these ADRs (~35%). Acetazolamide-related ADRs predominantly included metabolism, general, and neurological disorders.

Conclusions: These findings provide insights into pediatric safety of drugs potentially used off- label in myotonia, supporting real-world, evidence-based treatment.

Keywords: myotonic disorders, off-label use, pediatric pharmacovigilance, drug repurposing

Real-World Drug Safety in Pediatric Oncohematology: An Observational Pharmacovigilance Study in Two Sicilian Hospitals

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Aim: Although therapeutic advances in paediatric oncohaematology have improved prognosis through complex protocols, off-label use of adult drugs increases ADR risk. This study aimed to evaluate drug safety in real-world settings by analysing ADRs, stratifying safety data, assessing severity and outcomes, and evaluating impact on therapy and long-term toxicity. It also aimed to raise awareness among prescribing physicians in Sicily.

Methods: A 36-month observational pharmacovigilance study (2022–2025), funded by AIFA, was conducted in two pediatric oncohematology centers in Sicily. Clinical data, treatments administered, and ADRs were collected. Long-term toxicity was assessed in patients with ≥5 years follow-up. Outcomes included stratification by age, disease, and therapy, and analysis of ADRs by number, type, severity, outcome, and impact on treatment continuation. Descriptive statistics were applied.

Results: Overall, 292 patients were included (171 in Catania, 121 in Palermo), mean age 9.6 years, 57.2% male. A total of 2,400 prescriptions were issued, most commonly methotrexate (14%) and vincristine (11%). During the study, 393 reports were entered into the National Pharmacovigilance Network, yielding 1,131 ADRs: 929 (82.1%) in Catania, 556 serious (59.8%), and 202 (17.9%) in Palermo, 89 serious (44.1%). Most reported ADRs were anemia (10.4%) and vomiting (10.4%). Vincristine was the most frequently suspected drug (109 reports). Forty-four long-term pediatric oncology patients showed no significant late toxicity.

Conclusions: This real-world pharmacovigilance study confirmed intervention effectiveness by increasing ADR reporting. ADR severity classification improved, knowledge of drug safety and efficacy expanded, and healthcare professionals were more aware of ADR risks, while reporting quality improved.

Keywords: pediatrics, oncohematology, ADR, off-label, safety

Pharmacokinetics, Therapeutic Drug Monitoring, Biomarkers and Formulation Strategies for Therapy Optimization

Physicochemical and Thermal Characterization of Butyric Acid–Based DESs for Use in Self-Emulsifying Drug Delivery

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Growing environmental awareness has fostered the development of sustainable solvent alternatives. Deep eutectic solvents (DESs) have emerged as a promising green option due to their low toxicity, biodegradability, ease of preparation, and tunable physicochemical properties arising from hydrogen bond donor (HBD)–hydrogen bond acceptor (HBA) interactions [1]. When an active pharmaceutical ingredient (API) is incorporated as a component, the system is defined as a therapeutic deep eutectic solvent (THEDES). This work aims to develop a butyric acid–based DES for application as the lipidic phase of self- emulsifying drug delivery systems (SEDDS) intended for pediatric colon-targeted delivery. Limonene and cannabidiol (CBD) were investigated as HBAs. Butyric acid was selected as HBD due to its endogenous colonic production and its well-recognized physiological benefits, including anti-inflammatory activity, reinforcement of intestinal epithelial barrier integrity, and its role as an energy source for colonocytes. Additionally, DES formation was explored as a strategy to mitigate its characteristic pungent odor. The butyric acid–limonene DES exhibited odor reduction and slight variations in NMR chemical shifts, suggesting weak intermolecular interactions. Considering the established anti-inflammatory and analgesic properties of CBD [2], mediated through CB1/CB2 receptors involved in pain modulation and intestinal immune regulation, greater emphasis was placed on CBD-based systems. The combination of CBD and butyric acid led to the formation of a stable THEDES, potentially providing synergistic therapeutic effects for intestinal disorders. Several CBD:butyric acid molar ratios were evaluated; while some formulations recrystallized at room temperature, the 1:2 and 1:3 ratios yielded stable liquid systems.FTIR spectroscopy confirmed eutectic formation through shifts in characteristic absorption bands, indicative of hydrogen bonding. ^1H NMR analysis revealed chemical shift variations of the CH_2–COOH signals of butyric acid and the H6/H1 protons of CBD (Δδ 0.02–0.06 ppm), supporting intermolecular interactions. DSC analysis demonstrated the formation of a new eutectic phase with a reduced freezing point, while TGA revealed an intermediate thermal stability profile, relevant for pharmaceutical and nutraceutical applications.

Keywords: Thedes, SEDDS, Cannabidiol, Butyrric acid, colon delivery

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High resolution mass spectrometry for typing and quantification of transthyretin and immunoglobulin light chains in cardiac amyloidosis

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Aim: Amyloidosis is a rare disease caused by the deposition of misfolded proteins with a b-sheet conformation, leading to tissue damage. This study aimed to achieve accurate amyloid typing and quantification of transthyretin (TTR) and immunoglobulin light-chains (LC), the most common amyloidogenic proteins in cardiac amyloidosis [1], using high-resolution mass spectrometry.

Methods: Cardiac tissue samples were obtained from seven patients with cardiac amyloidosis. According to Canetti et al. [2], the tissue was collected from the slide and subjected to protein digestion. Peptides were analysed by liquid chromatography coupled to high-resolution mass spectrometry. Data processing was performed with Proteome Discoverer v.3.1 using a label-free quantification workflow. Protein abundance was calculated using the average intensity of the three most abundant peptides. Pairwise protein ratios were computed and statistical analysis was performed at the protein level using t-tests. Neonatal cardiac tissue was used as a negative control.

Results: Protein abundance profiles enabled accurate amyloid typing. In TTR samples, TTR abundance was at least two-fold higher than LC; whereas in LC samples LC abundance was at least two-fold higher than TTR. Compared to control tissue, a significantly high sample-to- control ratio was observed, with increases of at least 20-fold for TTR samples and 10-fold for LC samples. Significant protein abundance ratios were identified using an adjusted p-value threshold of <0.05.

Conclusions: High resolution mass spectrometry is a reliable and robust tool for amyloid protein typing and quantification in cardiac tissue. This study represents a translational approach for the clinical practice in other diseases.

Keywords Mass spectrometry, transthyretin, light chains immunoglobulin, amyloidosis, diagnostics

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Innovative Microencapsulation Strategy for Age-Appropriate Oral Delivery of Amphotericin B in Paediatric Cystic Fibrosis

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Cystic fibrosis (CF) is a rare paediatric disease caused by mutations in the CFTR gene, resulting in impaired ion transport and abnormal accumulation of highly viscous mucus in multiple organs, including the gastrointestinal tract (GIT). Intestinal manifestations in CF are often under-recognized, as current therapies primarily target respiratory symptoms. Amphotericin B (AmB), a potent antifungal agent, has been proposed to restore ion balance by forming non-selective ion channels; however, its oral use is limited by poor solubility, low permeability, and gastric instability. This study presents a novel microencapsulation system for oral AmB delivery, designed to enhance intestinal absorption and achieve site-specific release. The microcapsules feature a deep eutectic solvent (DES)-based mucolytic core that improves AmB solubility and reduces mucus viscosity, facilitating drug diffusion. A polymeric shell of Eudragit® L 30 D-55 and alginate protects AmB from gastric degradation and ensures release in the small intestine. Microcapsules were produced via prilling/vibration technique. After process optimization, they showed high encapsulation efficiency, uniform size, and flow properties suitable for oral dosage forms. Swelling and drug release studies confirmed gastric stability and intestinal release. Mucus diffusion assays demonstrated the formulation's ability to penetrate the viscous intestinal barrier characteristic of CF. Permeation studies on Caco-2/HT29 co-cultures confirmed that DES enhances mucosal penetration and drug absorption. This microencapsulation strategy represents a significant advancement for oral AmB therapy in CF, offering a stable, patient-friendly formulation that improves adherence and minimizes the risks associated with parenteral administration. The system provides a promising approach to address intestinal complications in CF, supporting more comprehensive disease management and potentially improving clinical outcomes in pediatric patients.

Keywords: Cystic fibrosis, Paediatric rare disease, Amphotericin B, Microencapsulation, Age-appropriate delivery system

3D Bioprinting of Alginate–Gelatine Hydrogels with Lipid Nanoparticles for Localized Drug Delivery in pediatric Glioblastoma Therapy

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Pediatric glioblastoma (p-GBM) is a rare tumor with biological and genetic features distinct from adult GBM. The use of adjuvant chemotherapy, maximal surgical resection followed by radiotherapy remains the standard treatment for children over the age of three (1). As far as known, therapeutic efficacy is limited by tumor heterogeneity and, critically, the blood–brain barrier. To address these challenges, localized delivery strategies are increasingly explored to deliver high drug concentrations directly at the tumor site (2). Therefore, the convergence of biomaterials and nanotechnology with additive manufacturing presents a promising paradigm for localized drug delivery. Sodium alginate (SA), an anionic polysaccharide, and gelatine (GEL), a denatured form of collagen, offer promising routes for engineering hydrogels suitable for 3D printing (3). This study investigates the use of SA and GEL in developing semisolid preparations for extrusion-based 3D printing and the incorporation of lipid nanoparticles (LNPs) into the hydrogel matrix for drug delivery. Solutions of 2% SA (w/v) and 10% GEL (w/v) in PBS buffer (pH 7.4) were mixed in (v/v) ratios (1:1, 2:1, 5:1, 1:5) to evaluate 3D printed scaffolds' printability, stability, and definition. The 2:1 SA:GEL ratio produced stable, well-defined structures. Other ratios compromised print fidelity or mechanical features. LNPs were added to the hydrogel matrix at polymer-to-lipid ratios (w/w) of 23:1, 25:1, and 28.5:1. The LNPs, consisting of lipids: ALC-0315, DSPC, cholesterol, and DMG-PEG2000 (4), were developed using microfluidic mixing. Various incorporation methods were tested, pre-incorporation into the SA phase yielded superior results in terms of homogeneity, scaffold shape fidelity, and crosslinking behaviour. Additionally, Rhodamine-labelled LNPs and empty LNPs were formulated to assess their localization and retention within the printed scaffolds using fluorescence microscopy and scanning electron microscopy (SEM). The current results confirmed the successful integration of LNPs, with notable red fluorescence signals distributed throughout the scaffold matrix. In summary, this research shows the feasibility of using SA and GEL formulations for bioprinting scaffolds with LNPs. The optimized SA:GEL ratio of 2:1 and incorporating LNPs into the SA solution first supports future drug/nucleic acid delivery development.

Keywords: Lipid Nanoparticles, Hydrogel, 3D Printing, Localized drug delivery.

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Immune alterations in genetic lysosomal acid lipase deficiency: data from naïve and ERT-treated patients

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Background and aim: Lysosomal acid lipase deficiency (LAL-D), a rare autosomal recessive disorder, impairs the hydrolysis of cholesteryl esters and triglycerides within the lysosomes, leading to multisystem lipid accumulation. Enzyme replacement therapy (ERT) has been shown to attenuate liver disease and dyslipidemia. Little is known about the impact of LAL-D, and consequently of ERT, on other organs and systems. Emerging evidence suggests that LAL-D may affect both innate and adaptive immune responses. Thus, aim of the project was to characterize the immunophenotype of ERT-naïve and ERT-treated LAL-D patients.

Methods: Immunophenotyping was performed by FACS analysis on blood samples from ERT-naïve and ERT-treated LAL-D patients matched with healthy controls. Natural killer (NK) cells were further characterized by in vitro functional assays.

Results: ERT-naïve LAL-D patients showed a trend toward reduced circulating leukocytes, with preserved T-cell frequencies, when compared to controls. Neutrophils were significantly increased in LAL-D naïve patients, compared to controls, and normalized by ERT. Notably, NK cells were reduced by 75% in LAL-D patients, with a shift in the distribution from CD56dim (cytotoxic) to CD56bright NK subset (cytokine-releasing). Consistently, the functional characterization showed an impaired cytotoxic and degranulation capacity of NK cells from ERT-naïve patients compared to controls. The frequency of NK population persists even in ERT-treated patients, with a partial correction of subset distribution.

Conclusions: Thus, our data suggest that LAL-D affects the distribution of immune cells and particularly that of NK cells, which seems to be only partially corrected by ERT. Further studies are needed to understand the molecular mechanisms linking LAL to NK biology.

Urinary microRNA profiling predicts hemodynamically significant patent ductus arteriosus and ibuprofen response in preterm infants: a prospective study

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Aim: Hemodynamically significant patent ductus arteriosus (hsPDA) is a frequent complication in preterm infants, and pharmacological closure with ibuprofen is ineffective in up to 30% of cases. This study aimed to investigate whether urinary microRNA (miRNA) signatures are associated with hsPDA development and ibuprofen response.

Methods: Fifty-two preterms (gestational age 23^{+0}–29^{+0} weeks or birth weight <1500 g) admitted to the neonatal intensive care unit (Careggi Hospital) were enrolled. Echocardiography was performed to diagnose hsPDA and to assess response to a first ibuprofen cycle (10–5–5 mg/kg/day). Urines were collected within 24 h of life and after treatment. Whole urinary miRNome profiling was performed using Agilent miRNA microarrays.

Results: At birth, 13 miRNAs were differentially expressed between hsPDA and controls ($p < 0.001$). Notably, hsamiR-483-3p, involved in renin–angiotensin–aldosterone system regulation and vascular remodeling, was markedly down-regulated in hsPDA. Responders showed higher expression of hsa-miR-582-3p, a regulator of vascular smooth muscle cells, whereas hsa-miR-7110-5p was overexpressed in nonresponders. In controls, temporal analysis revealed physiological up-regulation of hsa-miR-4685-3p at day 4 of life, linked to endothelial protection. Post-treatment analysis showed minimal miRNA modulation in responders, while non-responders exhibited significant modulation of 14 miRNAs ($p < 0.001$), including up-regulation of hsa-miR-3613-3p, an inhibitor of TGF-β/SMAD signaling. Hierarchical clustering demonstrated molecular similarity between responders and controls, with a distinct profile in non-responders.

Conclusions: Urinary miRNA profiling identifies molecular signatures associated with hsPDA and ibuprofen response, supporting urinary miRNAs as non-invasive biomarkers for early risk stratification and therapeutic prediction in neonatal PDA.

Keywords: Patent ductus arteriosus; microRNA; preterm infants; urinary biomarkers; ibuprofen response

Microfluidic Formulation of Precision Lipid Nanoparticles for Gene Delivery to Muscle Cells

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Aim: Scalable and reproducible formulation of lipid nanoparticles (LNPs) is essential for advancing nonviral gene delivery to skeletal muscle. This study aimed to develop a microfluidic-based formulation strategy to generate uniform LNPs optimized for plasmid DNA (pDNA) delivery to muscle cells, for gene therapy of muscle disorders such as Duchenne muscular dystrophy (DMD).

Methods: LNPs were produced using a staggered herringbone microfluidic mixer for organic phase containing SM-102, DSPC, cholesterol, and DMG-PEG2000 (35:35:27.5:2.5 molar ratio; 10 mM total lipid) and aqueous HEPES buffer (pH 4) containing pDNA. The flow rate ratio (organic: aqueous) was 1:3, (total flow rate 500 µL/min). Particle size, polydispersity index (PDI), and ζ-potential were measured by dynamic light scattering, and encapsulation efficiency by PicoGreen assay. Cytocompatibility was assessed using Cell Counting Kit-8. Transfection efficiency in an immortalized human dystrophic myoblast cell line was evaluated by flow cytometry (GFP expression), qRT-PCR, and immunofluorescence. LNP performance was compared with Lipofectamine and lentiviral vectors. Statistical analysis included one-way ANOVA with Bonferroni post hoc testing.

Results: Microfluidic LNPs had a mean diameter of 147.1 ± 2.5 nm, PDI 0.194 ± 0.031, encapsulation efficiency 81.95 ± 0.7%, and ζ-potential of −6.5 mV (empty) and −3.5 mV (pDNA-loaded). No cytotoxicity was observed after 24 h. LNPs achieved higher transfection efficiency than Lipofectamine and viral vectors, enabling robust channelrhodopsin-GFP expression.

Conclusions: Microfluidic formulation enables precise, reproducible LNP production as a scalable, lowimmunogenic platform for non-viral gene delivery to muscle cells, supporting future gene therapy strategies for DMD and related muscle diseases.

Keywords Microfluidics; Lipid nanoparticles; Plasmid DNA delivery; Skeletal muscle cells; Duchenne muscular dystrophy

Ketamine adverse reactions: mechanistic investigation and biomarker identification

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Ketamine is an effective and safe drug used in pediatric sedation. However, its use is associated with vomiting and recovery agitation. The project aims to investigate the role of miR-484 and miR- 18a-3p in ketamine-induced adverse reactions by using a rat model of schizophrenia, given the overlapping psychotic symptoms with recovery agitation, and in vitro studies of miR-484 molecular targets.

The expression levels of miR-484 and miR-18a-3p were quantified via quantitative real-time PCR (qPCR) in five brain regions of seventeen adult male Sprague-Dawley rats either treated with vehicle (n=8) or with MK-801 (n=9) for 14 days at 0.15 mg/kg. QPCR was also used to assess if MK-801 effects were reversed in eight adult male rats (n=8) after the administration of 5mg/kg of clozapine.

QPCR analysis was also employed to evaluate the expression of three bioinformatically identified targets after miR-484 over-expression in the immortalized neuroblastoma cell line SH-SY5Y.

MK-801 treatment increased expression of both miR-484 (p=0.042) and miR-18a-3p (p=0.001) in the ventral hippocampus and decreased expression of miR-484 (p=0.0001) in the dorsal hippocampus when compared to the control group. In the prefrontal cortex, dorsal striatum and nucleus accumbens there were no differences between groups.

In the ventral hippocampus, clozapine did not alter miR-484 or miR-18a-3p expression.

MiR-484 overexpression (p=0.046) showed a downregulation for all three targets, SLC6A4 (p=0.40), HIVEP2 (p=0.24) and DISC1 (p=0.36), supporting its regulatory potential despite not reaching statistical significance.

If confirmed, miR-484 could serve as a biomarker to predict ketamine-induced adverse reactions, enabling safer and more personalized pediatric sedation.

Role of dyslipidemia in Alagille Syndrome on cardiovascular and renal outcomes

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Aim: Alagille syndrome (ALGS) is a rare, autosomal dominant disease characterized by abnormal development of intrahepatic bile ducts, heart, arteries and kidneys due to disrupted Notch signaling. Although hypercholesterolemia is a hallmark feature of ALGS, studies on lipids are limited. The aim of the study is to characterize the lipid and lipoprotein profiles in ALGS and explore their relationship to disease phenotype focusing on liver involvement, renal and cardiovascular outcomes.

Methods: Twenty-five subjects affected by ALGS were enrolled with a median age of 13 years (IQR: 4.0–16.75), 20 patients with native livers and 5 liver transplant recipients.

Results: ALGS patients exhibited a distinctive lipid profile, characterized by high total cholesterol (42%), elevated free cholesterol (FC, 47.6%) and phospholipids (52%). LpX was identified in 62% of the cohort and was associated with markers of cholestatic liver disease. ALGS patients had IMT and PWV values exceeding the 95th percentile for age in 60% and 38% of cases, however, were not associated with dyslipidemia. In vitro, plasma from LpX-positive patients induced significant podocyte necrosis (p=0.03) and apoptosis (p=0.050), compared to LpX-negative patients. Additionally, LpX reduced the expression of podocin (p=0.005).

Conclusions: the results showed that ALGS patients present with a distinctive LpX- driven dyslipidemia, which reflects altered cholesterol homeostasis, likely due to increased FC release from the cholestatic liver and reduced LpX catabolism due to low LCAT activity. ALGS-associated dyslipidemia does not appear atherogenic. However, in vitro findings indicate a nephrotoxic role for LpX, with potential contribution to renal complications in ALGS.

Keywords Alagille syndrome; dyslipidemia; LpX; liver disease; renal disease

Uncovering immune and inflammatory involvement in Wolfram syndrome

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Aim: Wolfram syndrome (WS) is a rare autosomal recessive disorder caused by mutations in the WFS1 gene, clinically characterized by childhood-onset diabetes mellitus, optic nerve atrophy, deafness, diabetes insipidus, and neurological manifestations. WFS1 encodes Wolframin, an endoplasmic reticulum (ER) transmembrane protein essential for ER homeostasis. We previously reported a WS patient carrying two novel WFS1 mutations who exhibited immune dysregulation, with increased production of proinflammatory cytokines by peripheral blood mononuclear cells (PBMCs). The present study aimed to expand the patient cohort to investigate the impact of WFS1 mutations on the immune system.

Methods: PBMC subpopulations from seven WS patients harboring distinct WFS1 mutations and presenting heterogeneous clinical phenotypes were analyzed by multiparametric flow cytometry. Plasma cytokine levels were assessed by Luminex assay and PBMC gene expression was evaluated by RT-PCR.

Results: Compared to healthy donors (HD), WS patients showed evidence of systemic immune activation, including a significant increase in HLA-DR$^+$ cells, B cells (HD vs WS: $p < 0.01$), Th1 CD4$^+$ T cells, classical monocytes, and plasmacytoid dendritic cells (HD vs WS: $p < 0.05$). Moreover, PBMCs from WS patients produced significantly higher levels of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 compared to HD ($p < 0.05$).

Conclusions: Overall, these findings demonstrate a marked alteration of multiple immune cell subsets and the presence of systemic inflammation in patients with WS, suggesting a role of the immune dysregulation in the pathophysiology of the disease. Keywords: Wolfram Syndrome, inflammation, immune dysregulation

Preliminary Comparison of Two AUC_{0}–$_{12}$ Estimation Approaches for Mycophenolate in Paediatrics: A Retrospective Cohort Study

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Aim: To compare two paediatric AUC_{0} - $_{12}$ estimation methods for mycophenolate mofetil (MMF) in a preliminary cohort, assessing concordance between a reference four-point method historically used in our institution and a Bayesian approach across reduced sampling strategies (4, 3, 2, and single time points), with the aim of minimising the number of blood samples required for $AUC_{0}$$_{-12}$ estimation.

Methods: From a clinical care database (2018–2025), children with paediatric systemic immune-mediated inflammatory diseases receiving oral mycophenolate mofetil twice daily (mean dose 33.4 mg/kg/day) were identified at a tertiary paediatric hospital (IRCCS Burlo Garofolo). The cohort comprised 11 patients (mean age 13 years; median age 15 years), with a sex distribution of 4 males and 7 females, and European (White). For the identified patients, AUC_{0}–$_{12}$ was estimated using either the four-point concentration–time method (Filler et al.) or the Bayesian/population pharmacokinetic analysis (De Winter 1). Statistical analyses included the concordance correlation coefficient (CCC) and Pearson's correlation coefficient (r).

Results: CCC versus the four-point reference method was excellent for the 4-point strategy (t0124: CCC=0.990, 95%CI=0.960–0.997; r^2=0.992). Among reduced sampling strategies, 3-point sampling (t012) showed good concordance (CCC=0.940, 95%CI=0.779–0.985; r^2=0.906), while 2-point strategies performed very well, particularly t04 (CCC=0.974, 95%CI=0.901–0.994; r^2=0.960) and t02 (CCC=0.959, 95%CI=0.845–0.990; r^2=0.920). For single-point strategies, concordance was good for t0 (CCC=0.939, 95%CI=0.775–0.984; r^2=0.895) and t4 (CCC=0.904, 95%CI=0.665–0.975; r^2=0.900), whereas t2 showed only moderate concordance (CCC=0.798, 95%CI=0.380–0.945; r^2=0.650).

Conclusions Preliminary data from a small exploratory cohort suggest that the Bayesian approach provides very good agreement with the four- point reference method, including when applied to reduced sampling strategies, and may reduce the overall sampling burden; however, external validation in larger cohorts is required.

Keywords: mycophenolate; AUC; therapeutic drug monitoring; paediatric; pharmacokinetics

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New Drug Targets and Experimental Models for Drug Development

A novel cellular model for studying Ataxia-Telangiectasia pathophysiology

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Ataxia-Telangiectasia (A-T) is a rare pediatric neurological disorder caused by loss of functions mutations in the Atm gene encoding for ATM protein. The role of ATM as a master regulator of DNA damage response and cell cycle is widely described in literature, whereas less is known about cellular and molecular mechanisms underlying neuropathological alterations in A-T. Within the central nervous system (CNS), neural stem progenitor cells (NSPC) represent a population of undifferentiated cells endowed with several key properties: they are self-renewing and multipotent cells, and they can be pharmacologically modulated. For these reasons, studying ATM functions in NSPC represents a valuable approach for disclosing cellular and molecular pathophysiological mechanisms in untreated CNS disorders, including A-T. We used iPSC-derived hNPC to generate CRISPR/Cas9 mediated Atm gene knock out (KO). We demonstrated that ATM KO hNPC recapitulate canonical defects associated with ATM dysfunction, such as reduced survival rate, higher sensitivity to UVB-induced DNA damage and higher oxidative stress. Interestingly, we observed that ATM KO hNPC also display novel phenotypes, such as mitochondrial defects and reduced number of hNPC-derived neurons. Moreover, by proteomic analysis we identified an extensive number of proteins and pathways which are deregulated in ATM KO hNPC. In conclusion, hNPC represents a valuable model for identifying novel cellular and molecular pathways disrupted in absence of ATM.

Keywords: Ataxia-Telangiectasia, ATM, neural progenitor cells, rare disease, proteomics

Mitsugumin 29 as a novel modulator of SOCE and potential therapeutic target in Tubular Aggregate Myopathy

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Aim: Tubular aggregate myopathy (TAM) is an incurable rare inherited muscle disorder with tubular aggregates (TAs) as its pathological hallmark. The disease is caused by gain-of-function (GoF) mutations in Stim1 and Orai1, key regulators of Store-Operated Calcium Entry (SOCE), leading to excessive Ca^{2+} influx and hyperactive SOCE. However, the mechanisms linking altered Ca^{2+} signaling and TA formation remain unresolved. Mitsugumin 29 (MG29) is a muscle-specific protein localized to T-tubules and SR cisternae, where it interacts with key triad proteins. Muscle fibers from MG29 −/− mice exhibit fragmented T-tubules, vacuolated SR, misaligned triads, and reduced SOCE, indicating a role for MG29 in Ca^{2+} homeostasis. Therefore, we hypothesize that MG29 may be involved in TAM pathogenesis, acting synergistically with STIM1/ORAI1 mutations.

Methods: We performed molecular, biochemical, and functional analyses on healthy immortalized myotubes to evaluate MG29 interactions with Orai1/STIM1 and to assess the impact of MG29 silencing on SOCE and intracellular Ca^{2+} homeostasis.

Results: Co-immunoprecipitation followed by western blot and proximity ligation assays revealed that MG29 interacts exclusively with Orai1. Calcium imaging experiments demonstrated that MG29 silencing reduces both intracellular Ca^{2+} concentration and SOCE activity. Furthermore, transfection of myotubes with TAM-associated GoF mutations (L96V STIM1, G98S ORAI1) revealed that MG29 silencing attenuates Ca^{2+} overload and SOCE associated with these variants. To investigate the involvement of MG29 in the effects of candidate drugs for TAM treatment, we have initiated an in vitro pharmacological characterization using dantrolene, which suppresses uncontrolled rise in myoplasmic Ca^{2+} by inhibiting SOCE.

Conclusion: Here we provided the first evidence that MG29 modulates SOCE, thereby opening new avenues for targeted therapies in TAM.

Keywords: TAM, MG29, Stim1, Orai1, SOCE

Patientderived 3D muscleonchip platform for patientoriented preclinical studies in Duchenne muscular dystrophy

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Keywords: Duchenne Muscular Dystrophy, human skeletal muscle organoids, 3D platforms

Preclinical models for Duchenne muscular dystrophy (DMD) and other rare neuromuscular diseases are crucial to dissect disease mechanisms and evaluate targeted therapeutic strategies. In this framework, organonchip systems are attractive because they reproduce key aspects of human tissue complexity in vitro and enable rapid, ethically acceptable drug screening in line with the 3Rs principles. To be useful in translational research, these technologies must show high reproducibility and sufficient throughput to generate robust quantitative data. In this study, we established fibrinbased 3D skeletal muscleonchip bundles from immortalized myogenic precursors of DMD patients carrying distinct dystrophin mutations (DMD1, nonsense mutation in exon 59; DMD2, exon 48–50 deletion, from the Myoline biobank) and from a healthy donor control (hWT). The primary aim was an independent validation of the platform for phenotypic profiling and functional readouts. Comparative analyses revealed marked differences between healthy and dystrophic tissues in contractile performance, including force generation and fatigue resistance, confirming that the system captures diseaserelevant functional impairments. When muscle bundles were maintained over time in a stable physiological culture without continuous nervelike stimulation, the functional gap between healthy and dystrophic bundles progressively decreased. This suggests that an important component of the DMD phenotype in this setting may be delayed or impaired myogenic maturation. Given the central role of dystrophin and known aggravating factors in DMD onset and progression, additional triggers—such as contraction induced mechanical stress and/or a proinflammatory environment—may be required to fully unmask dystrophy related structural and functional alterations in 3D muscle organoids, enhancing their value for drug testing and personalized therapeutic development.

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Defining the cellular and metabolic signatures of SEPN1-Related Myopathy using patient- derived skeletal muscle cells for drug target identification

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Aim and methods. SEPN1-related myopathy (SEPN1-RM) is a rare congenital muscle disorder caused by loss-of-function mutations in the SEPN1 gene, which encodes the sarcoplasmic reticulum (SR) reductase SEPN1. Clinically, SEPN1-RM is characterized by axial muscle weakness, early- onset scoliosis, rigid spine, and respiratory insufficiency. SEPN1 reductase activity is crucial for maintaining redox homeostasis, also in terms of its interaction with the Endoplasmic Reticulum Oxidoreductase 1 Alpha (ERO1A), a key mediator of the unfolded protein response. Currently, no effective treatment is available for SEPN1-RM and the use of taurodeoxycholic acid (TUDCA), an FDA-approved bile acid derivative, has been recently proposed as potential therapeutic approach (Germani et al., Cell Rep Med 2024). To gain insight into the underlying mechanisms of SEPN1- RM, we here conducted a comprehensive characterization of myoblasts and myotubes derived from a paediatric patient carrying the SEPN1 c.827_829dup variant, using a range of techniques including real-time PCR, Western blotting, high-content imaging, and NMR-based metabolomic analysis.

Results. SEPN1-mutant myoblasts exhibited reduced proliferative capacity compared to controls, while showing enhanced differentiation into myotubes. Gene/protein expression analysis of myogenic markers (Pax7, Myf5, Myod, Mef2c, Myog, Dmd), along with key regulator of calcium handling (Serca, MCU, VDAC, Csq) indicated alteration in myogenesis and calcium homeostasis in SEPN1-mutant myotubes. The increased expression of ERO1A further supports its role as an important mediator in the SEPN1-RM pathogenesis. Metabolic profiles revealed significant differences between SEPN1-mutant and control myotubes, with higher levels of citrate, pyruvate, and taurine in the mutant cells. Notably, very similar molecular alterations have been observed in association with another SEPN1 variant (c.66_73dup), which we have recently begun to characterize. We are currently evaluating the potential beneficial effects of TUDCA, focusing on functional parameters such as mitochondrial respiration and cytoplasmic and mitochondrial calcium levels.

Conclusions. Our findings define distinct cellular and metabolic signatures associated with SEPN1-RM, providing new insights into the pathophysiology of this rare disease and support the identification of novel therapeutic targets.

Dysregulation of the HGF/c-MET signaling axis in Duchenne muscular dystrophy: a new potential therapeutic target

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Introduction. Duchenne muscular dystrophy (DMD) is a rare paediatric disease caused by dystrophin deficiency, leading to destabilization of dystrophin-associated glycoprotein complex. This results in chronic inflammation, myofiber damage, progressive exhaustion of regenerative capacity, culminating in fibro-adipose tissue replacement. Current corticosteroid treatment provides only symptomatic benefit highlighting the need for novel therapeutic approaches. This study focuses on the hepatocyte growth factor (HGF)/c-MET axis, involved in cell survival, tissue regeneration, and modulation of inflammation and fibrosis.

Methods. HGF/c-MET expression was analysed in gastrocnemius muscle and cardiac tissue from two dystrophic mouse models, the classic C57BL/10ScSn-Dmdmdx/J (BL10-mdx) and the hyper fibrotic D2.B10-Dmdmdx/J (D2-mdx), compared to their wildtypes (BL10-WT, D2-WT) across lifespan (n=5 per cohort), alongside disease-related marker genes. In addition, qPCR analyses were performed on immortalized human myogenic cells from a healthy donor and a DMD patient carrying a spanning deletion in exons 48-50, during differentiation (24 hours, 5, 10 days).

Results. Dystrophic mice showed altered HGF/c-MET expression during disease progression, with increased HGF in both models and consistently higher MET levels in D2-mdx mice than BL10-mdx and controls, in agreement with the more severe phenotype. Similarly, DMD muscle cells presented elevated HGF/c-MET expression, which paralleled their myogenic impairment, evidenced by delayed expression of differentiation markers, reduced fusion index, and lower MF20 signal intensity, suggesting a compensatory response.

Conclusions. These findings disclosed a pathological dysregulation of HGF/c-MET signaling in dystrophic conditions, likely reflecting an attempt to sustain regeneration and identify this pathway as a potential target for mitigating inflammation, tissue damage, and fibrosis in DMD.

KEY WORDS: Duchenne muscular dystrophy, HGF/C-MET axis, regeneration, therapy

AQP3 and AQP9 are pivotal in human leukocyte motility and show pharmacological relevance as drug targets in immune disorders

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Blood leukocytes can migrate to the inflamed tissue, and to engulf and kill invading microbes. This requires rapid changes of cell shape through fast movements of water into or out of the cell across aquaporin membrane channels (AQPs). Here, we study AQP3 and AQP9 in phagocytosis and killing of the pathogenic bacterium K. pneumoniae by human leukocytes, and in LPS-induced cell migration. AQP3 mRNA was seen in peripheral blood mononuclear cells (PBMC) but not in polymorphonuclear white blood cells (PMN). AQP9 was in PBMC and PMN. Immunofluorescence confirmed AQP3 in monocytes and in lymphocytes. AQP9 was in PBMC and neutrophils. Specific inhibitors of AQP3 (DFP00173; DFP) and AQP9 (RG100204; RG) were used for bacterial phagocytosis and killing studies. No role of individually blocked AQP3 or AQP9 was seen in the phagocytosis of K. pneumoniae by neutrophils or monocytes after 60 min of bacterial infection. Impairment of phagocytic capacity of monocytes but not neutrophils was seen only when both AQPs were inhibited simultaneously and when the infection lasted 60 min. No reduction in bacterial killing was seen when the two AQPs were individually or simultaneously blocked. PBMC migration was impaired after exposure to RG in presence or absence of LPS. DFP reduced PBMC migration only under LPS. Neutrophil migration was reduced by RG regardless of LPS challenge or not. AQP3 and AQP9 exert critical but distinct roles in leukocyte motility while no involvement is seen in bacterial killing. Pharmacological gating of AQP3 and/or AQP9 may represent a novel therapeutic strategy in immune disorders, potentially reducing reliance on corticosteroids that have significant safety concerns, especially in pediatric age.

Keywords: white blood cells; aquaporin inhibitors; cell motility; bacterial killing; inflammation; innate immunity; immune dysregulation

Genotype-dependent mitochondrial and myogenic defects in 2D cellular models of Duchenne Muscular Dystrophy

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Aim: Duchenne Muscular Dystrophy (DMD) is a genetic X-linked neuromuscular disorder caused by heterogeneous mutations in dystrophin gene, yet their contribution to phenotypic variability remains unclear. This study aimed to investigate genotype–phenotype correlations in patient- derived muscle precursors, focusing on mitochondrial function and myogenic differentiation.

Methods: Two immortalized dystrophic satellite cell lines (Myoline) were used: HDMD1, carrying a stop codon mutation in exon 59, and HDMD2, with a deletion of exons 48–52. An immortalized healthy cell line (HWT) served as control. Mitochondrial respiration was assessed by Seahorse mitostress assays at different stages of differentiation (48 h, 96 h, and 17 days). Electrophysiological properties were evaluated using automated patch clamp (Patchliner-Nanion). Gene expression analyses of myogenic markers, mitochondrial-related genes, and ion channels were performed by molecular biology techniques.

Results: HDMD1 cells showed a marked and progressive reduction in mitochondrial respiration compared to HDMD2 and HWT, affecting basal respiration, ATP-linked respiration, and maximal respiratory capacity, particularly during differentiation. Electrophysiological analysis revealed reduced membrane capacitance and delayed maturation of inward and outward currents in dystrophic myocytes, more pronounced in HDMD1. Molecular analyses confirmed a mutation-dependent delay in the myogenic program and dysregulation of mitochondrial and ion channel-related genes, with HDMD1 displaying the less differentiated phenotype.

Conclusions: These findings demonstrate mutation-specific defects in mitochondrial function and myogenic differentiation in DMD muscle precursors, supporting the existence of genotype–phenotype correlations at the cellular level and highlighting novel targets for personalized therapeutic strategies.

Keywords: DMD, myogenesis, mytochondria, cellular models

Anti-atrophic effects of JMV2894, a growth hormone secretagogue, in Duchenne muscular dystrophy: novel insights from a preclinical study in D2-mdx mice

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Background & Aim – Growth hormone secretagogues (GHSs) are gaining interest as multi-target therapeutic candidates for Duchenne muscular dystrophy (DMD). This study investigated the effects of JMV2894, a pseudopeptide GHS, in the severe D2.B10-Dmd mdx /J (D2-mdx) mouse model, featuring extensive muscle atrophy and fibrosis.

Methods – Four-week-old D2-mdx mice were treated subcutaneously with JMV2894 (640 or 1280 µg/kg/day) for six weeks. Age-matched wildtype and untreated D2-mdx mice served as controls. Treatment efficacy was assessed using a set of clinically-oriented in vivo/ex vivo outcome measures.

Results – JMV2894 was well tolerated and exerted pronounced anti-atrophic effects, especially at the lower dose. In vivo, this was evidenced by improved hind limb ultrasound volume, with a recovery score (RS) >100% towards wildtypes. Ex vivo, it was confirmed by increased gastrocnemius (GC) myofiber size (RS 33%), and downregulation of atrophy-related genes Atrogin- 1 and MuRF-1 (RS ≥ 54%). JMV2894 also tended to modulate inflammation, reducing alternative complement pathway genes (C3, C3AR1, CFb, CFd), while increasing C1q (RS up to 75%). Fibrosis was mildly improved histologically (RS 19%), despite the observed reductions in GC echodensity (RS 25%) and matrix-remodeling genes (TGF-β1, COL1A1, MMP-9, ADAMTS-5; RS up to 95%). Increased IGF-1 transcript and plasma levels (RS >100%), along with receptor and downstream signaling upregulation, suggest GH-mediated actions of JMV2894. Benefits occurred despite limited muscle exposure, suggesting the need for improved formulations.

Conclusions – These findings disclosed novel, phenotype-specific effects of JMV2894 in dystrophic settings, highlighting the challenges of translating preclinical results into broadly effective therapies for DMD and other rare paediatric neuromuscular disorders.

Keywords – Duchenne muscular dystrophy; preclinical study; D2-mdx; growth hormone secretagogues; muscle atrophy

Targeting intestinal epithelial homeostasis in paediatric ulcerative colitis: an organoid-based drug discovery approach

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This project aims to identify therapies that restore intestinal epithelium homeostasis in ulcerative colitis (UC) using intestinal organoids.

Adult stem cell-derived organoids were generated from biopsies of UC paediatric patients (n=6, mean age 12.8 years). Additional models included LS180 and NF-κB reporter-Jurkat cells. Viability of 314 natural/semi-synthetic compounds (1μM, 72h) was assessed by CellTiter-Glo3D assay, identifying 11 molecules increasing stem organoid viability (≥10%) without compromising differentiated organoids (reduction ≤20%). Gene expression was evaluated by TaqMan assays, protein expression by immunoblotting, and chemokines by ELISA. Stem cell-enriched organoid treatment (1μM, 24-48h) showed that all compounds reduced MUC2 mRNA, indicating decreased goblet cell differentiation, while none reduced stemness markers (LGR5, ATAD2, STMN1). Notably, 4 compounds increased STMN1, suggesting enhanced proliferation and stemness promoting epithelial regeneration. Nine compounds demonstrated anti- inflammatory effects reducing CXCL1 and/or IL8 secretion, with vincanol and quercetin showing the most consistent reduction of both chemokines at both timepoints (p<0.05), without affecting mRNA levels, indicating post-transcriptional regulation. NF-κB signaling remained unchanged. Since lysergol showed promising effects on viability and IL8 reduction independently of NF-κB, proteomic analysis by mass spectrometry (1μM, 24h) revealed downregulation of TRAF2 (padj=0.0065) and EPS15 (padj=0.012), and upregulation of CDX2 (padj=0.024), suggesting alternative mechanisms affecting chemokine stability and epithelial differentiation.

These findings demonstrate that intestinal organoids enable the identification of dual-action molecules restoring epithelial homeostasis through regenerative and anti-inflammatory pathways, representing promising candidates for paediatric UC therapy.

Keywords: ulcerative colitis, intestinal organoids, paediatric patients, drug discovery, intestinal regeneration

Five variants in the voltage sensing domain of kv7.2 potassium channels cause developmental and epileptic encephalopathy by gain-of-function effects

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Aim: Variants in KCNQ2 or KCNQ3, encoding for Kv7.2 and Kv7.3 voltage-gated potassium channel subunits, respectively, have been identified in patients with developmental and epileptic encephalopathies (DEE). DEE represents a heterogeneous group of rare disorders, characterized by drug-resistant seizures and developmental delay or regression. Structurally, each Kv7 subunit contains six transmembrane segments, with the S1–S4 region forming the voltage-sensing domain (VSD), whereas segments S5-S6 constitute the pore region. In the present work, we describe the clinical features of five DEE patients carrying KCNQ2 variants and investigate their in vitro functional consequences. All variants are located within the VSD of the Kv7.2 channel.

Methods: Wild-type Kv7.2 channels or channels carrying the pathogenic variants were transiently expressed in mammalian CHO cells and analyzed using whole-cell patch-clamp recordings.

Results: Patch-clamp recordings showed that, when expressed as homomeric channels, all five variants exhibited a hyperpolarizing shift in the voltage dependence of activation, and only three exhibited an increased current density. Similar results were also described in the heteromeric configuration with Kv7.3 subunits. Interestingly, the variant within the S4 segment dramatically affects Kv7.2 kinetics and the slope of the activation curve. These electrophysiological properties are consistent with a gain-of-function pathogenic mechanism.

Conclusions: Overall, this study expands our catalogue of gain-of-function Kv7.2 variants and further supports the existence of a distinct clinical phenotype associated with Kv7.2 GoF mutations, quite distinct from that observed in patients carrying Kv7.2 LoF variants.

Keywords: Kv7.2; Developmental and epileptic encephalopathies; voltage-sensor domain

Preclinical evaluation of conjugated linoleic acid dietary supplementation on antioxidant pathway restoration in SOD1-G93A mouse model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease for which there is currently no cure. Both motor-neurons and skeletal muscle are functionally impaired1. Many factors contribute to the onset of the disease and the aetiology is complex. Oxidative stress plays a critical role and accelerates cell damage. In ALS patients the activity of the nuclear factor erythroid 2-related factor (Nrf2), the main transcriptional regulator of antioxidant and anti-inflammatory responses and mitochondrial biogenesis2, is compromised. We investigated the effects of conjugated linoleic acid (CLA), an activator of Nrf2 signalling pathway by chronic administration in SOD1-G93A mouse model of ALS. Animals were fed a CLA-enriched diet (600mg/kg/day) starting at early stage of life (60 days) to prevent the symptoms of the pathology. Age-matched WT were included as controls. Experimental cohorts consisted of WT (n=8), SOD1-G93A (n=10) and SOD1-G93A+CLA (n=12) mice. qRT-PCR and Western blot analysis were performed to assess the expression of G6PD, NQO1 and PGC-1a which support antioxidant activity and Nrf2 activation. CLA supplementation promoted an increase in gene expression of G6PDX by 356% (P=0.03), NQO1 by 168% (P=0.0710) and PPARGC1A by 55% (P=0.0426) vs. untreated mice. Accordingly with the mRNA expression we found an increase in protein expression of G6PD by 53% (P=0.0275) and NQO1 by 35% (P=0.0949) toward the WT value. Statistical analysis was performed using Student's t-test. These findings suggest that dietary CLA supplementation can activate Nrf2 pathway and may support and improve current ALS therapies. (Funded by European Union-Next Generation EU project PRIN-PNRR 2022 P202224WKC).

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Drug repurposing of SGLT2 inhibitors for Lafora Disease: bridging preclinical research and clinical evidence

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Aims and Methods. Lafora disease (LD) is a fatal, currently untreatable neurodegenerative disorder characterized by progressive myoclonus epilepsy and severe neurological decline. Sodium-glucose cotransporter-2 inhibitors (SGLT2i), known as gliflozins, have been proposed as candidate drugs for repurposing based on extensive evidence of neuroprotective effects reported in literature (Imbrici et al, Pharmacol Res 2024). We conducted a single-center, first-in-human observational pilot study in two LD patients treated with empagliflozin (10 mg/day) for six months. Safety and tolerability were primary outcomes. Exploratory efficacy measures included seizure frequency, EEG features, neurological and motor assessments, and brain imaging using MRI and FDG-PET. In parallel, to assess whether gliflozins reduce glycogen accumulation, an immunofluorescence (IF)-based assay was performed in SH-SY5Y neuroblastoma cells.

Results. Empagliflozin was safe and well tolerated, with expected pharmacodynamic effects (glucosuria and mild ketonuria with normal glycemia). Clinically, seizure frequency, myoclonus, and global motor function remained largely stable, with variable cognitive outcomes. Notably, while brain MRI showed stable mild cortical atrophy, FDG-PET revealed progressive and spatially expanding cerebral hypometabolism, suggesting ongoing metabolic dysfunction despite clinical stabilization. In vitro IF-based assay showed that none of the tested gliflozins decrease glycogen levels (percentage of red spot area sum at 30μM = 91.3%, 93.6% and 88.4% for dapagliflozin, empagliflozin and canagliflozin vs 28.6% for glycogen synthase inhibitor MZ-101 at 2 mM).

Conclusions. These findings suggest that empagliflozin may exert symptomatic or functional effects at the neuronal level, possibly via pharmacometabolic mechanisms, without halting neurodegeneration. Larger biomarker-driven trials are needed to assess the long-term therapeutic potential of SGLT2 inhibitors in this ultra-rare disease.

Keywords: Lafora disease, gliflozins, SGLT2, epilepsy, glycogen

In vitro cytotoxicity effects of Tyrosine Kinase Inhibitors (TKIs) on Diffuse Intrinsic Pontine Glioma

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Aim: DIPG (Diffuse Intrinsic Pontine Glioma) is a rare pediatric high-grade glioma, which treatment represents a global challenge [1]. Due to the known dysregulation of tyrosine kinases in DIPG [2], we evaluated Tyrosine Kinase Inhibitors (TKI) through cytotoxicity assays.

Methods: Our aim is to assess the activity of TKIs on DIPG-36(H3.1K27M) and DIPG-50(H3.3K27M) cells. We performed Crystal Violet (CV), CCK-8 assays on DIPG-36 to evaluate the activity of dasatinib (DASA), everolimus (EVE), crizotinib (CRIZO), lapatinib (LAPA), erlotinib (ERLO), perifosine (PERIF) and midostaurin (MIDO) after 48-72h. Clonogenic assay was performed on the most effective drugs. Midostaurin was investigated through DiBAC4(3), which correlates cell fluorescence and intracellular voltage; MIDO was also evaluated on DIPG-50 cells through CV and CCK-8. Statistical analysis used was one-way ANOVA.

Results: In DIPG-36, CV showed a strong cell's survival reduction after 48h, particularly for EVE 100μM (0%), CRIZO 100 μM(6.08%) and 50μM MIDO (2.10%). After 72h, we obtained 0% cell survival using DASA 100μM, EVE 100μM and MIDO 50μM. In CCK-8, DASA, CRIZO 100μM and MIDO 50μM shown 0% survival after 4872h; 0% was also obtained using PERIF 100μM and EVE 100μM after 72h. Clonogenic assay showed complete colony inhibition with 2.14 μM MIDO, 100 μM EVE, LAPA and DASA. Midostaurin eliminated colony formation at 2.14μM; so, it was investigated in DIPG-50 after 48-72h obtaining 19% and 0% cell survival. IC50(M) were in nanomolar concentration after 72h in CCK-8.

Conclusions: Therefore TKIs, particularly midostaurin, possess a cytotoxic action in DIPG. Future aims will be to further investigate target proteins to better understand mechanisms of cell death in these cell lines.

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Keywords: DIPG (Diffuse Intrinsic Pontine Glioma), TKIs (Tyrosine Kinase Inhibitors), Cytotoxicity, Pediatric Tumors, Brain Tumors

Early renal fibrotic alterations in D2 mdx mouse model of Duchenne muscular dystrophy and potential protective effects of the growth hormone secretagogue JMV2894

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Aim: Duchenne muscular dystrophy (DMD) is a rare pediatric-onset disorder increasingly recognized as a multisystem disease. Recent therapeutic advances have extended survival in patients with Duchenne muscular dystrophy (DMD), worsening late complications such multiorgan dysfunction. However, early involvement of tissues other than skeletal muscle can contribute to pathological progression. In this frame we aimed to characterize early renal structural alterations in the severe D2-mdx mouse model of DMD. In addition, we evaluated the potential renoprotective effects of the growth hormone secretagogue (GHS) peptide JMV2894, that showed beneficial effects on D2-mdx muscle pathology in previous studies.

Methods: Thirty-two four-week-old male mice were divided into four groups: D2 wild type, D2-mdx treated with vehicle, and D2-mdx treated with JMV2894 at 640 or 1280 µg/kg/day administered subcutaneously for six weeks. Renal architecture and fibrosis were assessed ex vivo by hematoxylin–eosin and Sirius Red staining for collagen deposition; renal weight was normalized to mouse body weight. Plasma insulin-like growth factor-1 (IGF-1) levels were assessed in vivo by ELISA. Statistical analyses were performed using t-tests and one-way ANOVA, with $p < 0.05$ considered significant.

Results: D2-mdx mice showed increased normalized renal weight, glomerular and tubular alterations, and significantly enhanced renal fibrosis compared to controls. JMV2894 treatment further increased normalized renal weight, while improving renal histoarchitecture particularly at the lower dose. Collage staining and percent of fibrotic tissue was significantly reduced at both doses, with recovery scores of 63% and 85% for the low and high doses, respectively. IGF-I levels were slightly reduced in D2 mdx vs. wt and increased after treatment with 640 µg/kg of IMV2894.

Conclusions: These findings demonstrate for the first time an early renal involvement in the D2-mdx model and support a potential protective effect of JMV2894 on renal structure and fibrosis, likely via IGF-1.

Keywords: Duchenne muscular dystrophy; kidney; fibrosis; JMV2894; IGF-1

Metachromatic Leukodystrophy with Dystonia - Successful Management with Cannabidiol

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Aim: Metachromatic leukodystrophy (MLD) is a rare lysosomal storage disorder in which epilepsy occurs in more than 70% of patients, yet seizure semiology remains poorly characterized. This study aimed to describe seizure types associated with MLD through a systematic review and to report an original clinical case evaluating the response to conventional antiseizure medications (ASMs) and adjunctive cannabidiol (CBD), which has also shown efficacy in treating dystonia, a frequent comorbidity in these patients.

Methods: A systematic review was conducted according to PRISMA guidelines using PubMed, SCOPUS, Web of Science, and ClinicalTrials.gov. Studies including patients with MLD and epilepsy and reporting seizure types, treatments and outcomes were considered. Of 142 screened studies, 6 met the inclusion criteria.

Results: Epilepsy in MLD showed heterogeneous phenotypes, including generalized tonic–clonic seizures (45%), epileptic spasms (20%), and myoclonic seizures (15%). No studies reported the use of CBD for MLD-associated epilepsy. We also describe a pediatric patient with MLD, refractory myoclonic seizures, and dystonia despite treatment with levetiracetam, clonazepam and baclofen. The addition of CBD to ASMs resulted in significant seizure control. To our knowledge, this represents the first reported case of successful CBD use in epilepsy associated with MLD.

Conclusions: This review highlights the high prevalence and heterogeneous presentation of epilepsy in MLD, as well as the absence of evidence regarding CBD use in this context. Our findings suggest that CBD may represent a promising adjunctive option for refractory epilepsy in MLD, supporting the need for further investigations

Keywords: Metachromatic Leukodystrophy, Epilepsy, Dystonia, Antiepileptic drugs, Cannabidiol

Beneficial effects of SS-31 peptide on cardiac mitochondrial morphology and dysfunction in a murine model of Barth Syndrome

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Barth Syndrome (BTHS) is a rare, life-threatening, X-linked disease, which mainly causes infancy or childhood-onset cardiomyopathy and skeletal myopathy, but also growth delay, neutropenia, organic aciduria. BTHS results from loss-of-function mutations of the TAFAZZIN (TAZ) gene, encoding for an acyltransferase enzyme (tafazzin), which is required for remodelling of the mitochondrial phospholipid cardiolipin (CL) towards its highly symmetrical acyl composition. Deregulation of CL maturation results in a dramatically increased monolysocardiolipin (MLCL)/CL ratio in BTHS mitochondria associated with abnormal inner membrane ultrastructure and respiratory chain dysfunction. The development of an effective therapy remains challenging, particularly because of extraordinary phenotype variability and unpredictable clinical course of BTHS patients. The aim of our study is an investigation on the effects of SS-31 peptide in vivo treatment in a BTHS murine model. This small CL-targeted peptide is one of the most promising therapeutic approaches for mitochondrial diseases as it improves inner membrane stability and bioenergetics functions. Our results showed that SS-31 treatment of tafazzin-knockdown (TAZ-KD) mice (4-month-old male mice, 3 mg/Kg/day for 10 weeks) promoted supercomplexes organization and improved respiratory capacity in isolated heart mitochondria, without changes in the MALDI-TOF/MS phospholipid profiling. The beneficial effects of the treatment on tafazzin-deficient dysfunctional mitochondria were associated with restoration of defective mitophagy and amelioration of mitochondrial ultrastructural morphology observed by TEM. In conclusion, our study indicates that the pharmacological treatment with SS-31 significantly improves cardiac mitochondrial dysfunction and altered morphology in a BTHS animal model, suggesting the peptide as future well-suited therapeutic candidate for patients with BTHS.

Keywords: mitochondrial rare disease, cardiac dysfunction, tafazzin, cardiolipin remodeling, SS-31 peptide.

From the First FDA-Approved Therapy to Next-Generation ClpP Activators: Deconstructing Dordaviprone (ONC201) for Pediatric H3K27-Altered Diffuse Midline Glioma

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Background: Diffuse Midline Glioma (DMG) harboring H3K27 alteration is one of the most aggressive and therapeutically challenging pediatric brain tumors. H3K27M mutation induces profound epigenetic dysregulation, leading to global chromatin remodeling and aberrant transcriptional programs that drive tumor progression and drug-resistance. Despite extensive clinical efforts, long-sought effective treatment options remained unavailable, highlighting a critical unmet medical need in pediatric oncology. Mitochondrial proteostasis has recently emerged as a druggable target in DMG. In particular, the mitochondrial caseinolytic protease ClpP is essential for mitochondrial protein quality control, and its hyperactivation by small molecules induces uncontrolled degradation of mitochondrial matrix proteins, resulting in metabolic collapse and tumor cell death. Dordaviprone (ONC201), a first-in-class imipridone and ClpP activator, has demonstrated clinical efficacy in H3K27M-mutant DMG, although limited to a few cases, receiving the Fast Track approval from the U.S. FDA as the first treatment for this rare pediatric brain tumor.

Aim: While this milestone validates ClpP activation as a clinically feasible strategy, heterogeneous patient responses and disease progression underscore the need to identify alternative drug-candidates for non-responding patients.

Methods: By deconstructing the chemical structure of the imipridone core, depriving it of its rigid tricyclic scaffold, we rationally designed and synthesized a new generation of compounds with novel chemical frameworks, then subjected to biological and pharmaceutical profiling, including stability, cytotoxicity, membrane permeability, ClpP activity, and affinity.

Results: The newly drug-candidates, initiated to a comprehensive preclinical study, retain high selectivity and activity toward ClpP, resulting in cell death in patient-derived DMG cells. The detailed results of such investigation, regarding the development of new drug-candidates along with their pharmaceutical profile, will be presented and discussed.

Keywords: DMG, ClpP, Mitochondria, ONC201, ClpP activators

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Targeting inflammation and redox imbalance in Duchenne muscular dystrophy using a corticosteroid–H_2S hybrid

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Aim: deflazacort (DFZ) represents the gold-standard pharmacological therapy for Duchenne Muscular Dystrophy (DMD), but its long-term use is limited by adverse effects. We demonstrated that hydrogen sulfide (H_2S) supplementation improves skeletal muscle (SKM) function in dystrophic mice 2. The present work investigates the therapeutic potential of DFZ–H_2S donor hybrids in DMD.

Methods: In vitro experiments were performed in C2C12 myotubes pre-treated with DFZ–H_2S donor hybrids, 21-DDF (the active principle of DFZ), or vehicle before exposure to LPS+IFN-γ for 24 hours. Then NOx production, intracellular ROS, and inflammatory/redox gene expression were evaluated. In vivo experiments were performed on mdx mice treated for 12 weeks with the selected H_2S hybrid, DFZ or vehicle for evaluating SKM performance and muscle redox status.

Results: In myotubes, LPS+IFN-γ induced a robust inflammatory response, evidenced by increased Nox production and upregulation of TNFα, iNOS, and IL-6 (***p<0.001; n=4). Pre-treatment with the reference compound 21-DDF significantly reduced inflammatory markers. Among the hybrids tested, 21-ADT lowered NOx levels (**p < 0.01; n=4), displaying a response profile comparable to 21-DDF. Gene expression analysis confirmed that 21-ADT showed the strongest anti-inflammatory effect compared to the other hybrids (*p < 0.05). In mdx mice, ADT-hybrid treatment improved SKM performance (**p < 0.01; n=4), achieving functional recovery comparable to DFZ but at half the dose. Unlike DFZ, the ADT-hybrid markedly reduced H_2O_2 levels and restored the GSH/GSSG ratio (*p < 0.05).

Conclusions: ADT-hybrid represents a promising option for improving SKM function and redox balance in DMD**.**

Keywords: DMD, H 2 S-DFZ hybrid, SKM

(1) Biggar et al; J Neuromuscul Dis.

(2) Panza et al., 2021. Redox Biol.

Protective Role of FHR-2 in the Glomerular Microenvironment: Regulation of the Terminal Complement Pathway and Its Pathogenetic Implications in Pediatric aHUS.

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Background: The FHR2, the smallest protein in the FH-FHRs family, inhibits the alternative complement pathway. Mutations are linked to aHUS and C3GN. Despite its role, this factor has been insufficiently investigated.

Aims and Methods: We aimed to evaluate FHR2's binding capabilities in the kidney microenvironment by analysing its interactions with HUVECs and GBM components. We assessed the inhibition of C5b-9 cell deposition and C3b cofactor activity using serum from a aHUS patient (2337) with FHR2 deficiency, who developed aHUS as previously described (1). We compared this serum to Normal Human Serum with standard FHR2 levels. To test FHR2 dependence, we added recombinant FHR2 at 140 ng, produced in HEK-293 cells and purified via affinity chromatography. Binding and regulatory activities were examined using confocal microscopy, FACS, ELISA, and Western blot.

Results and Conclusions: We show that FHR2 has low-affinity binding to intact HUVECs but binds strongly to necrotic cells at SCR1-2. This suggests FHR2 may differentiate healthy from damaged tissues, affecting immune responses in the kidney. While FHR2 binds to all GBM components dose-dependently, it has a higher affinity for laminin-521, also at SCR1-2. Functionally, FHR2 supports FH in C3b cofactor activity and blocks the deposition of C5b-9, suggesting a synergistic role controlling complement activation. Supplementing FHR2 at 140 ng/ml in serum from a patient with complete FHR2 deficiency markedly decreases C5b-9 deposition on HUVECs, demonstrating FHR2's ability to restore impaired complement regulation. This change highlights the potential of exogenous FHR2 as a therapeutic option.

1. Stea ED, Skerka C, Accetturo M, et al. Case report: Novel FHR2 variants in atypical Hemolytic Uremic Syndrome: A case study of a translational medicine approach in renal transplantation. Front Immunol. 2022; 13:1008294. doi:10.3389/fimmu.2022.1008294

L-Citrulline exerts diaphragm-specific effects in mdx mice alone or in combination with gold standard steroids: insight into its potential as adjuvant therapy in Duchenne muscular dystrophy

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Aim: The absence of functional protein dystrophin in Duchenne Muscular Dystrophy (DMD) leads to progressive muscle weakness, paralleled by failing regeneration and deregulation of nitric oxide (NO) signaling. Here, we focused on L-Citrulline (L-Cit), a precursor of L-arginine (L-Arg) required for NO production in muscle and reduced in dystrophic mdx muscle.

Methods: L-Cit was administered (2 mg/g/die), through diet, alone, in comparison and/or combination with the gold standard prednisolone (PDN; 1 mg/kg, 5 days/week subcutaneously) to 4–5-week-old mdx mice for 8 weeks. Outcomes were evaluated on in/ex vivo readouts.

Results: L-Cit supplementation effectively increased the levels of L-Arg, L-Cit and L-Ornithine in plasma and quadriceps of mdx mice. Interestingly, L-Cit, alone or plus PDN, significantly improved maximal forelimb force in vivo, while ameliorating DIA movement amplitude and reducing DIA echodensity. In parallel, ex vivo, we detected a significant improvement of DIA force and contraction kinetics in mice treated with L-Cit alone or in combination with PDN. LCit was also able to restore the expression of genes involved in Ca2+ handling during contraction (RyR1, RyR3 and SERCA), while reducing the markers of inflammation and fibrosis (CD68 and TGFb1), and ameliorating mitochondrial biogenesis-associated genes (PGC1-α and MEF2C). No effect was observed on S-nitrosylation levels of HDAC2 and on DIA and GC nNOS gene expression, suggesting a NO-independent mechanisms underlying the positive outcome observed.

Conclusions: Our results revealed the ability of L-Cit supplementation to ameliorate in vivo and ex vivo function of DIA muscle, highlighting novel metabolic and calcium-related mechanisms of potential clinical interest. Supported by Duchenne Parent Project The Netherlands (DPP-NL) and by PRIN MUR (Research Projects of National Relevance- Ministero dell'Università e della Ricerca) 2020.

Keywords: Duchenne muscular dystrophy; nitric oxide; L-citrulline; L-Arginine; diaphragm; calcium handling