JIM
JIM 2025; 2 (4): e1052
DOI: 10.61012_202511_1052
Category: *Opinion paper*

PEGZILARGINASE AND BEYOND: ITALIAN REAL-WORLD EXPERIENCE AND PROPOSAL OF INNOVATIVE TOOLS FOR COMPREHENSIVE MANAGEMENT OF ARG1-D

S. GASPERINI[1], D. MARTINELLI[2], F. PORTA[3,4]

[1]Inherited Metabolic Disorders Unit, Department of Pediatrics, Fondazione San Gerardo dei Tintori IRCCS, Monza, Italy
[2]Inpatient Unit of Metabolic Diseases, Unit of Metabolic Diseases and Hepatology, Bambino Gesù Children's Research Hospital IRCCS, Rome, Italy
[3]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy
[4]Department of Pediatrics, AOU Città Della Salute e Della Scienza di Torino, Turin, Italy

Corresponding Author
Diego Martinelli, MD; e-mail: diego.martinelli@opbg.net

***ABSTRACT* –** Arginase 1 deficiency (ARG1-D) is a rare urea cycle disorder primarily affecting the central nervous system with progressive spasticity, cognitive decline, and variable seizures, despite often mild hyperammonemia. Conventional treatment fails to maintain optimal metabolic control, leading to irreversible functional impairment. Pegzilarginase, a pegylated recombinant human ARG1 enzyme, has recently emerged as a causal therapy capable of reducing plasma arginine and improving motor outcomes, marking a new era in disease-modifying interventions.

This expert opinion paper, authored by an Italian multidisciplinary panel – including clinicians with real-world and trial-based experience – aims to harmonize diagnostic and follow-up strategies in ARG1-D. Based on literature review, clinical experience, and evidence from the PEACE trial, the panel proposes a multidomain clinical framework integrating core, advanced, and exploratory assessments across biochemical, neuromotor, imaging, hepatic, musculoskeletal, and quality-of-life domains.

The proposed model includes essential tools such as plasma arginine and guanidino compounds monitoring, standardized motor function scales (GMFM, GMFCS), MRI, and cognitive assessments. Optional investigations like cerebrospinal fluid (CSF) arginine, proton magnetic resonance spectroscopy (^{1}H-MRS), and motor evoked potentials offer mechanistic insights, while exploratory biomarkers [e.g., growth differentiation factor 15 (GDF15), neurofilament light chain] may refine understanding of redox-autophagic mechanisms and functional recovery. The paper highlights unmet needs, including muscle fibrosis and autophagy modulation by arginine, with implications for both pathogenesis and treatment response.

This framework serves as a foundation for future national guidelines and research. By fostering standardized, evidence-based care and embedding advanced tools in routine practice, it aims to improve outcomes for current and future patients with ARG1-D, transforming the natural history of this previously relentless disorder through expert collaboration and innovation.

Keywords: Arginase 1 deficiency, Urea cycle disorders, Pegzilarginase, Real-world evidence, Multidomain clinical monitoring, Multidisciplinary management, Functional biomarkers, Real-world multidomain monitoring framework, Neurometabolic disease management, Expert consensus in rare disorders.

INTRODUCTION

Urea Cycle Disorders: General Framework and Clinical Significance

Urea cycle disorders (UCDs) are a group of rare inherited metabolic conditions resulting from defects in enzymes or transporters involved in the urea cycle, the primary pathway for detoxifying and excreting excess nitrogen in the form of urea. The disruption of this pathway leads to the accumulation of nitrogenous waste products, causing episodic or chronic hyperammonemia and associated neurotoxicity[1]. The clinical spectrum of UCDs is heterogeneous, ranging from neonatal-onset classical forms to later-onset variants characterized by intermittent hyperammonemia, gastrointestinal symptoms, cognitive decline, behavioral disturbances, or motor impairment[1,2]. While the overall incidence of UCDs is estimated to be between 1 in 35,000 and 1 in 50,000 live births, their diagnosis remains challenging due to symptom overlap with more common neurological or hepatic conditions[1]. The introduction of newborn screening (NBS) programs has improved early detection for some UCDs, although limitations persist for certain forms not reliably detectable by this approach[1,2].

Arginase 1 Deficiency: Clinical Burden and Diagnostic Challenges

Arginase 1 Deficiency (ARG1-D) is the most distal and rarest UCD, with an estimated global prevalence ranging between 1:726,000 and 1:950,000[1,2]. Unlike other UCDs, which are primarily characterized by life-threatening hyperammonemia, ARG1-D manifests predominantly during early childhood with spastic paraplegia. Additional clinical clues include slowing of linear growth, progressive neurological involvement, gait abnormalities, plateauing and subsequent regression of cognitive development, intellectual disability, and epilepsy, often mimicking cerebral palsy[2,3]. Seizures occur in over 60% of cases and may be resistant to treatment, while hyperammonemia, though possible, is typically mild and episodic[1,4]. Cognitive function is variably affected, with some patients showing intellectual disability and behavioral issues such as irritability, inattention, or hyperactivity[3,4].

Biochemically, ARG1-D is characterized by chronically elevated blood arginine levels, typically three to fourfold above the upper limit of normal. Accumulation of arginine and its downstream metabolites, including guanidino compounds (GCs), plays a central role in disease pathophysiology, contributing to neuronal dysfunction and myelination defects through mechanisms such as gamma-aminobutyric acid (GABA) receptor inhibition and oxidative stress[2,3,5].

The diagnostic process relies on biochemical evidence of persistent hyperargininemia and is confirmed by biallelic pathogenic variants in the *ARG1* gene. Additionally, reduced arginase activity in red blood cells can be detected[3]. However, diagnosis is often delayed due to the absence of specific symptoms in the neonatal period, poor disease awareness, and overlapping features with non-metabolic neurodevelopmental disorders such as cerebral palsy[1,3].

Despite current dietary and pharmacological interventions aimed at lowering plasma arginine levels and preventing hyperammonemia (i.e., protein restriction and ammonia scavengers), patients hardly achieve optimal metabolic control (blood Arg <200 µmol/L), especially due to the endogenous production of arginine[1,5]. As a result, individuals with ARG1-D face progressive loss of functional mobility, communication abilities, and independence, ultimately leading to substantial caregiver burden and impaired quality of life[2,5].

Recent data from the Pegzilarginase Effect on Arginase 1 Clinical Endpoints (PEACE) trial[5] demonstrated that pegzilarginase, cobalt-substituted, pegylated human ARG1 enzyme replacement therapy, is capable of rapid and sustained reduction of blood arginine concentrations, with meaningful clinical improvements in gross motor function. This highlights a new era in disease-modifying therapy, shifting the focus from merely slowing progression to enabling potential recovery of function and proactive long-term management.

Prior Italian Experience in Diagnosis, Management, and Follow-Up of ARG1-D

In Italy, the clinical experience with ARG1-D has progressively expanded, although the extremely low prevalence – estimated at approximately 0.61 cases per million inhabitants in 2021 – has contributed to heterogeneous awareness and clinical practices across centers and specialties[2]. Diagnosis in Italy predominantly relied on clinical recognition of hallmark neurological features of ARG1-D, including progressive lower-limb spasticity, developmental delay or regression, cognitive impairment, and persistently elevated plasma arginine levels, often prompting re-evaluation of patients initially diagnosed with cerebral palsy or hereditary spastic paraplegia[2,4].

The standard of care for Italian patients traditionally included a combination of dietary protein restriction and oral nitrogen-scavenging agents. Management also includes symptomatic treatment of seizures, spasticity, and neuromotor complications, often requiring multidisciplinary input from metabolic specialists, neurologists, and rehabilitation teams. Follow-up protocols across Italian centers have included regular biochemical assessments, clinical neuromotor evaluations, and, in select cases, neuroimaging or neurophysiological studies, although standardization of monitoring strategies remains an area of unmet need[2]. Disease follow-up includes monitoring of bone metabolism, growth, and nutritional balance, particularly in light of the markedly protein-restricted diet. From February 2025, pegzilarginase became available as an etiologic treatment for ARG1-D patients aged two years and older[2,3].

The PEACE Trial: Design, Outcomes, and Clinical Relevance

The PEACE trial[5] represented the first randomized, double-blind, placebo-controlled phase 3 study specifically designed to assess the efficacy and safety of pegzilarginase in individuals with ARG1-D. The trial enrolled 32 patients across seven countries, including Italy, and incorporated a 24-week blinded treatment phase followed by a long-term open-label extension (LTE) of up to 150 weeks. Participants were randomized 2:1 to receive pegzilarginase or placebo, in addition to individualized standard of care, including dietary and pharmacological measures.

Pegzilarginase demonstrated statistically significant and clinically meaningful reductions in plasma arginine, with mean concentrations decreasing from 354.0 µmol/L at baseline to 86.4 µmol/L after 24 weeks – a 76.7% reduction compared to placebo. Notably, over 90% of treated patients achieved normal plasma arginine levels (40-115 µmol/L), a target that is unachievable with standard therapy alone. In parallel, patients showed improvements in functional mobility assessed by validated outcome measures such as the Gross Motor Function Classification System (GMCFS), Gross Motor Function Measures – 88 (GMFM), Gross Motor Function Measure – Parts E and D (GMFM-E; GMFM-D), the Modified Ashworth Scale (MAS), the Gillette Functional assessment, the 9-PEG Hole test, the Functional Mobility Scale (FMS), the Vineland Adaptive Behaviour Scales (VABS) and the 2-Minute Walk Test (2MWT). These gains were maintained and enhanced during the LTE phase, with continued improvement observed at 48 weeks. Pegzilarginase also led to significant reductions in key neurotoxic guanidino compounds, such as guanidinoacetic acid and argininic acid, while increasing plasma ornithine, thereby targeting the pathophysiological drivers of neurologic dysfunction in ARG1-D[5].

The safety profile of pegzilarginase was favorable, with most adverse events being mild or moderate in severity, and no new safety signals emerged during long-term exposure. No treatment discontinuations were reported due to hypersensitivity or metabolic decompensation. Collectively, PEACE provides the first high-quality evidence that enzyme replacement therapy can modify the natural history of ARG1-D, offering a therapeutic approach that not only corrects the metabolic defect but also has the potential to restore functional capacity and improve long-term outcomes for affected individuals[5].

RATIONALE AND OBJECTIVES OF THIS EXPERT OPINION PAPER

The introduction of pegzilarginase as the first etiological therapy for ARG1-D marks a pivotal advancement in the management of this rare and progressive disorder. However, its arrival also highlights the urgent need for a harmonized national framework to guide clinical practice. Until now, the diagnosis and care of patients with ARG1-D in Italy have largely been fragmented across centers, with variability in diagnostic workups, metabolic management strategies, and functional follow-up approaches[1,2].

This heterogeneity, while partially attributable to the rarity and clinical complexity of the disease, could result in inconsistent standards of care and unequal access to optimal treatment pathways. In light of recent therapeutic developments, a nationally shared perspective is critical to ensure equitable, timely, and evidence-based care for individuals with ARG1-D.

This expert opinion paper was conceived as a platform for critical reflection and shared clinical advancement in the management of ARG1-D in the era of enzyme replacement therapy. The initiative brings together the perspectives of a diversified group of clinicians to stimulate dialogue across complementary clinical profiles. The panel also includes clinicians with direct participation in the PEACE trial[5] as well as those with real-life experience managing differently impacted patients, thereby encompassing both investigational rigor and pragmatic clinical insight.

With causal therapy now available, it becomes imperative to re-evaluate the approach to ARG1-D beyond the structured environments of controlled trials. This paper aims to lay the groundwork for national guidance on diagnosis, therapeutic decision-making, and long-term monitoring, with the goal of building consensus both on core clinical practices and on exploratory research parameters.

Ultimately, this collaborative effort aims to serve not only as a practical contribution to national clinical practice but also as a starting point for a broader scientific conversation about the evolving natural history of ARG1-D in the post-pegzilarginase era – an era where patient management must evolve alongside therapeutic innovation.

PROPOSED CLINICAL PARAMETERS AND MONITORING FRAMEWORK

Given the complexity and multisystemic nature of ARG1-D, and in light of the paradigm-shifting therapeutic availability of pegzilarginase, the panel proposes a structured, multidomain framework to support the longitudinal characterization and monitoring of affected individuals. This model integrates assessments across biochemical, metabolic, neuromotor, neuroimaging, neurophysiological, hepatic, musculoskeletal, and quality-of-life domains, aiming to capture both disease mechanisms and individual therapeutic trajectories, and ultimately to inform personalized follow-up strategies.

Importantly, the phenotypic variability of ARG1-D demands a flexible approach to clinical monitoring that should consider age-specific and stage-specific presentation. As highlighted in recent expert consensus[2], certain "red flags", such as progressive lower limb spasticity, developmental regression, seizures, and cognitive or behavioral decline, should prompt further metabolic evaluation, particularly when standard neurological diagnoses (e.g., cerebral palsy or hereditary spastic paraplegia) do not fully explain the clinical course. In pediatric patients, early signs such as tip-toe walking, frequent tripping, growth delay, and feeding difficulties with spontaneous avoidance of protein-rich foods may precede overt neurological symptoms[2]. Conversely, in adolescents and adults, the disease may present with gait disturbances, loss of functional autonomy, or spastic paraplegia misclassified under more common neurodegenerative phenotypes.

To ensure broad applicability across clinical settings, the panel has identified for each domain a set of essential core assessments, readily implementable in most centers involved in the care of patients with inherited metabolic disorders, as well as optional advanced investigations, which may provide deeper mechanistic insights or support specialized therapeutic decision-making. In addition, the framework highlights a selection of emerging or exploratory research parameters, considered promising for future validation, including biomarkers of neuroinflammation and neurodegeneration, novel metabolic readouts, and parameters of muscle damage and the reversibility of these manifestations, which may further enhance our ability to stratify patients, monitor subclinical progression, and personalize long-term care strategies.

Crucially, because many first-line encounters with ARG1-D patients occur outside of specialized metabolic settings, this proposed framework is designed to be multidisciplinary in nature and translatable to general neurology, pediatrics, rehabilitation, and neuropsychiatry. By fostering shared awareness and diagnostic readiness across specialties, we aim to improve early detection and proactive management of this underrecognized, yet treatable, condition.

A comprehensive overview of the proposed assessments across all domains, including their categorization into essential, optional, and exploratory tools, is summarized in Table 1.

1. Biochemical and Metabolic Domain

Essential Core Assessments

Peripheral arginine levels longitudinal monitoring represents the key metabolic parameter for correlating metabolic control and clinical outcomes before and after treatment with the aim of normalizing arginine plasma levels.

Moreover, assessment of guanidino compounds could be additional longitudinal informative markers of metabolic modifications during treatment. These include guanidinoacetic acid (GAA), argininic acid (ARGA), α-keto-δ-guanidinovaleric acid (GVA), and α-N-acetylarginine (NAARG), all measurable by Ultra-High Performance Liquid Chromatography – Tandem Mass Spectrometry (UHPLC-MS/MS) and previously associated with seizure risk and white matter injury in ARG1-D[6,7].

Table 1. Summary of recommended clinical parameters in ARG1-D monitoring, by domain and level of recommendation.

Domain	Category	Recommended Parameters/Tools
Biochemical and Metabolic	Essential core assessments	Plasma arginine levels; guanidino compounds (GAA, ARGA, GVA, NAARG) measured by UHPLC-MS/MS.
	Optional advanced investigations	CSF arginine quantification; neurofilament light chain (NfL) in plasma/CSF; assessment of ureagenesis.
	Exploratory research parameters	Brain-acting hepatokines: GDF15 and FGF21, associated with mitochondrial stress, inflammation, and liver-brain signaling.
Neuromotor	Essential core assessments	GMFCS; GMFM-88; GMFM-D and GMFM-E; Modified Ashworth Scale (MAS); Gillette FAQ; 9-Hole Peg Test; Functional Mobility Scale (FMS); VABS; 2-Minute Walk Test (2MWT).
	Optional advanced investigations	Motor evoked potentials (MEPs) to assess descending corticospinal conduction.
	Exploratory research parameters	Ultrasound-based elastography; muscle MRI to assess stiffness, fibrosis, and reversibility of muscle damage.
Imaging	Essential core assessments	Brain and spinal cord MRI to assess baseline and longitudinal structural integrity, particularly of the corticospinal tracts.
	Optional advanced investigations	Proton magnetic resonance spectroscopy (^{1}H-MRS) for central arginine levels; diffusion tensor imaging (DTI); tractography; NODDI.
Liver Function	Essential core assessments	Albumin, INR, AST, ALT, GGT, total/direct bilirubin; liver ultrasound with elastography (Fibroscan®) for fibrosis assessment.
Cognitive, QoL, Behavioral	Essential core assessments	Age-appropriate cognitive tools (e.g., Wechsler scales, exectuve function tests); Pediatric Quality of Life Inventory (PedsQL); SF-36 (adults); CBCL; VABS; MetabQoL 1.0.
Musculoskeletal	Optional advanced investigations	DXA/MOC and QUS of the calcaneus; biochemical markers: osteocalcin, PTH, 25(OH)-Vitamin D, serum calcium and phosphorus, bone-specific ALP, NTX.
	Exploratory research parameters	Ultrasound-based elastography; muscle MRI for structural and compositional analysis.

Guanidinoacetic acid (GAA), argininic acid (ARGA), α-keto-δ-guanidinovaleric acid (GVA), α-N-acetylarginine (NAARG), Ultra-High Performance Liquid Chromatography – Tandem Mass Spectrometry (UHPLC-MS/MS), cerebrospinal fluid (CSF), Gross Motor Function Classification System (GMFCS), Gross Motor Function Measure – 88 (GMFM-88) Gross Motor Function Measure – Parts E and D (GMFM-E; GMFM-D), Vineland Adaptive Behavior Scales (VABS), international normalized ratio (INR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), Child Behavior Checklist (CBCL), dual-energy X-ray absorptiometry (DXA/MOC), quantitative ultrasound (QUS), parathyroid hormone (PTH), alkaline phosphatase (ALP), N-terminal telopeptide of type I collagen (NTX).

Optional Advanced Investigations

As part of a comprehensive baseline and follow-up assessment, the expert panel also proposes the inclusion of lumbar puncture for cerebrospinal fluid (CSF) arginine quantification as a complementary diagnostic and monitoring tool. Central arginine levels may offer a more accurate reflection of neuronal exposure to metabolic toxicity and help elucidate the relationship between biochemical normalization in plasma and clinical response. Recognizing the logistical and ethical considerations associated with lumbar puncture, the procedure could be strategically performed in conjunction with brain MRI, particularly under sedation in pediatric patients, thereby minimizing procedural burden while maximizing diagnostic yield. This approach would support a more integrative and mechanistic understanding of treatment effects, particularly in early-phase or borderline clinical cases.

Assessment of ureagenesis before and after initiation of pegzilarginase therapy is advisable[8] but not all metabolic centers have access to this test. Recently proposed biomarkers of neurodegeneration, such as neurofilament light chain (NfL), could be informative on axonal impairment and improvement, as confirmed in several other neurological disorders characterized by spastic paraplegia[9].

Exploratory Research Parameters

Additionally, the panel suggests the inclusion of brain-acting hepatokines GDF15 and FGF21 as exploratory biomarkers. GDF15, a stress-induced cytokine associated with mitochondrial dysfunction and inflammation, and FGF21, a key regulator of energy balance and metabolism by acting as a mediator for liver-to-brain communication, may offer insight into systemic energy expenditure, metabolic stress, and neuroinflammatory activation in ARG1-D[10-12].

2. Neuromotor Domain

Essential Core Assessments

To objectively monitor motor function before and during treatment, the use of validated clinical outcome measures is essential. The expert panel recommends regular assessment using the same instruments employed in the PEACE trial[5]:

- Gross Motor Function Classification System (GMFCS) to stratify overall motor impairment severity and assess functional ambulation categories.
- Gross Motor Function Measure – 88 (GMFM-88) for detailed evaluation of gross motor skills across multiple dimensions.
- Gross Motor Function Measure – Parts E and D (GMFM-E; GMFM-D) specifically targeting domains of walking/running/jumping and sitting/standing, respectively.
- Modified Ashworth Scale (MAS) for semi-quantitative assessment of muscle spasticity.
- Gillette Functional Assessment Questionnaire to capture mobility performance in everyday contexts from the caregiver's perspective.
- 9-Hole Peg Test (9-HPT) for upper limb dexterity and fine motor control evaluation.
- Functional Mobility Scale (FMS) to assess ambulatory ability across different environmental distances (5, 50, and 500 meters).
- Vineland Adaptive Behavior Scales (VABS) to measure adaptive behaviors in communication, daily living skills, and socialization.
- 2-Minute Walk Test (2MWT) as a standardized metric of functional endurance and walking capacity.

These scales have shown sensitivity in patients with ARG1-D and provide quantifiable endpoints aligned with therapeutic goals[5].

Optional Advanced Investigations

Functional neurophysiological studies, especially motor evoked potentials (MEPs), are proposed to assess the integrity of corticospinal tract pathways, offering a dynamic and objective measure of descending motor conduction over time.

3. Imaging Domain

Neuroimaging represents a crucial tool for characterizing structural and functional changes in the central nervous system.

Essential Core Assessment

The panel recommends performing brain and spinal cord MRI both before and after initiation of pegzilarginase therapy, with the aim of assessing structural involvement of the central nervous system, particularly the integrity of the corticospinal tracts. This imaging modality serves as a key tool to detect and monitor pre-existing or evolving cerebral and spinal injury, enabling clinicians to evaluate whether structural damage is present at baseline and to track its stability, progression, or potential reversibility under treatment.

Optional Advanced Investigations

As part of the optional advanced investigations suggested, the use of proton magnetic resonance spectroscopy (^{1}H-MRS) is proposed to non-invasively assess cerebral arginine levels before and after the initiation of pegzilarginase therapy. Spectroscopic sequences with intermediate echo times (TE ~144 ms) have demonstrated the capacity to isolate and quantify the arginine peak at approximately 3.8 ppm, providing a potential biomarker of central biochemical exposure in ARG1-D. This technique may be particularly valuable in exploring the relationship between plasma arginine normalization and persistent neurological symptoms, especially in patients with evidence of progressive spasticity or cognitive plateauing despite metabolic control. In this regard, ^{1}H-MRS offers a complementary and non-invasive approach to cerebrospinal fluid sampling and could be strategically integrated into routine brain MRI protocols, including during sessions conducted under sedation in pediatric patients. Previous studies[13,14] have confirmed the visibility of this peak in affected individuals and support the utility of spectroscopy as a surrogate marker of arginine neurotoxicity and treatment response.

Diffusion tensor imaging (DTI) and tractography are also advised in order to evaluate corticospinal tract integrity longitudinally. Additional functional sequences, such as neurite orientation dispersion and density images (NODDI), would be able to assess the microstructural complexity of dendrites and axons and the integrity of white matter.

All these modalities have the potential to detect subclinical changes and to correlate neuroanatomical recovery with clinical improvement in response to pegzilarginase.

4. Liver Function Monitoring

Given the impact of ARGD-1 on the liver, proposed liver function monitoring includes:
- Synthetic parameters: including albumin and international normalized ratio (INR)
- Cytolytic parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT)
- Cholestatic markers: gamma-glutamyl transferase (GGT), alkaline phosphatase, total/direct bilirubin
- Liver fibrosis assessment: Ultrasound scan with elastography (Fibroscan®)

5. Cognitive Function and Quality of Life and Behavioral Assessment

Monitoring patient-centered outcomes is essential to contextualize biochemical and functional data. The expert group recommends regular use of standardized cognitive behavioral and quality-of-life assessment tools, including:
- Standardized age-appropriate cognitive assessment tools (Wechsler scales)
- Age-specific test for executive functions
- Pediatric Quality of Life Inventory (PedsQL)
- SF-36 QoL assessment for adults
- Child Behavior Checklist (CBCL)
- Vineland Adaptive Behavior Scales (VABS)
- MetabQoL 1.0

These instruments allow for systematic evaluation of emotional regulation, attention, adaptive functioning, and caregiver-reported well-being, with high relevance in chronic adult and pediatric neurological conditions[15-17].

Particularly, to comprehensively capture the burden of disease in children and adolescents affected by intoxication-type inborn errors of metabolism (IT-IEM), the MetabQoL 1.0 questionnaire was developed within the framework of the European Network and Registry for Intoxication Type Metabolic Diseases (E-IMD). Unlike generic or chronic-generic instruments, MetabQoL 1.0 is the first disease-specific health-related quality of life (HrQoL) assessment tool tailored to this patient population. Moreover, it is positioned as a robust and responsive instrument for monitoring patient-reported outcomes in clinical trials and long-term care. It is particularly suited for evaluating the multidimensional impact of emerging therapies, such as pegzilarginase, in ARG1-D, and it represents a critical step toward integrating patient-centered endpoints into the routine management of rare metabolic diseases[18].

EXPERT OPINION AND DISCUSSION

The therapeutic advent of pegzilarginase introduces a transformational shift in the natural history of ARG1-D, allowing for the first time sustained normalization of plasma arginine levels and improvements in motor function in the majority of patients[5,7]. This unprecedented biochemical and functional efficacy has simultaneously revealed the need for a deeper and more nuanced understanding of the disease's pathophysiological heterogeneity, especially in relation to neurodegeneration, residual disease activity, and long-term outcomes.

The Italian expert group proposes a framework for interpreting the real-world clinical data that will be gathered following this publication, with the aim of informing precision medicine approaches and establishing shared national guidance for the diagnosis and longitudinal management of patients with ARG1-D.

FROM BIOCHEMICAL CORRECTION TO FUNCTIONAL UNDERSTANDING

The normalization of plasma arginine levels, as achieved in over 90% of patients treated with pegzilarginase in the PEACE trial[5], constitutes a major biochemical breakthrough in the treatment of ARG1-D. This therapeutic milestone has demonstrated clear clinical benefits, particularly in terms of functional stabilization or improvement. However, despite these advances, a wide range of pathophysiological mechanisms remain unexplored, limiting our ability to fully understand the determinants of response and long-term disease trajectory.

Indeed, the discrepancy often observed between biochemical normalization and residual neurological impairment in some patients suggests that plasma arginine may not reliably mirror CNS arginine burden. Given the bidirectional but transporter-modulated equilibrium between plasma and CSF, the expert panel underscores the importance of systematic CSF arginine and guanidino compound (GC) profiling, including GAA, ARGA, GVA, and NAARG, as an investigative tool to assess the extent of persistent neurotoxicity, despite adequate peripheral control[2,7].

In parallel, neurofilament light chain (NfL) in both plasma and CSF is proposed as a surrogate marker of axonal damage, enabling clinicians to distinguish biochemical response from true neuroprotective efficacy. This distinction may be particularly relevant in individuals with longstanding disease or incomplete clinical recovery, in whom structural CNS damage might already be established.

The optional advanced investigations and exploratory research parameters proposed in this paper were deliberately selected to probe the fundamental mechanisms of disease, extending well beyond routine clinical endpoints. These investigations aim to bridge current knowledge gaps and support the evolution of a real-world, mechanism-informed management model, paving the way toward an increasingly multidisciplinary and individualized care strategy.

One of the key challenges in ARG1-D is the observed phenotypic heterogeneity, even among patients carrying identical genotypes. This observation strongly suggests a role for epigenetic regulation and genetic modifiers, which may shape not only the severity of the neuromotor phenotype but also the capacity for recovery following treatment. The pathophysiological role of hypomobility-induced muscle damage, and its potential reversibility under therapy, also emerges as a critical but insufficiently understood dimension.

The panel proposes that future research should address whether functional improvements observed after pegzilarginase initiation are mediated by recovery of axonal integrity, white matter structure, or myelin plasticity. In this context, it can be hypothesized that both injury and repair may be governed by overlapping inflammatory and redox-sensitive pathways, which could be differentially activated across patients.

Furthermore, the paper highlights the muscle compartment as an area of unmet clinical need. In particular, a better understanding is required regarding the onset and evolution of muscle fibrosis and whether this process can be halted or reversed by targeted metabolic correction and rehabilitation strategies. To this end, the panel identifies three principal biomarker domains to guide future exploration: cellular lysis markers, reflecting direct tissue injury; inflammatory status, with a focus on GDF15 as a sensor of mitochondrial and metabolic stress; and myelin integrity, as indicated by neurofilament light chain levels, serving as a dynamic index of central nervous system structural damage.

Collectively, these lines of investigation aim to dissect the multifactorial nature of ARG1-D and provide real-world, mechanism-driven evidence that may inform both clinical decisions and the design of next-generation therapeutic strategies. The current paper, by framing these open questions, aspires to lay the groundwork for future translational research efforts in this evolving field.

UNSOLVED QUESTIONS IN ARG1-D: FROM MUSCLE STIFFNESS TO ARGININE-DRIVEN AUTOPHAGIC MODULATION

While the hallmark features of ARG1-D remain centered on progressive neuromotor dysfunction, cognitive impairment, and biochemical dysregulation, clinical observations increasingly point to a constellation of secondary manifestations, notably muscle damage and growth retardation, which, although not defining elements of the disease, may significantly contribute to long-term disability. These features are likely the result of chronic neurodisability, prolonged spasticity, nutritional compromise, and metabolic inefficiency, and deserve greater attention as part of an expanded disease model.

In particular, clinicians have reported segmental muscle weakness and acquired stiffness, especially in patients with longstanding mobility limitations[2]. Analogous findings[19,20] in cerebral palsy (CP) suggest that two pathophysiological mechanisms may underlie this phenomenon: an increase in extracellular matrix viscosity, which reduces elasticity and heightens resistance to stretch, and a fibrotic substitution of contractile muscle fibers, ultimately compromising functional output and the reversibility of motor deficits. These changes, although secondary, may become self-perpetuating and resistant to recovery even after correction of the underlying biochemical defect, especially if not detected and addressed early.

To better characterize these alterations, the expert panel highlights the potential of ultrasound-based elastography as a non-invasive technique to assess muscle stiffness and fibrosis *in vivo*. This modality, which measures the mechanical properties of tissues by quantifying their elastic response to an applied force or vibration, offers the possibility of detecting early structural changes before overt clinical symptoms arise. While elastography has shown promise in neuromuscular disorders and in the evaluation of spastic muscles, it is not yet widely available in most centers, and no established clinical experience currently exists for its application in ARG1-D. Nonetheless, the panel recommends this tool as a future implementation priority, particularly in research contexts, to better elucidate the pathophysiological mechanisms of muscle damage and remodeling in ARG1-D and to explore potential biomarkers of tissue reversibility under enzyme replacement therapy.

In this context, the expert panel proposes the use of muscle magnetic resonance imaging (muscle MRI) as a non-invasive tool to evaluate the morphological and structural integrity of skeletal muscle. This includes the detection of fatty infiltration, atrophy, inflammation, or fibrosis, which may aid in differentiating primary neuromuscular involvement from secondary adaptations due to prolonged disuse or spasticity. Monitoring these parameters before and after the initiation of pegzilarginase therapy could yield crucial insights into the degree of muscle reversibility and inform targeted rehabilitation strategies.

In parallel, the potential involvement of the skeletal system must not be overlooked. Patients with ARG1-D may experience subclinical disturbances of bone metabolism, arising from chronic dietary protein restriction, reduced mechanical loading, or systemic metabolic derangements. To assess bone health, the panel recommends a dual approach combining instrumental techniques, including dual-energy X-ray absorptiometry (DXA/MOC) and quantitative ultrasound (QUS) of the calcaneus, with biochemical profiling of key markers. These include osteocalcin, parathyroid hormone (PTH), 25-hydroxyvitamin

D, serum calcium and phosphorus, bone-specific alkaline phosphatase, and N-terminal telopeptide of type I collagen (NTX). Such evaluations may uncover early signs of osteopenia or mineral imbalance, which, if identified and addressed proactively, may reduce the risk of fracture or skeletal fragility, especially during critical growth periods.

Incorporating both muscle and bone monitoring into the clinical routine expands the perspective from a purely neurological focus to a more comprehensive neuromusculoskeletal model of disease. This multidimensional approach recognizes that functional outcomes in ARG1-D are the result not only of central metabolic correction but also of peripheral tissue integrity, which may be modifiable through early intervention and longitudinal surveillance.

An additional pathophysiological dimension that deserves deeper exploration in ARG1-D is the role of arginine in the modulation of autophagic processes. Autophagy represents a fundamental cellular mechanism responsible for the maintenance, repair, and turnover of damaged organelles and proteins, playing a critical role in neuronal homeostasis and network integrity[21]. In several inborn errors of metabolism, including urea cycle disorders, disturbances in nitrogen handling and amino acid flux have been shown to alter autophagy, either activating or suppressing this pathway depending on the metabolic context.

In ARG1-D, chronic elevation of arginine and guanidino compounds may have a dual effect: while contributing directly to neurotoxicity and oxidative stress, they may also interfere with cellular regulatory pathways such as nitric oxide synthesis, protein arginylation, and lysosomal turnover, all of which are tightly connected to the autophagic machinery[21,22]. Indeed, high levels of arginine have been shown to modulate autophagy in cancer cells and neurodegenerative models, through mechanisms involving epigenetic control, argininosuccinate synthase expression, and redox signaling cascades[21].

These mechanisms are not unique to ARG1-D but appear to converge across several urea cycle disorders, including hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome and P5CS deficiency, where either arginine deficiency or ammonia excess contributes to autophagic deregulation. The shared neurodegenerative phenotype observed in these disorders, particularly in spastic paraparesis, may therefore reflect a common vulnerability of long axonal tracts to impaired autophagic flux and metabolic stress[21].

Understanding how arginine modulates autophagy is not only relevant for explaining disease progression, but also potentially critical for interpreting the functional recovery observed in some patients after pegzilarginase therapy. It is plausible that the amelioration of clinical symptoms is mediated, at least in part, by the reactivation of endogenous neuronal repair mechanisms, including remyelination and axonal regeneration, both of which depend on efficient autophagic regulation.

In this context, the expert panel underscores the importance of integrating targeted biomarker profiling into clinical follow-up protocols. Specifically, the combined assessment of markers of cellular lysis [e.g., lactate dehydrogenase (LDH), creatine-kinase (CK)], neuroinflammation (e.g., GDF15), and myelin integrity (e.g., neurofilament light chain) may offer insights into the relative contributions of necrotic, inflammatory, and redox-autophagic pathways in individual patients, guiding therapeutic expectations and supporting stratified care approaches.

Ultimately, these unresolved clinical dimensions underscore the need for ongoing observational research and interdisciplinary collaboration to refine monitoring strategies, define clinically meaningful secondary endpoints, and ensure that every aspect of disease burden, both primary and secondary, is addressed in a timely and proactive manner.

SOCIAL COSTS AND BURDEN OF DISEASE IN ARG1-D

The impact of ARG1-D extends well beyond the clinical and biochemical domains, imposing a profound burden on patients, caregivers, and society at large. Recent European cross-sectional surveys[23] have provided quantitative insight into this burden, highlighting how patients with ARG1-D, particularly those with combined mobility and cognitive impairments, experience a marked loss of autonomy, reduced quality of life, and significant reliance on formal and informal caregiving resources. From a societal perspective, the estimated annual cost per patient has been reported to exceed £68,000, with wide variation depending on functional severity: patients with severe cognitive impairment or advanced motor dysfunction (GMFCS levels 3-5) incur annual costs surpassing £100,000, compared to ~£49,000 in those with mild impairment[24]. The impact on caregiver quality of life and workforce participation is equally notable, with a significant proportion experiencing moderate-to-severe caregiver burden[23].

By mitigating clinical progression and preserving function, pegzilarginase could not only improve individual health outcomes but also substantially reduce the long-term socioeconomic impact associated with ARG1-D.

This multidomain framework has the potential to enable clinicians and researchers to:

1. Identify early biological signals of treatment response or failure, allowing timely therapeutic adjustments;
2. Correlate biochemical and imaging markers with clinical trajectories, supporting predictive modeling;
3. Uncover residual disease activity despite normalized plasma arginine, prompting reconsideration of treatment goals;

Contribute to harmonized national and international registries, like the well-established E-IMD (https://www.e-imd.org), enabling multicenter data pooling and comparative analysis.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

The structured data collection proposed in this paper will allow the Italian community to generate hypothesis-driven real-world evidence, addressing key open questions in ARG1-D, like:

- What is the minimal effective reduction in arginine or GCs needed to halt CNS damage?
- When initiated early, how much does pegzilarginase positively impact patients' quality of life? And how much does it differ when compared to patients starting it later in their history?
- Can neurocognitive, neurophysiological and imaging markers guide prognosis better than static biochemical targets?

These questions are no longer theoretical. As enzyme therapy becomes accessible, there is an urgent need to match therapeutic innovation with diagnostic precision and real-world evidence. The multidimensional framework proposed here aims to ensure that clinical practice does not lag behind the therapeutic possibilities now available for this rare but increasingly manageable disorder.

CONCLUSIONS

The availability of pegzilarginase introduces a new opportunity for patients with ARG1-D. For the first time, clinicians have access to a disease-modifying therapy capable of normalizing plasma arginine, reducing neurotoxic metabolites, and translating these biochemical changes into clinically meaningful improvements in functional mobility. This shift represents not merely a therapeutic milestone, but a fundamental redefinition of what is possible, nowadays, for individuals living with ARG1-D.

However, this transformation cannot be achieved by pharmacology alone. It requires a coordinated, multidisciplinary collaboration among expert centers to redefine standards of diagnosis, monitoring, and care delivery. The consensus framework proposed in this paper serves as a foundation for a new era of proactive and precision-based management.

By fostering national alignment around real-world clinical practices and embedding advanced biomarker strategies into routine care, this initiative has the potential to impact not only the present of patients already diagnosed, but also to transform the future for families yet to receive a diagnosis. With earlier recognition, better tools for monitoring, and an effective therapy now available, we can envision a paradigm where ARG1-D is no longer a relentlessly progressive disorder, but a manageable condition with preserved function, autonomy, and hope.

This is the power of expert collaboration and scientific innovation working in synergy. As a community, we now stand at the threshold of a future where the trajectory of ARG1-D may be permanently altered for the better, benefiting generations to come.

AI Disclosure:

No AI-assisted technology was used for the production of this manuscript.

Authors' Contributions:

Serena Gasperini, Diego Martinelli, and Francesco Porta contributed equally to the conception, drafting, and critical revision of the manuscript. All authors reviewed and approved the final version for submission.

Availability of Data and Materials:

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Conflict of Interest:

The authors have served as consultants for Immedica Pharma.

Ethics Approval:

Not applicable.

Funding:

The editorial project was provided through an Immedica Pharma unconditional grant. Immedica Pharma had no role in the project design and conduct, collection, management, analysis, and interpretation of data, or the preparation and review of the manuscript.

Informed Consent:

Not applicable.

ORCID ID:

Serena Gasperini: 0000-0001-8743-4407
Diego Martinelli: 0000-0002-9324-2871
Francesco Porta: 0000-0003-3899-4867

REFERENCES

1. Bin Sawad A, Jackimiec J, Bechter M, Trucillo A, Lindsley K, Bhagat A, Uyei J, Diaz GA. Epidemiology, methods of diagnosis, and clinical management of patients with arginase 1 deficiency (ARG1-D): A systematic review. Mol Genet Metab 2022; 137: 153-163.
2. Burlina A, Ardissone A, Battini R, Burlina A, Gasperini S, Pession A, Porta F, Vici CD. Arginase 1 deficiency: a treatable form of spastic paraplegia. Neurol Sci 2025; 46: 4219-4228.
3. Sun A, Crombez EA, Wong D. Arginase Deficiency. 2004 Oct 21 [updated 2020 May 28]. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025.
4. Carvalho DR, Brum JM, Speck-Martins CE, Ventura FD, Navarro MM, Coelho KE, Portugal D, Pratesi R. Clinical features and neurologic progression of hyperargininemia. Pediatr Neurol 2012; 46: 369-374.
5. Russo RS, Gasperini S, Bubb G, Neuman L, Sloan LS, Diaz GA, Enns GM; PEACE Investigators. Efficacy and safety of pegzilarginase in arginase 1 deficiency (PEACE): a phase 3, randomized, double-blind, placebo-controlled, multi-centre trial. EClinicalMedicine 2024; 68: 102405.
6. Deignan JL, De Deyn PP, Cederbaum SD, Fuchshuber A, Roth B, Gsell W, Marescau B. Guanidino compound levels in blood, cerebrospinal fluid, and post-mortem brain material of patients with argininemia. Mol Genet Metab 2010; 100 Suppl 1: S31-S36.
7. Diaz GA, Bechter M, Cederbaum SD. The role and control of arginine levels in arginase 1 deficiency. J Inherit Metab Dis 2023; 46: 3-14.
8. Schmidt C, Hofmann U, Kohlmüller D, Mürdter T, Zanger UM, Schwab M, Hoffmann GF. Comprehensive analysis of pyrimidine metabolism in 450 children with unspecific neurological symptoms using high-pressure liquid chromatography-electrospray ionization tandem mass spectrometry. J Inherit Metab Dis 2005; 28: 1109-22.
9. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, Barro C, Kappos L, Comabella M, Fazekas F, Petzold A, Blennow K, Zetterberg H, Kuhle J. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 2018; 14: 577-589.
10. Assadi A, Zahabi A, Hart RA. GDF15, an update of the physiological and pathological roles it plays: a review. Pflugers Arch 2020; 472: 1535-1546.
11. Mulderrig L, Garaycoechea JI, Tuong ZK, Millington CL, Dingler FA, Ferdinand JR, Gaul L, Tadross JA, Arends MJ, O'Rahilly S, Crossan GP, Clatworthy MR, Patel KJ. Aldehyde-driven transcriptional stress triggers an anorexic DNA damage response. Nature 2021; 600: 158-163.
12. Ha NT, Lee CH. Roles of Farnesyl-Diphosphate Farnesyltransferase 1 in Tumour and Tumour Microenvironments. Cells 2020; 9: 2352.
13. Carvalho DR, Farage L, Martins BJ, Brum JM, Speck-Martins CE, Pratesi R. Brain MRI and magnetic resonance spectroscopy findings in patients with hyperargininemia. J Neuroimaging 2014; 24: 155-160.
14. Enokizono M, Aida N, Yagishita A, Nakata Y, Ideguchi R, Kurokawa R, Kono T, Moritani T, Mori H. Neuroimaging findings of inborn errors of metabolism: urea cycle disorders, aminoacidopathies, and organic acidopathies. Jpn J Radiol 2023; 41: 683-702.
15. Varni JW, Limbers C, Burwinkle TM. Literature review: health-related quality of life measurement in pediatric oncology: hearing the voices of the children. J Pediatr Psychol 2007; 32: 1151-1163.

16. Price JA, Morris ZA, Costello S. The Application of Adaptive Behaviour Models: A Systematic Review. Behav Sci (Basel) 2018; 8: 11.
17. Achenbach TM, Ivanova MY, Rescorla LA, Turner LV, Althoff RR. Internalizing/Externalizing Problems: Review and Recommendations for Clinical and Research Applications. J Am Acad Child Adolesc Psychiatry 2016; 55: 647-656.
18. Zeltner NA, Baumgartner MR, Bondarenko A, Ensenauer R, Karall D, Kölker S, Mühlhausen C, Scholl-Bürgi S, Thimm E, Quitmann J, Burgard P, Landolt MA, Huemer M. Development and Psychometric Evaluation of the MetabQoL 1.0: A Quality of Life Questionnaire for Paediatric Patients with Intoxication-Type Inborn Errors of Metabolism. JIMD Rep 2017; 37: 27-35.
19. Raghavan P. Muscle physiology in spasticity and muscle stiffness. Toxicon 2025; 259: 108350.
20. Boulard C, Gross R, Gautheron V, Lapole T. What causes increased passive stiffness of plantarflexor muscle-tendon unit in children with spastic cerebral palsy? Eur J Appl Physiol 2019; 119: 2151-2165.
21. Panza E, Martinelli D, Magini P, Dionisi Vici C, Seri M. Hereditary Spastic Paraplegia Is a Common Phenotypic Finding in ARG1 Deficiency, P5CS Deficiency and HHH Syndrome: Three Inborn Errors of Metabolism Caused by Alteration of an Interconnected Pathway of Glutamate and Urea Cycle Metabolism. Front Neurol 2019; 10: 131.
22. Giordani GM, Diniz F, Fussiger H, Gonzalez-Salazar C, Donis KC, Freua F, Ortega RPM, de Freitas JL, Barsottini OGP, Rosemberg S, Kok F, Pedroso JL, França MC Jr, Saute JAM. Clinical and molecular characterization of a large cohort of childhood onset hereditary spastic paraplegias. Sci Rep 2021; 11: 22248.
23. Olofsson S, Löfvendahl S, Widén J, Jacobson L, Lindgren P, Stepien KM, Arnoux JB, Luz Couce Pico M, Leão Teles E, Rudebeck M. Disease burden among patients with Arginase 1 deficiency and their caregivers: A multinational, cross-sectional survey. JIMD Rep 2024; 65: 450-460.
24. Olofsson S, Löfvendahl S, Widén J, Rudebeck M, Lindgren P, Stepien KM, Arnoux JB, Luz Couce Pico M, Leão Teles E, Jacobson L. Societal costs and quality of life associated with arginase 1 deficiency in a European setting - a multinational, cross-sectional survey. J Med Econ 2024; 27: 1146-1156.