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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

EDITORIAL

Strengthening our role in Medical Research and Education

Victor Oliveira [1]
EDITOR-IN-CHIEF

1 MD, PhD. Neurologist; Board Member of the Sociedade das Ciências Médicas de Lisboa; Principal Investigator at Faculdade de Medicina – Universidade de Lisboa

We resumed contact with our readers by establishing a quarterly publication schedule, with issues to be released in February, June and October.

As one of the oldest scientific journals in the world, it is inevitable that, over nearly two centuries (the first issue was published in January 1835), our journal has been influenced by the social context in which it exists.

We reaffirm our commitment to making *Jornal da Sociedade das Ciências Médicas de Lisboa* a key platform for disseminating current knowledge across the broad field of medical and health sciences. Our intention is not to compete with other publications, but to carve out our own path in reaching all areas of health sciences.

We will also seek contributions from prominent individuals whose careers and work are of significance to our readers.

As the *Jornal da Sociedade das Ciências Médicas de Lisboa* is the official publication of the medical schools, Faculdade de Medicina da Universidade de Lisboa and Nova Medical School – Faculdade de Ciências Médicas, we are pleased to feature scientific papers from both undergraduate and postgraduate authors, including academic theses and other works from all areas of health sciences.

These objectives are not exclusive to these institutions. Contributions from any other sources, whether national or international, are welcome. The key criterion is quality, ensured through external peer review, providing our readers with up-to-date information that adheres to the highest standards.

We believe that by offering this scientific journal in open-access format, at no cost to the authors and with easy electronic availability, we will facilitate the dissemination of health sciences and enhance the recognition of their authors.

Victor Oliveira
Editor-in-Chief (JSCMed)

> *We reaffirm our commitment to making Jornal da Sociedade das Ciências Médicas de Lisboa a key platform for disseminating current knowledge across the broad field of medical and health sciences.*

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

Transforming Healthcare Through Integration, Innovation and Training: The Medicina ULisboa-Torres Vedras Campus Concept

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ABSTRACT: The Medicina ULisboa-Torres Vedras Campus is an innovative, multidisciplinary academic healthcare model that integrates clinical care, academic training, research, and innovation. Committed to shaping the future of healthcare, the Campus focuses on developing new approaches supported by interdisciplinary care and advanced technologies to tackle both current and emerging challenges. Situated in Torres Vedras—a region characterized by an aging population and environmental health vulnerabilities—the Campus operates on key foundational pillars, each with a distinct mission, yet working synergistically to achieve a shared vision. These pillars include the Academic Family Health Unit, Public Health Unit, Interdisciplinary Unit, Clinical Research Center, Health Technology Research Center, Academic Center, and the University Center for Disaster Resilience and Emergency Management.

The Campus employs a three-pronged strategy to address healthcare needs: leveraging clinical expertise in chronic disease management to refine training methodologies and develop innovative care models; driving groundbreaking research to conceptualize and validate transformative care frameworks; embedding academic education within real-world clinical environments, providing students with practical, hands-on learning opportunities.

Through its initiatives, the Medicina ULisboa-Torres Vedras Campus—a partnership between Faculdade de Medicina da Universidade de Lisboa and Municipality of Torres Vedras—aims to establish itself as a global leader in community-centered care. By seamlessly integrating innovation, academic excellence, and community engagement, the Campus provides a scalable and replicable model for addressing complex health challenges, transforming healthcare systems, and thriving in an ever-evolving global landscape.

KEY WORDS: Interdisciplinary Care, Primary Health Care, One Health, Health Technology, Academic Center

INTRODUCTION

Healthcare systems worldwide are confronting transformative challenges, including the rising burden of chronic diseases—such as cardiovascular, musculoskeletal, and neurodegenerative disorders—a growing shortage of healthcare professionals, and the imperative to align education and practice within integrated systems (1). These complex issues necessitate innovative care models that emphasize prevention, comprehensive health, and sustainability.

While countries like the United Kingdom, Denmark, and Germany excel in specific aspects of healthcare (2), they often lack integrated frameworks that seamlessly combine academic innovation with holistic, patient-centered care. Torres Vedras, in alignment with the 2030 Agenda for Sustainable Development (3–5), exemplifies a regional commitment to addressing these challenges. Initiatives such as the "Health and Quality of Life Development Plan" (6) focus on reducing health inequities, enhancing preventive care, and addressing the social determinants of health. The region's aging population underscores the urgent need for solutions that tackle chronic diseases and mitigate their societal and economic impacts (6). The Medicina ULisboa-Torres Vedras Campus—a partnership between the Faculdade de Medicina da Universidade de Lisboa (FMUL) and the Municipality of Torres Vedras—responds to these challenges by integrating high-quality healthcare, research, and education. At its core, the Campus addresses three critical priorities through a targeted strategy: managing the rising burden of chronic diseases by leveraging real-world insights to inform innovative care models; fostering integrated, patient-centered solutions by advancing research in interdisciplinary care frameworks; and preparing a skilled healthcare workforce by merging education, research, and practical training within real-world clinical environments. A cornerstone of this approach is the One Health framework, which recognizes the interdependence of human, animal, and environmental health. In parallel, the University Center for Disaster Resilience and Emergency Management will focus on equipping healthcare professionals with advanced skills to respond to emergencies and disasters through comprehensive training in risk assessment, crisis management, and coordinated interventions. Together, these entities create synergies that enable the Campus to address emerging health threats (7,8) while driving proactive healthcare innovation.

Furthermore, the Campus will actively foster synergies with the existing Units and Institutes of FMUL in Lisbon to enhance interdisciplinarity in research, education, and population care. This integrated approach will ensure a cohesive institutional strategy and drive impactful scientific and social innovations across both campuses.

By addressing both local and global health challenges, the Medicina ULisboa-Torres Vedras Campus establishes a new benchmark for integrated, equitable, and sustainable healthcare systems. Its visionary approach offers a replicable model for regions worldwide, demonstrating how academia, healthcare, and community engagement can transform the future of global health.

Historical Summary

Since its establishment in the 16th century, the history of the former "Convento do Barro", later known as "Hospital Dr. José Maria Antunes Júnior", has been closely tied to the adoption of cutting-edge approaches to patient care and the integration of multidisciplinary methods into the rehabilitation process—a revolutionary concept at the time. In 1956, the site was transformed into one of the last sanatoriums constructed in Portugal. The facility was designed to deliver advanced care for tuberculosis patients and introduced innovative multidisciplinary approaches to treatment. However, within just a few years, a shift in public health strategy prioritized outpatient care for tuberculosis, leading to the decline of sanatoriums as the preferred treatment model.

More recently, after being incorporated into the Centro Hospitalar do Oeste as part of hospital service restructuring, a decision was made to permanently close the facility, which was finalized in May 2015. Recognizing the historical and strategic importance of this site to the Torres Vedras region, the Municipality took decisive steps to preserve its heritage. This culminated in the signing of an agreement in April 2021, between the Portuguese State and the Municipality of Torres Vedras, granting the municipality management authority over the property for 50 years (9).

This transfer was based on a memorandum of understanding, signed in 2019, between the Municipality of Torres Vedras and the Faculty of Medicine of the University of Lisbon (FMUL). The aim was to transform the former Dr. José Maria Antunes Júnior Hospital into a state-of-the-art healthcare and academic center, dedicated to providing high-quality care, training health

professionals, and advancing teaching and research in Medicine and other Biomedical Sciences. FMUL's commitment to this project is rooted in its mission to transcend traditional boundaries, bringing healthcare, teaching and research into the community. The center will not only serve as a hub for education and research but also as a bridge between the historical heritage of "Convento do Barro" and the future of integrated healthcare, honoring its past while embracing a modern, inclusive, and interdisciplinary approach.

CHALLENGES

Global healthcare systems, while effective in certain areas, continue to face significant challenges in managing chronic diseases, addressing workforce shortages, integrating advanced technologies, and providing community-centered care. By integrating care, education, research, and technology, the Campus creates a transformative ecosystem aimed at overcoming these critical healthcare challenges (10,11).

THREE KEY CHALLENGES

Emerging Health Problems – A critical challenge facing healthcare systems is the rising prevalence of chronic diseases, especially among aging populations. For instance, dementia is projected to affect 78 million people globally by 2030 and 139 million by 2050 (12). Environmental factors, such as exposure to pesticides in regions like Torres Vedras, further exacerbate these risks, underscoring the urgent need for preventive strategies and innovative care models that address both societal and individual impacts (6,13). Additionally, in an era marked by the increasing frequency and intensity of natural and anthropogenic disasters, and aligned with the principles of interdisciplinary education and One Health, the Medicina ULisboa-Torres Vedras Campus will host the University Center for Disaster Resilience and Emergency Management —a multidisciplinary hub dedicated to training professionals to respond effectively to crises, that will equip healthcare and emergency professionals with advanced skills in prevention, intervention, and recovery across diverse scenarios.

Redefining Models of Care – Fragmented care models often fall short in addressing the complex interplay between clinical, social, and environmental determinants of health. The Medicina ULisboa-Torres Vedras Campus champions integrated care by combining advanced technologies, community-based interventions, and interdisciplinary collaboration. Key functional units, such as the Academic Family Health Unit and the Interdisciplinary Unit, serve as platforms for developing, refining, and testing innovative care strategies (5,10,11).

Training the Healthcare Workforce – Addressing global workforce shortages requires transformative education strategies. The Medicina ULisboa-Torres Vedras Campus places a strong emphasis on training healthcare professionals, including allied health professionals such as psychologists, nutritionists, physiotherapists, and speech therapists. By embedding training within real-world clinical settings, the Campus equips these professionals with the skills necessary to navigate modern healthcare challenges, while fostering interdisciplinary expertise and driving innovation.

ADDRESSING THE CHALLENGES

Vision for the Future

The Medicina ULisboa-Torres Vedras Campus exemplifies the practical implementation of One Health principles, addressing regional health challenges while providing a replicable model for global healthcare innovation. By fostering interdisciplinary collaboration, the Campus promotes resilient and sustainable healthcare systems capable of tackling complex and evolving health needs.

Core Pillars of the Concept

1. **Integrated Philosophy of Care**: The Campus bridges the traditional gap between primary and secondary care. Units such as the Academic Family Health Unit and the Interdisciplinary Unit provide personalized, continuous management of complex conditions, such as dementia and cardiovascular disorders, specifically tailored to the needs of the "Oeste" region.
2. **Leadership in Chronic Disease Management**: Inspired by landmark studies like the Framingham Heart Study (14), the Campus employs advanced tools such as predictive analytics and community-based interventions to develop targeted strategies for managing chronic diseases. This approach extends beyond isolated care initiatives to address the broader social and environmental determinants of health.
3. **Workforce Development and Innovation**: To address global healthcare workforce shortages, the Campus integrates training with practice, equipping professionals with cutting-edge methodologies, including

AI-assisted diagnostics and patient-centered care models. This comprehensive educational framework not only builds local capacity but also fosters innovation.

4. **Technology-Driven Care**: Leveraging advanced technologies, the Campus enhances clinical decision-making, optimizes workflows, and enables seamless communication among care teams, ensuring more efficient, precise, and personalized healthcare delivery

5. **Community-Centric Health**: Rooted in the socioeconomic and environmental context of Torres Vedras, the Campus aligns its services with local needs. This approach addresses broader health determinants while promoting equity and sustainability, exemplifying the principles of the One Health framework.

OPERATIONALIZING ONE HEALTH AT THE CAMPUS

Bridging Disciplines: A Holistic Framework

Functional units such as the Academic Family Health Unit, Interdisciplinary Unit, Public Health Unit, and Clinical Research Unit collaborate closely to implement the One Health approach. By fostering interdisciplinary teamwork, the Campus ensures that complex health challenges are addressed from multiple perspectives, creating innovative solutions that benefit both communities and ecosystems.

Addressing Future Challenges Through One Health

1. **Environmental and Climate Impacts on Health:** Environmental factors, including pesticide exposure and climate-related health risks, are closely monitored and analyzed within the Campus's integrated data framework. Research teams work to develop adaptive strategies to mitigate these risks, ensuring both human health and ecosystem resilience.

2. **Innovation, Research, and Education in One Health**: The Health Technology Research Center plays a pivotal role in advancing One Health initiatives by developing cutting-edge tools for data collection, analysis, and cross-sector integration. Digital platforms enable seamless collaboration between researchers, clinicians, and policymakers, facilitating evidence-based decision-making. Educational programs at the Campus integrate One Health principles into their curricula, offering students hands-on opportunities to engage with interdisciplinary projects. Through these real-world challenges, students and trainees gain the skills necessary to navigate the complexities of global health systems.

ORGANIZATIONAL MODEL: HEALTH/ RESEARCH UNITS

The Medicina ULisboa-Torres Vedras Campus addresses unmet needs in healthcare by combining innovative academic, clinical, and research approaches. The Campus is designed to respond to contemporary challenges, including the growing burden of chronic diseases, the necessity of integrating public health with healthcare delivery, and the need for preparedness in the face of emergencies and disasters. These challenges emphasize the importance of fostering interdisciplinary collaboration, innovative care models, and the integration of cutting-edge research into clinical practice.

Central to this vision is the alignment with the national strategy for healthcare, with a strong emphasis on primary healthcare innovation, public health initiatives, and emergency preparedness. This includes:

– **Primary Healthcare**: Implemented with an innovative academic concept, under the coordination of the Faculty of Medicine of the University of Lisbon, and staffed by health professionals dedicated to integrating clinical activity with research and teaching. It will also serve as a pilot unit for the implementation and evaluation of new models for primary healthcare provision and organization.

– **Interdisciplinary Care:** Focused on individuals with chronic illnesses that significantly impact and burden the population's health, providing comprehensive care across disciplines.

– **Health Professional Training**: Offering training for medical students, doctors, and other health professionals in a real clinical practice environment, ensuring practical, hands-on experience.

– **Clinical Research**: Involving individuals enrolled at the Campus in a prospective cohort study, which will monitor their health over time.

– **Health Technology Research:** Incorporating research groups in areas such as Digital Health, computer science, machine learning, and the design and evaluation of medical devices.

– **Public Health**: A clinical component that integrates health care delivery to the population with research in the area of One Health.

– **Center for Disaster Resilience and Emergency Management:** Integrated into a national framework for training and activating responses in disaster situations.

The organizational model emphasizes core functional units, collectively known as Health/Research Units, each contributing to the overarching vision of transforming healthcare. With an innovative and interdisciplinary approach, the Medicina ULisboa-Torres Vedras Campus integrates a Healthcare hub, an Academic and Research hub, and a hub for Humanitarian Medicine, Emergency and Disaster Management into a single location. These interconnected units include the Academic Family Health Unit, Public Health Unit, Interdisciplinary Unit, Clinical Research Center, Health Technology Research Center, Academic Center, and University Center for Disaster Resilience and Emergency Management (Figure 1).

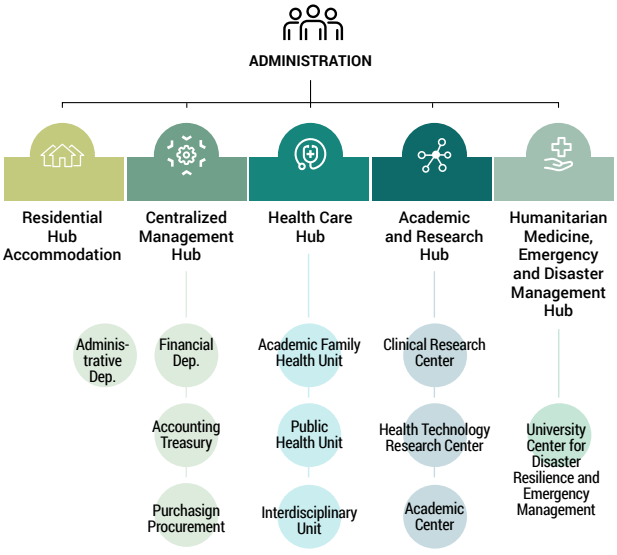

FIGURE 1. Organizational chart of Medicina ULisboa | Torres Vedras Campus

HEALTH/RESEARCH UNITS

We will start with an overview of the Academic Family Health Unit, followed by a comprehensive exploration of the additional health and research units that form the foundation of the Medicina ULisboa-Torres Vedras Campus (Figure 2).

ACADEMIC FAMILY HEALTH UNIT (AFHU): REDEFINING PRIMARY HEALTHCARE

The **Alma-Ata Declaration** of 1978, adopted by the World Health Organization (WHO), established primary healthcare as the cornerstone of effective health systems worldwide (15). In Portugal, **Family Health Units (USFs)** were introduced in 2006 as a transformative model for personalized healthcare, supported by multidisciplinary teams that emphasize autonomy, organizational maturity, and collaborative practices (16).
Building on these successes, the **Medicina ULisboa-Torres Vedras Campus** is pioneering the development of an **Academic Family Health Unit (AFHU)**. This initiative aims to integrate clinical care, education, and research, serving as a national and international model for primary healthcare. Coordinated by the Faculty of Medicine of the University of Lisbon (FMUL), the AFHU bridges gaps in family healthcare while fostering interdisciplinary collaboration and knowledge transfer.

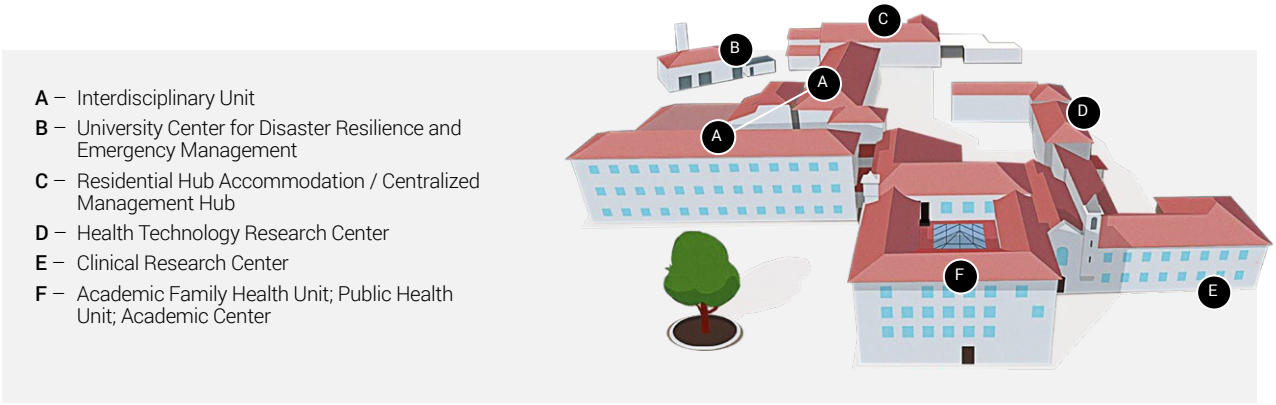

FIGURE 2. The Medicina ULisboa | Torres Vedras Campus facilities [Campus Location: Barro, Torres Vedras; GPS: 39.06456, -9.26102]

KEY FEATURES OF THE AFHU

Academic Excellence:

- The AFHU hosts students, interns, and researchers, making it a hub for training healthcare professionals and integrating research into daily clinical practice.
- Strong partnerships with FMUL enhance its capacity for scientific advancement, ensuring that the unit remains at the forefront of healthcare innovation.

Integration with the Campus Ecosystem:

- Aligned with the Medicina ULisboa-Torres Vedras Campus's vision of interdisciplinary collaboration, the AFHU works closely with units such as the Interdisciplinary Care Unit and the Health Technology Research Center.
- This synergy drives healthcare innovation, ensuring high-quality care while advancing groundbreaking research and education.

Addressing Healthcare Gaps:

- The AFHU addresses the local shortage of family doctors in Torres Vedras, delivering timely, comprehensive care to underserved populations.
- By applying risk stratification models, care can be tailored to the complexity and specific needs of different patient groups, ensuring efficient and equitable resource allocation.

Research and Training Initiatives:

Clinical Research:

- The AFHU will conduct clinical trials in primary healthcare, supported by the on-campus Clinical Research Center.
- These activities generate financial resources to support further clinical and research projects while providing compensation for human resources.

Health Technology Integration:

- Collaboration with the Health Technology Research Center leverages expertise in computer science, electronics, robotics, and other fields to attract external investment.

Prospective Cohort Study:

- All individuals receiving care on the Campus will be invited to join a prospective cohort study.
- A digital platform supporting this initiative is under development to optimize its implementation.

This integrated approach ensures the AFHU remains a cornerstone for advancing primary healthcare while addressing critical local and national challenges.

PUBLIC HEALTH UNIT

A **Public Health Unit** will be integrated into the Campus, enhancing its capacity for research and healthcare delivery in areas such as epidemiology and the One Health approach. This integration addresses the interconnectedness of human, animal, and environmental health, aligning seamlessly with the Campus's vision for sustainable and interdisciplinary healthcare solutions.

ACADEMIC CENTER

The **Academic Center (AC)**, coordinated by the Faculty of Medicine of the University of Lisbon, will oversee all pre- and post-graduate training activities. Focused on postgraduate medical training and the education of health professionals in primary healthcare and interdisciplinary chronic disease care, the Center will gradually expand to include medical student training in primary care. These educational activities will complement clinical and research efforts, ensuring a cohesive approach to healthcare education.

INTERDISCIPLINARY UNIT

The **Interdisciplinary Unit** focuses on managing chronic diseases with significant societal impacts. Its multidisciplinary team includes physicians, nurses, physiotherapists, speech therapists, occupational therapists, psychologists, nutritionists, and social workers. Collaborating with other units like the **Academic Family Health Unit**, the **Academic Center**, the **Clinical Research Center**, and the **Health Technology Research Center**, this unit ensures a holistic approach to care. Key intervention areas include cardiology and vascular risk factors, neurosciences, and musculoskeletal pathologies, aiming to enhance population health and well-being. The Campus will incorporate comprehensive rehabilitation strategies, particularly in cardiovascular care, as part of its core offerings. This focus on

rehabilitation will be essential in the management of chronic diseases and will address the national shortage of Prevention and Rehabilitation Centers, positioning Torres Vedras as a key reference in this area

CLINICAL RESEARCH CENTER

As a cornerstone of the Medicina ULisboa-Torres Vedras Campus, the **Clinical Research Center** seamlessly integrates research, education, and clinical practice to drive healthcare innovation. It conducts clinical studies and trials in close collaboration with other Campus units, including the **Academic Family Health Unit**, **Public Health Unit**, and **Interdisciplinary Unit**. By focusing on underrepresented areas such as primary healthcare, the Center generates critical data to improve health outcomes and bridges the gap between research and clinical care.

At the heart of the Clinical Research Center's mission is the development of a **prospective cohort study**, a unique initiative that integrates human, animal, and environmental health data through the **One Health** approach. This pioneering project aims to uncover the complex relationships between chronic diseases, environmental factors, and public health, supported by a dedicated digital platform for seamless data collection and analysis. Leveraging its collaboration with the **Health Technology Research Center**, the Clinical Research Center harnesses expertise in artificial intelligence, data science, and medical device development, fostering innovation and attracting external investment. By addressing pressing health challenges and advancing interdisciplinary research, the Center sets a new standard for clinical research and embodies the Campus's vision of transformative healthcare solutions.

HEALTH TECHNOLOGY RESEARCH CENTER

Health Technology Research Center, supported by a close multidisciplinary collaboration with the LASIGE Computer Science and Research Centre situated at ULisboa's Science School, bridges clinical needs with technological innovation, creating a unique space for the development and evaluation of cutting-edge solutions in fields such as biomedical engineering, computer science, artificial intelligence, and medical devices. Situated on a campus with active clinical and research activities, it provides a collaborative environment where research groups can address real clinical challenges. Partnerships with other academic institutions will strengthen the Center's ability to attract investment and accelerate technological advancements.

UNIVERSITY CENTER FOR DISASTER RESILIENCE AND EMERGENCY MANAGEMENT

The **University Center for Disaster Resilience and Emergency Management**, established through a collaboration between the Faculty of Medicine of the University of Lisbon and Harvard Medical School, is dedicated to training healthcare professionals to respond effectively to emergencies and disasters. Its mission is to address the increasing frequency and intensity of risk scenarios and natural disasters, ensuring a more efficient and coordinated response. It will offer also direct training in disaster scenarios to the community, as it is expected that in such extreme events most of the add needed in the first few hours will be dependent on citizens own initiative, as professional help may suffer unprecedent delays due to overwhelming demand. It will have autonomy in terms of electricity, sanitation, communications and internet, so that it can function as a national backup center for crisis and emergency management.

DISCUSSION

The **Medicina ULisboa-Torres Vedras Campus** represents a proactive response to the pressing global challenges in healthcare, addressing the interplay between emerging health problems, the evolution of care models, and the urgent need for skilled healthcare professionals. By integrating research, education, and clinical practice within a cohesive ecosystem, this innovative campus provides a framework for tackling these challenges while redefining healthcare delivery both locally and globally.

Addressing Emerging Health Problems

Chronic and neurodegenerative diseases, exacerbated

by aging populations and environmental factors, pose significant threats to global health. The Campus adopts a **One Health** approach, to address these challenges holistically. A central component of this vision is the inclusion of a **prospective cohort study**. By collecting and analyzing data from multiple health domains, the study creates a unique research platform to uncover the relationships between environmental exposures, lifestyle factors, and disease progression. This evidence-based approach facilitates the development of tailored prevention and treatment strategies, particularly for conditions like dementia and cardiovascular disease, which significantly impact the Torres Vedras region.

Redefining Models of Care

Current healthcare systems often operate in silos, leading to fragmented care that fails to meet the complexities of modern health challenges. The **Medicina ULisboa-Torres Vedras Campus** overcomes this limitation by fostering interdisciplinary collaboration across its Health/Research Units, including the **Academic Family Health Unit (AFHU)**, **Interdisciplinary Unit**, and **Clinical Research Unit**. These units serve as experimental hubs for testing and refining integrated care models that emphasize prediction, prevention, and patient-centered care. The use of advanced technologies such as **artificial intelligence, predictive analytics**, and **telemedicine** enhances precision in diagnostics and care delivery. This seamless integration of technology ensures efficient coordination across disciplines, enabling scalable solutions for managing chronic diseases and other health challenges. Furthermore, the Campus's focus on **community-based interventions** aligns healthcare delivery with local socioeconomic and environmental needs, setting a replicable standard for sustainable and equitable care. Through the integration of advanced prevention and rehabilitation programs, the Torres Vedras Campus will strengthen its ability to tackle challenges associated with chronic diseases, establishing itself as a strategic and innovative leader within the national healthcare system.

Training Healthcare Professionals for the Future

One of the most significant barriers to effective healthcare is the global shortage of skilled healthcare professionals. The Campus addresses this challenge through its comprehensive approach to training, integrating education, research, and clinical practice. By embedding training programs within real-world settings, the Campus ensures that students and professionals are equipped with the skills needed to navigate the complexities of modern healthcare. Innovative teaching methodologies, such as **simulation-based learning** and **interdisciplinary team training**, prepare future healthcare providers to work collaboratively and adapt to evolving health challenges. As an integral part of FMUL, the Campus embodies its commitment to academic excellence, ensuring that graduates are equipped to address emerging health needs with innovation and expertise.

Advancing Research and Innovation

Research is a cornerstone of the Campus's mission, with a focus on addressing gaps in primary healthcare and chronic disease management. The **Clinical Research Unit** plays a pivotal role in conducting clinical trials and generating data to inform evidence-based practices. By fostering collaborations between clinical researchers and technology experts at the **Health Technology Research Center**, the Campus accelerates the development of innovative solutions, such as **AI-driven diagnostics** and new **medical devices**. The **prospective cohort study** based on **One Health** data exemplifies the Campus's commitment to innovative research that transcends traditional disciplinary boundaries. This approach not only enhances scientific understanding but also positions the Campus as a leader in global healthcare innovation.

A Vision for the Future

The **Medicina ULisboa-Torres Vedras Campus** serves as a forward-thinking model for the future of healthcare, combining prediction, prevention, and community engagement to address complex health challenges. By integrating education, research, and care delivery, the Campus creates a transformative ecosystem that not only meets immediate health needs but also anticipates and mitigates future challenges. This approach ensures that the Campus remains at the forefront of healthcare innovation, establishing a global standard for sustainable, equitable, and innovative healthcare systems.

CONCLUSION

The **Medicina ULisboa-Torres Vedras Campus** drives healthcare innovation by integrating clinical care, education, and technology, establishing a benchmark for

community-centered and sustainable healthcare. Built on three key pillars:

1. **Addressing Emerging Health Challenges** with a **One Health** approach, the Campus recognizes the interconnectedness of human, animal, and environmental health, positioning itself at the forefront of tackling global health issues. Complementing this vision, the University Center for Disaster Resilience and Emergency Management combines theoretical knowledge with practical, scenario-based simulations to prepare professionals for effective crisis response. Focused on prevention, intervention, and recovery, it aims to strengthen national and regional resilience in managing complex and unpredictable events, ensuring a coordinated and efficient approach to disaster scenarios.

2. **Redefining Care Models** for integration and sustainability, the Campus fosters interdisciplinary collaboration and leverages advanced technologies to create innovative, scalable solutions that enhance the efficiency and effectiveness of healthcare delivery.

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The Campus's **prospective cohort study** highlights its commitment to interdisciplinary research, connecting human, animal, and environmental health data. By aligning healthcare delivery with both local and global priorities, the **Medicina ULisboa-Torres Vedras Campus** not only transforms care in the "Oeste" region but also serves as a scalable model for tackling complex health challenges. The focus on **prediction**, **prevention**, and **sustainability** ensures that the Campus contributes to a more **equitable** future in healthcare.

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Frailty and nutritional status in institutionalized elderly patients with neurodegenerative disorders

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ABSTRACT: **Introduction:** Frailty is a geriatric syndrome defined as a state of increased vulnerability to negative health outcomes that is considered the most powerful predictor of disability, dependence, institutionalization and death, and so considered a major health burden. Malnutrition has been described to be independently associated with frailty. **Objectives:** Primary objective was to describe the frequency of frailty in institutionalized patients with neurodegenerative disorders. Secondary objectives were to describe the frequency of undernutrition and to evaluate the correlation between frailty and nutritional status. **Methods:** A cross-sectional observational pilot study was performed. All patients aged 65 years and older with at least one neurodegenerative disorder admitted in CNS- Campus Neurológico were included. A nutritional assessment, through the Mini Nutritional Assessment (MNA), anthropometric measurements and the Edinburgh Feeding Evaluation in Dementia Questionnaire (EdFED-Q), and a frailty assessment, through the Marigliano-Cacciafesta Polypathological Scale (MCPS) and the Clinical Frailty Scale (CFS), were conducted. **Results:** 76 participants were included with a mean age of 76±6.8 years. Parkinsonian syndromes represented 82.9% of the sample. The frequency of frailty was 71.1%. Patients with atypical parkinsonism were significantly frailer than patients with Parkinson's disease (PD) (85.7 and 60%, respectively). 69.3% of the patients with dementia were frail. The frequency of undernutrition (and risk of) was 73.7%. Although not statistically significant, undernutrition was more frequent in dementia syndromes, followed by atypical parkinsonism and PD (30.8, 21.2 and 10%, respectively). Significant correlations were found between all the nutritional assessment parameters and the MCPS, being the strongest with the MNA and the EdFED-Q. **Conclusions:** The prevalence of frailty in institutionalized patients with neurodegenerative disorders is high, along with the prevalence of undernutrition. Frailty and nutritional status parameters share significant correlations.

KEY WORDS: Frailty; Elderly; Nutritional status; Parkinsonism; Dementia

INTRODUCTION

Frailty is a common geriatric syndrome that results mostly from the cumulative decline of multiple physiological systems and their reserves that are associated with the ageing process[1-6]. This decline manifests as a state of increased vulnerability to negative outcomes when facing a stress, this increased vulnerability is due to the decreased ability to regain homeostasis and functional abilities[1,5-10].

Common symptoms of frailty are extreme fatigue, unintended weight loss, frequent infections, slow gait, muscle weakness, and low energy expenditure[1,7,11]. Balance and gait impairments, fluctuating confusion, delirium, and impaired awareness are considered major features of frailty and may occur as outcomes of frailty after a stress event[1,12-14].

The prevalence of frailty increases with age: it is estimated that 7% of adults aged 65 years are frail worldwide, while in those over 80 years it increases to 20%[1,4,11,15]. In healthcare institutions this prevalence is expected to be higher, and despite the heterogeneous results from the few studies that have been conducted, it is estimated that frailty affects nearly half the residents of healthcare institutions[16-18].

The physiopathology of frailty is a complex multifactorial process[1,11]. Although universal consensus regarding the operational criteria for assessing frailty is lacking, the physical model, known as the Frailty Phenotype [FP], is the most used and cited instrument to assess frailty, mainly in community-dwelling settings[19-22]. To assess all diagnostic criteria from the FP, the person must be able to comply, both physically and mentally, to perform the required tasks, consequently the validation study excluded patients with Parkinson's disease [PD], stroke, a history of depression, and cognitive impairment [CI][7,23].

Most of the validation studies for other instruments that assess frailty excluded dementia or CI and/or PD, making it difficult to assess frailty with validated instruments in this population[24].

Amici and colleagues designed an 11-item scale, the Marigliano-Cacciafesta Polypathological Scale [MCPS], that assesses the presence and severity of frailty by identifying and classifying the possible severity of disorders related to 11 physiological systems (such as neurological disorders, respiratory, renal, metabolism and nutritional status, and cognitive state and mood)[2].

Frailty and neurodegenerative diseases, such as PD and dementia syndromes, share common symptoms like balance and gait impairments, delirium, fluctuating confusion, impaired awareness, and disability that fluctuates over time[7,11,25,26]. For these reasons, it seems reasonable to hypothesize that the prevalence of frailty in this population is high[7,11,25,26].

The prevalence of malnutrition in the elderly is heterogeneous and rises as the level of care increases[27-30]. Nutritional status and frailty share a close relationship, being estimated that 90% of community-dwelling elders at risk of malnutrition are either prefrail or frail[31]. Malnutrition seems independently associated with frailty[32].

The primary objective is to describe the frequency of frailty in institutionalized patients with neurodegenerative disorders in the moment of admission in a healthcare institution. The secondary objectives are: *a)* To describe the frequency of undernutrition and risk of undernutrition; *b)* To evaluate the correlation between frailty and nutritional status; *c)* To evaluate the correlation between the MCPS and CFS.

MATERIALS AND METHODS

Study design and population

A cross-sectional observational pilot study was performed. All patients 65 years and older who were consecutively admitted to CNS - Campus Neurológico with at least one of the following neurodegenerative disorders were included:

a) Dementia syndromes, such as Alzheimer's disease (AD), frontotemporal dementia (FTD), vascular dementia (VD), or other non-specified dementia syndromes;
b) Parkinsonian syndromes, such as PD, Lewy body dementia (LBD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), multiple system atrophy (MSA), or vascular parkinsonism (VP);
c) Motor neuron disease (MND).

This study was approved by both the Scientific Board of the Faculty of Medicine of the University of Lisbon (FMUL) and by the Ethics Committee of CNS-Campus Neurológico (CNS) in 21st November 2017 and 1st February 2018, respectively.

Written informed consent to participate in the study was provided by all patients who met the inclusion criteria. This consent was obtained from a legal representative if the patient had dementia.

Data collection and Assessment protocol

Data collection was performed for 4 months within the first 24-48 hours after the patient was admitted. Sociodemographic information was collected, as was the main neurodegenerative disorder that had been diagnosed and its severity rated according to:

a) The Hoehn & Yahr scale (H&Y) for parkinsonian syndromes[33]
b) The Clinical Dementia Rating (CDR) for dementia syndromes[34]

Frailty was assessed with the MCPS and the Clinical Frailty Scale (CFS). Global frequency of frailty according to the MCPS was considered as the sum of medium-severe, severe and very severe states of the assessment tool, while for CFS a score of 4 or more.

Nutritional status was assessed with the Mini Nutritional Assessment (MNA), Body Mass Index (BMI) and the Edinburgh Feeding Evaluation in Dementia Questionnaire (EdFED-Q).

Statistical analysis

The distribution of the data was analysed by skewness and kurtosis, and normal distribution was considered when the variable followed a symmetric and mesocuric presentation.

All data was analysed using descriptive statistics: categorical variables through relative frequencies, and continuous variables through mean and standard deviation.

The Spearman's correlation test was used to assess the following correlations:

a) The correlation between the MCPS and the CFS
b) The correlation between the CFS and the BMI, MNA, and the EdFED-Q

The Pearson's correlation test was used to assess the following correlations:

a) The correlation between the MCPS and the H&Y (severity of parkinsonian syndromes)
b) The correlation between the MCPS and the CDR (severity of dementia syndromes)
c) The correlation between the MCPS and the BMI, MNA and the EdFED-Q
d) The correlation between the H&Y and the MNA and the EdFED-Q

To assess differences between the mean values of independent groups, the Mann- Whitney test was used for the following variables: age, MCPS score, MNA score, BMI value, and the EdFED-Q. The difference between median values of categorical variables such as the H&Y and the CFS with the Chi square test.

Statistical significance was considered ≤0.05 for all tests.

RESULTS

A total of 76 participants (69.7% males) were included in this study at the moment of admission, from which 82.9% were Parkinsonian syndromes. No participant with MND was included. Due to the low number of participants with PSP, LBD, MSA, CBD, VP, and non-specified parkinsonian syndromes, all these diseases were grouped in a single group: "Atypical parkinsonism"[35].

Frailty

According to the MCPS and the CFS most of the participants were medium-severely frail (46.1%) and severely frail (44.7%), respectively (table 1).

Table 2 displays data regarding the sociodemographic information, clinical data, and descriptive analysis of frailty and nutritional status.

TABLE 1. Frailty frequency according to the Marigliano-Cacciafesta Polypathological Scale and to the Clinical Frailty Scale.

Marigliano-Cacciafesta Polypathological Scale (n=76)		Clinical Frailty Scale (n=76)	
Classification	**Frequency (%)**	**Classification**	**Frequency (%)**
Slight	6.6 (n=5)	Managing well	3.9 (n=3)
Medium	22.4 (n=17)	Vulnerable	9.2 (n=7)
Medium-severe	46.1 (n=35)	Mildly frail	10.5 (n=8)
Severe	15.8 (n=12)	Moderately frail	27.6 (n=21)
Very severe	9.2 (n=7)	Severely frail	44.7 (n=34)
		Very severely frail	3.9 (n=3)

TABLE 2. Sociodemographic and clinical data of the participants in the admission moment.

	All participants (n=76)	Parkinsonian syndromes (n=63)									Dementia syndromes (n=13)			p [b]
		Parkinson's disease (n=30)	Atypical parkinsonism (n=33)	p [a]	Atypical parkinsonism diagnoses						Alzheimer's disease (n=5)	FTD (n=4)	Non-specified dementia syndrome (n=4)	
					LBD (n=12)	PSP (n=5)	MSA (n=4)	Corticobasal degeneration (n=3)	Vascular parkinsonism (n=2)	Non-specified parkinsonian syndrome (n=7)				
Age (years)	76±6.8	75.1±5.5	75.8±7.4	0.68[1]	78.4±8.4	76.6±7.8	72±6.6	74.7±4.0	72.0±1.4	74.6±7.7	80.0±6.6	82.5±10.7	73.0±2.9	0.18[1]
Gender (female/male)	23/53	7/23	–	–	2/10	1/4	0/4	½	1/1	4/3	4/1	2/2	1/3	–
Severity of the disease														
Hoehn & Yahr	4 (4)	3 (4)	5 (4)	0.05 [2*]	4 (4)	5 (2)	4.5 (2)	5 (0)	4.5 (1)	5 (3)	–	–	–	–
Clinical dementia rating	2 (2.5)	–	–	–	–	–	–	–	–	–	2 (1)	2.5 (1)	2 (2.5)	–
Frailty														
MCPS	38.3±21.0	31.8±18.1	45.2±22.5	0.01 [1*]	43.6±19.9	37.6±17.5	4 9.5±21.1	76.0±26.9	42.0±29.7	38.1±23.4	26.2±14.8	48.3±24.2	35.0±17.5	0.76[1]
Clinical Frailty Scale	3 (5)	3 (5)	2 (5)	0.31[2]	2 (4)	2 (2)	2 (1)	2 (1)	3 (0)	2 (4)	3 (3)	2.5 (1)	2.5 (2)	0.89[2]
Nutritional status														
MNA	20.3±5.0	21.3±4.7	19.8±5	0.18 [1]	19.7±4.1	21.4±3.9	17.8±6.6	14.3±6.3	25.0±0.7	20.9±5.3	18.6±6.5	19.9±6.9	19.5±3.2	0.46[1]
Body mass index	26.1±5.3	26.3±5.1	26.3±5.8	0.69 [1]	26.9±7.3	26.2±2.6	25.9±4.9	21.2±5.0	31.3±10.3	26.4±4.2	22.1±2.2	28.5±6.8	25.3±2.9	0.51[1]
EdFED-Q	3.7±3.7	2.6±3.4	4.5±4	0.01 [1*]	4.1±3.1	2.8±3.1	4.8±4.1	8.7±6.4	1.5±0.7	5.4±5.0	4.2±1.9	6.0±4.2	2.5±1.9	0.22[1]

Mean values ± standard deviation; Median values (interquartile range); **MCPS** (Marigliano-Cacciafesta Polypathological Scale); **MNA** (Mini Nutritional Assessment); **SGA** (Subjective Global Assessment); **EdFED-Q** (Edinburgh Feeding Evaluation in Dementia Questionnaire); **LBD** (Lewy Body Dementia); **PSP** (Progressive Supranuclear Palsy); **MSA** (Multiple Systems Atrophy); **FTD** (Frontotemporal dementia).

[a] p value for the comparison between Parkinson's disease and atypical parkinsonism groups;

[b] p value for the comparison between parkinsonian and dementia syndromes groups;

[1] p value for the Mann-Whitney test for independent samples;

[2] p value for the Chi square test for independent samples;

[*] Significant

Severe and very severe frailty was higher in atypical parkinsonism, followed by dementia syndromes (figure 1).

The MCPS score and the CFS classification were statistically significant correlated (rs=-0.665; p=0.000). This correlation was stronger in dementia syndromes (rs= -0.773; p=0.002), followed by atypical parkinsonism (rs= -0.635; p=0.000) and PD (rs= -0.501; p=0.005).

Nutritional status

The global frequency of undernutrition (and risk of) was 73.7% according to the MNA. Despite no statistically significant differences between parkinsonian and dementia syndromes, undernutrition seem more frequent in dementia (84.6%) followed by atypical parkinsonism (75.7%) (figure 2).

Most of the patients with PD were obese (30%) according to the BMI, while atypical parkinsonism were more frequently normal (30.3%) and dementia syndromes undernourished (23.1%) (figure 3).

Frailty and Nutritional status

Significant correlations were found between the nutritional assessment parameters and the MCPS. The MNA and the EdFED-Q scores both showed a strong correlation with the MCPS (table 3).

DISCUSSION

Frailty

In the present study, the frequency of frailty in institutionalized patients with neurodegenerative disorders is high (71.1%).

Due to the lack of studies examining frailty in institutionalized patients with neurodegenerative disorders it is difficult to compare our results[16]. However, the prevalence found in our study was considerably higher than in previous studies with community-dwelling older adults, since the overall prevalence of frailty in 10 European countries is 17%[15,31].

There is scant evidence on the prevalence of frailty in nursing homes, possibly due to the practical limitations of using screening tools: the majority of tools require the physical or mental collaboration of the patient, which may be difficult in institutionalized individuals. The high levels of dependence, comorbidity, disabilities, and malnutrition over long-term care may contribute to making such screening difficult[16].

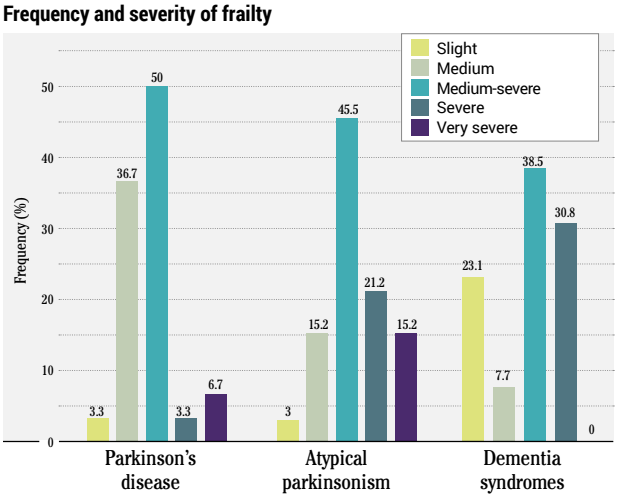

FIGURE 1. Frequency of the severity of frailty assessed by the Marigliano-Cacciafesta Polypathological Scale (MCPS) according to the neurodegenerative disorder (n=76).

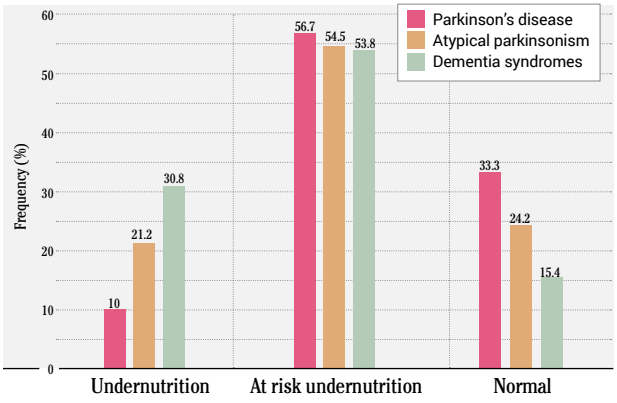

FIGURE 2. Frequency of nutritional status according to the Mini Nutritional Assessment (MNA) for the different neurodegenerative disorders (n=76).

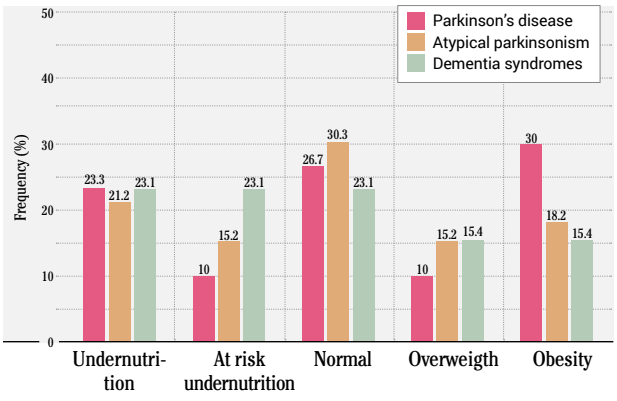

FIGURE 3. Frequency of nutritional status according to the body mass index (BMI) for the different neurodegenerative disorders (n=76).

TABLE 3. Correlations between frailty scales and nutritional parameters.

	Body mass index	MNA	EdFED-Q
MCPS	r= -0.363**	r= -0.732**	r= 0.714**
CFS	rs=0.227*	rs=0.629**	rs= -0.689**

MCPS (Marigliano-Cacciafesta Polypathological Scale), **CFS** (Clinical Frailty Scale), **MNA** (Mini Nutritional Assessment), **EdFED-Q** (Edinburgh Feeding Evaluation in Dementia Questionnaire). * $p<0.05$ ** $p<0.01$

A systematic review published by Kojima and colleagues assessed 9 studies of institutionalized elderly patients and estimated the prevalence of frailty to be 52%, and prefrailty to be 40% according to different assessment criteria (Frailty Phenotype, Clinical Frailty Scale, Edmonton Frail Scale, and two others)[16]. In our institutionalized elderly sample (average age of 76±6.8 years), the frequency of frailty was considerably higher, despite our sample being younger than those in the aforementioned systematic review (80.3 years)[16]. This suggests that the load of neurodegenerative disorders may influence the risk and/or severity of frailty even in those below the age of 80 years. However, it is impossible to make any conclusions as the clinical characteristics of the population pooled by the systematic review were not described[16].

Although few studies using the MCPS have been published, this tool has been considered useful for screening frailty and to program an intervention/rehabilitation as it allows for the severity of frailty to be stratified[36,37].

In our sample, participants with atypical parkinsonism presented more severe frailty than PD. Also, the atypical parkinsonism participants had greater disease severity compared to those with PD. These differences are in concordance with the literature[38-42]. Atypical parkinsonism usually has a faster and more severe progression than PD, with a poor response to dopaminergic treatment, a worse prognosis, shorter survival, and more complications in the early stages[35,42]. Motor features such as early postural instability and falls, early dysarthria and dysphagia, dystonia, and impaired response to levodopa treatment are frequent in atypical parkinsonism, along with early and severe cognitive and behavioral changes, apraxia, hallucinations, orthostatic hypotension, and urinary dysfunction[35].

In our study, the frequency of frailty in patients with Parkinsonian syndromes was 70.6% (corresponds to the sum of medium-severe, severe, and very severe frailty). Specifically, in PD the frequency was 60% and in atypical parkinsonism 85.7%.

In a sample of 133 patients in an acute hospital setting with an average age of 74 years, the frequency of frailty was 75.9%, which is similar to our results although assessed with different criteria[43]. Also, 76.7% of those patients were malnourished and at risk of malnutrition[43].

Although moderate, we found a statistically significant correlation between frailty and the severity of Parkinsonian syndromes[44]. A small number of studies have described the prevalence of frailty in PD, and some have demonstrated that women with PD have a higher risk of frailty than men[25,26,45,46]. Despite this, the prevalence of frailty in PD has been reported to be high (69.4%)[47]. Furthermore, the severity of PD assessed with the unified Parkinson's disease rating scale and levodopa dose seems higher in frail patients[22,46,48].

The high frequency of frailty found in our study was expectedly high since some of the clinical features of Parkinsonian and dementia syndromes are considered major risk factors for frailty and are part of several assessment tools. Slow gait speed is a common feature of Parkinsonian syndromes, along with postural instability, risk of falls, and balance impairment[49-51]. Depression, cognitive decline, malnutrition, and urinary dysfunction may also occur especially in advanced stages[46,52-54]. Since most of our participants were rated as having a high severity of the neurodegenerative disease, this frequency seems reasonable.

Frailty in this specific population is frequent and particularly more severe in patients with atypical parkinsonism. These results highlight the importance of an early screening for frailty.

Nutritional status

The frequency of undernutrition and the risk of undernutrition according to the MNA in our study is also high, and in concordance with a previous study performed in similar population (73.7 versus 77.1% respectively[55]), and, in general, higher than published studies in nursing homes or community[28,29,31,32,55-58].

Besides the wide variation, depending on the applied methodology, in PD patients the general prevalence of malnutrition varies between 0-24% while 3 to 60% are estimated to be at risk[59]. When assessed with the MNA, the variation between studies decreases to 0-2% of malnourished and 20-34% at risk[59]. Our results

in PD patients regarding undernutrition and risk of undernutrition (66.7%) were similar to the ones obtained in a sample of 34 institutionalized PD elders, where 62% were malnourished or at risk at the admission according to the MNA[55].

Body weight and PD share a relation that is still unexplained[52]. Weight loss is frequent, especially in advanced stages of the disease, and it has been shown that weight loss and low body weight (and BMI) are associated with a higher risk of developing dyskinesia due to the higher ratio of levodopa dose per kilogram (>6mg/kg)[60]. Also, weight loss is associated with mortality and poor quality of life[60,61].

The frequency of undernutrition or risk of undernutrition in patients with LBD in our study (n=12) was 83.3%, which is higher than the one found by Roque and colleagues in a community setting (77.3%)[62].

Regarding dementia syndromes, 84.6% of the patients were undernourished or at risk according to the MNA. Specifically, in AD patients (n=5), 80% was undernourished or at risk of undernutrition. Despite the small number of patients with AD included, this frequency is higher than the one found in community-dwelling AD elders (varies from 14.1 to 55.9%)[62].

Frailty and Nutritional Status

Interestingly, the general frequency of undernutrition (or risk of) is very similar to the frequency of frailty. This goes in favour of the strong correlation between MNA and MCPS that was demonstrated in our study ($r = -0.732$; $p<0.01$) and in line with previous studies regarding the correlation between nutritional status and frailty[31,43]. The MNA assesses several risk factors for frailty, namely weight loss and low BMI, reduced mobility, and low nutritional intake. In the parkinsonian syndromes, the undernourished participants were also the ones with more severe frailty while the patients at risk of undernutrition were also medium-severely frail. In dementia syndromes similar tendency was verified.

On the other hand, the correlation between BMI and MCPS was weak ($r = -0.363$; $p<0.01$). In the MCPS, nutritional status can be assessed with the MNA or the BMI, however the considered BMI cut-offs are commonly used for adults and not for elders. This means that an elder can be mistakenly considered overweight instead of normal since the reference value for normal in older adults is 24-26.9kg/m2 that is close to overweight cut-offs in adults (25-29.9kg/m2). Despite this, in our study we also found a U-shaped relation between frailty and BMI[63-65]. This relation was more obvious in parkinsonian syndromes than in dementia syndromes possibly due to the differences in the number of participants in both groups.

CONCLUSIONS

The frequency of frailty in institutionalized patients with neurodegenerative disorders is, as expected, high. Similar frequency of undernutrition (or risk of) was found. Nutritional status and frailty seem to be significantly correlated. Since inadequate nutrition and/or poor nutritional status are potentially treatable causes for frailty, it seems reasonable to further investigate the effects of therapeutic nutritional interventions to prevent and to treat frailty.

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Medical Students' Attitudes, Perceptions, and Usage of Large Language Models in Education: A Questionnaire-based Study

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ABSTRACT: Objectives: This study examined medical students' attitudes, perceptions, and usage patterns of AI, particularly large language models (LLMs), in medical education. The goal was to explore how these tools are used for academic purposes and their potential integration into medical curricula. **Methods:** A cross-sectional questionnaire was distributed to medical students across six academic years at a Portuguese institution during autumn 2024. Respondents rated their study habits, the relevance of digital resources, frequency of engagement with LLMs, trust in AI-generated content, and opinions on curricular integration. **Results:** A total of 306 students (13.4% response rate) completed the survey. AI was used by 87% of respondents, primarily for resolving theoretical doubts (84%), while its application in complex academic tasks was limited. Freely available models (GPT-3.5) were the most commonly used, whereas only 17% had experience with paid versions such as GPT-4. Trust in AI-generated clinical recommendations was low, with only 16% considering them reliable in a clinical case-based scenario. Limited familiarity (69%) and cost (58%) were identified as key barriers to broader adoption. No substantial evidence suggested widespread use of AI for academic misconduct. Despite scepticism regarding its reliability in clinical contexts, most respondents supported AI integration into the curriculum, with 65% favouring an optional course. **Significance:** Students frequently use AI for theoretical learning but remain sceptical of its reliability in medical decision-making. Addressing concerns through AI literacy and reducing cost barriers may encourage responsible adoption in medical education.

KEY WORDS: Medical education, artificial intelligence, survey, GPT

INTRODUCTION

Artificial intelligence (AI) has transitioned from a niche curiosity into a widely used, transformative force across numerous fields, including medicine and medical education. A key advancement in this domain is the development of Large Language Models (LLMs), exemplified by OpenAI's GPT-3 and GPT-4, though similar models such as Claude 3, Gemini, and DeepSeek are rapidly emerging[1,2]. LLMs process and generate human-like text using deep learning architectures and self-attention mechanisms, enabling tasks such as summari-

zation, translation, and answering medical licensing exam questions with accuracy comparable to human professionals[3,4]. While debates persist about whether these models rely on "simple" statistical patterns (the so-called stochastic parrot, or "glorious autocomplete") rather than genuine understanding[1], their growing influence in medical education, for instance as study aids, simulation tools, and clinical reasoning supports, is evident. Examples include enhancing case-based learning, exam preparation, and clinical documentation. At NYU Grossman School of Medicine, AI tools facilitate interactive learning [5], while studies highlight their role in generating question banks for standardized exams[6,7].

This integration aligns with broader trends in technology-driven learning. Medical students increasingly rely on digital platforms like Osmosis, AMBOSS, and Lecturio, which supplement traditional resources with interactive content[8–10]. Tools like ChatGPT, though not designed for medical education, are widely used to clarify concepts, assist with scientific writing, and provide feedback[11]. Notably, GPT-4 has achieved scores exceeding 86% on medical licensing exams[3], and it has also addressed ethical and professional topics with competence[4], which further increases the interest about its role in exam preparation and clinical reasoning. However, concerns persist about over-reliance, critical thinking shortfalls and bias, and inaccuracies in AI-generated content, particularly when confabulated responses mimic seemingly accurate statements[12].

Despite these developments, medical students' perceptions and usage patterns of AI tools like GPT-3/4 remain understudied. Existing research indicates that students recognize AI's potential but express concerns about ethical limitations and risks of dehumanizing clinical practice[13,14]. While interest in AI is high, foundational understanding of its principles and applications is often lacking[15,16]. Additional debates focus on privacy, trust, and the role of human oversight in AI-augmented workflows[17,18]. As medical curricula adapt to new technologies, understanding student perspectives is key to ensuring AI enhances rather than replaces essential clinical skills.

This study aims to explore medical students' attitudes, beliefs, and usage patterns regarding AI, focusing on LLMs such as GPT-3.5 and GPT-4 at the Faculty of Medicine, University of Lisbon (FMUL), Portugal.

METHODS

Study Design and Population:

This cross-sectional questionnaire-based study targeted medical students (years 1–6, n = 2,278) at the Faculty of Medicine, University of Lisbon (FMUL).

Recruitment and Data Collection:

Students were recruited via institutional emails and WhatsApp groups representing each cohort. Invitations were distributed in three waves: initial emails to year representatives (Week 1), reminders (Week 3), and direct emails to students in Years 2–4 (Week 6). The anonymous questionnaire, administered in Portuguese via Google Forms (Google LLC, Mountain View, CA, USA), was available from November 4 to December 12, 2024. Eligibility was restricted to FMUL students using institutional email addresses, with a one-response-per-account limit to prevent duplicates.

Questionnaire Design:

The 13-item questionnaire (Supplementary file and Table S1 for full questionnaire translated into English) assessed two domains: (I) Study Habits: Relevance of resources (e.g., in-person classes, AI platforms) for semester-long study and exam preparation; (II) AI Engagement: Usage frequency, perceived utility in medical contexts, trust in outputs, and opinions on curricular integration. Questions utilized Likert scales (1–7), multiple-choice, and open-ended formats (Table 1).

TABLE 1. Summary of Key Questionnaire Domains

Question	Focus	Response Type	Key Options/Scale
1	Year of study	Multiple-choice	Years 1–6
2	Study methods (semester)	Likert scale (1–7)	11 resources (e.g., AMBOSS, AI platforms)
5	AI platform usage frequency	Ordinal scale	ChatGPT-3.5, GPT-4, Gemini, Copilot
8	Trust in AI clinical recommendation	Scenario-based	5 options reflecting trust/skepticism
12	AI curriculum integration	Multiple-choice	5 strategies (e.g., mandatory courses)

Academic year (Q1, Years 1–6), study methods (Q2, rating resources like AMBOSS and AI platforms on a Likert scale), AI usage frequency (Q5, use of ChatGPT-3.5, GPT-4, Gemini, and Copilot), trust in AI recommendations (Q8, scenario-based responses), and curriculum integration (Q12, preferences for AI incorporation into FMUL's curriculum). The full questionnaire is available in Supplementary file and summarised in Table S1.

Ethical Considerations:

Data were anonymized and restricted to FMUL-affiliated emails. Ethical approval was granted by the CAML Ethical Committee (Ref. 193/24, September 2024).

Data Analysis:

All responses were exported from the online platform as CSV files, checked for completeness, and prepared for analysis. Sample characteristics were summarized using frequencies and percentages for categorical variables and medians with interquartile ranges (IQRs) for ordinal Likert scale items. Categorical variables were analysed using chi-square tests to assess differences in distribution across groups, with Cramér's V as a measure of effect size. A binomial test was used to determine whether the proportion of sixth-year students differed significantly from the expected value. Likert-scale responses were visualized using boxplots, where medians were marked by red lines, boxes represented IQRs, whiskers extended to 1.5 × IQR from Q1 and Q3, and outliers were displayed as dots. Given the non-normal distribution of Likert-scale data, comparisons between independent groups were performed using the Mann-Whitney U test. Effect sizes were calculated with Cliff's Delta to quantify the magnitude of observed differences. All statistical analyses and visualizations were conducted using Python 3.11.0 on macOS 12.7.6.

RESULTS

Of the 2,278 medical students at FMUL, 306 completed the survey (55% in preclinical and 45% in clinical years), yielding a 13.4% response rate (margin of error: ±5.2%). Response distributions varied across all 6 years, with significant deviations ($p < 0.001$, Cramér's $V = 0.33$, moderate effect; Figure 1). Due to weak effect sizes in preclinical vs. clinical comparisons ($p = 0.02$, Cramér's $V = 0.13$, weak effect), analyses were conducted at the preclinical versus clinical level. Response rates for most individual questions exceeded 97%, except for open-ended items (Supplementary Table S1). These received responses from as few as 3.2% of students and thus were excluded from further analysis.

Several traditional learning methods, e.g. textbooks and theoretical classes lost importance while a shift towards digital media, particularly video platforms, was observed (Supplementary Figures S1, S2). Learning platforms such as AMBOSS and other online resources received moderately positive ratings (Figure 2), though with a wide IQR (2–7). For exam preparation, these resources were pooled into a single composite, which showed a narrowing of the IQR to 4–6 while the median remained unchanged (Figure 2). However, each individual platform was less used during the semester than the composite for examen preparation (both $p < 0.001$; AMBOSS: $\delta = -0.23$, other online study platforms: $\delta = -0.16$, small effect sizes). Doubt-resolution tools (Figure 2), such as Google/Wikipedia or AI platforms, were

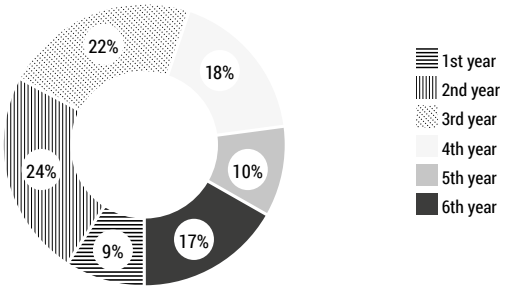

FIGURE 1. Participation in survey by year.

Striped patterns represent preclinical years (1st–3rd, left side), and solid shades represent clinical years (4th–6th, right side). Yearly participation differed significantly from an equal distribution ($p < 0.001$, Cramér's $V = 0.33$, moderate effect size), with 1st (-7.7%, $p < 0.001$) and 5th-year students (-6.7%, $p = 0.002$) underrepresented, while 2nd (+7.3%, $p < 0.001$) and 3rd-year students (+5.3%, $p = 0.012$) were overrepresented. Preclinical students (Years 1–3) outnumbered clinical students (Years 4–6) (56.5% vs. 43.5%, $p = 0.02$, Cramér's $V = 0.13$, weak effect size).

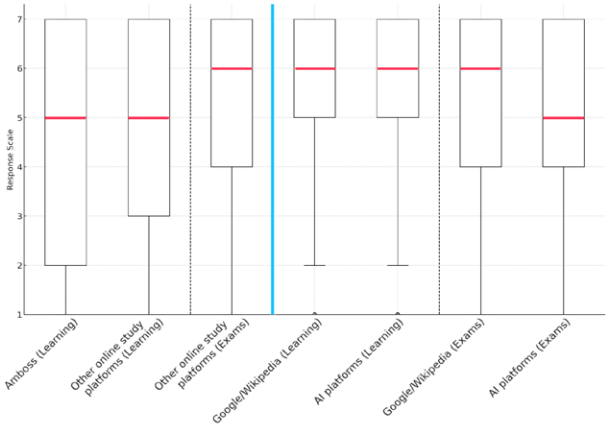

FIGURE 2. Relevance ratings of study methods.

Students rated study methods on a scale from 1 ("Not relevant at all") to 7 ("Extremely relevant"). A blue vertical line separates online study platforms (left) from doubt-resolution tools (right). Dashed grey lines distinguish semester-long study methods (left) from exam preparation tools (right). For exam preparation, semester-long resources are grouped under a single "online platform" category. Red lines indicate medians, boxes represent interquartile ranges (IQR), whiskers extend to 1.5 × IQR from Q1 and Q3, and outliers are shown as dots.

consistently rated highly. AI platforms demonstrated a slight but statistically significant decline in perceived relevance from semester-long learning to exam preparation (median: 6 to 5, IQR: 5–7 to 4–6; $p < 0.001$, $\delta = 0.19$, small effect size). In contrast, ratings for Google/Wikipedia increased slightly during exam preparation; however, the difference compared to AI platforms was not statistically significant ($p = 0.14$). Preclinical-year students preferred practical and theoretical-practical classes, *Sebentas* (study materials prepared by students from previous years), and online videos, whereas clinical years students favoured university-provided video lectures and AMBOSS (Supplementary Table S2). The strongest effect was observed for AMBOSS ($\delta = -0.52$, $p < 0.001$, medium to large effect), indicating a notable increase in its relevance for clinical year students.

Students expressed scepticism about AI's role in more practical tasks, rating it more useful for acquiring general knowledge than for developing practical skills (Supplementary Figure S3). Similarly, AI was perceived as helpful for checking basic medical questions and medical fact-checking but notably less useful during clinical rotations, where more complex clinical uncertainties arise (Supplementary Figure S2). Clinical-year students rated AI slightly higher for answering medicine-related questions ($p = 0.02$, $\delta = 0.15$, small effect size), but its utility during clinical rotations was rated similarly across groups ($p = 0.9$). Specific responses on AI's use in medical education reflected scepticism about its practical applicability (Figure 3, section A). While AI was perceived as useful for generating exam questions, its usefulness for creating practical simulations was rated lower. Trust in AI was limited, as shown in a dual-purpose question assessing both medical knowledge and confidence in AI-generated recommendations. When presented with GPT-4's suggestion of intravenous artesunate as the first-line treatment for severe malaria, only 16% of students trusted the AI's recommendation (details in Supplementary file)[19].

Students perceived AI as similarly useful for text correction and drafting, though drafting was rated slightly higher (Figure 3, section B; Supplementary Figure S4). Correction was viewed as moderately useful, with clinical students rating it slightly higher than preclinical students, though the difference was not statistically significant ($p = 0.120$; Supplementary Figure S5). In contrast, AI's usefulness for drafting was rated higher overall, particularly among clinical students, but again, the difference was not significant ($p = 0.409$; Supplementary Figure S5).

Astonishingly, 13% of students reported lacking both interest in and knowledge of AI. However, this may be an underestimate given the low 13.4% response rate. While 87% expressed interest, 69% had little prior knowledge, and only 18% actively engaged in independent learning. Most AI platforms saw minimal use, with 83%–90% of students reporting "never/almost never" using ChatGPT-4 (paid), Google Gemini, or Microsoft Copilot (Supplementary Figures S6). In contrast, ChatGPT-3.5 (free) was used more frequently with 58% of students using it less than once per day (Figure 4). The majority supported integrating AI into the FMUL curriculum through at least one approach. An optional AI course was the most preferred option (65%), while 30% supported integration into existing courses and 49% favored its inclusion in medical ethics subjects. Additionally, 58% wanted FMUL to fund AI tools, such as ChatGPT Plus subscriptions.

DISCUSSION

Most students in the sample used GPT-3.5 (the free version of ChatGPT) primarily as a general doubt-resolution tool, similar to Google or Wikipedia. Approximately 80% had never used more advanced models such as GPT-4 or alternative (paid) premium models, which may contribute to their scepticism regarding the reliability of AI and its limited recognition of its superior accuracy, as evidenced for example by premium model performance in medical examinations[3,6]. The reliance on free AI tools for quick academic queries suggests that access to more advanced models may be perceived as unnecessary. Concerns regarding AI-generated "hallucinations" (more accurately, confabulations) may also contribute to scepticism, as also reflected in the low acceptance rate of GPT-4's correct clinical case management recommendations in this survey[19]. Previous research indicates that negative experiences with AI can create a persistent bias against subsequent accurate responses[20,21]. Furthermore, AI-generated recommendations are often disregarded in favour of initial human judgments[22], reinforcing scepticism. If AI errors are recalled more readily than its correct outputs, this may further discourage its integration into clinical decision-making.

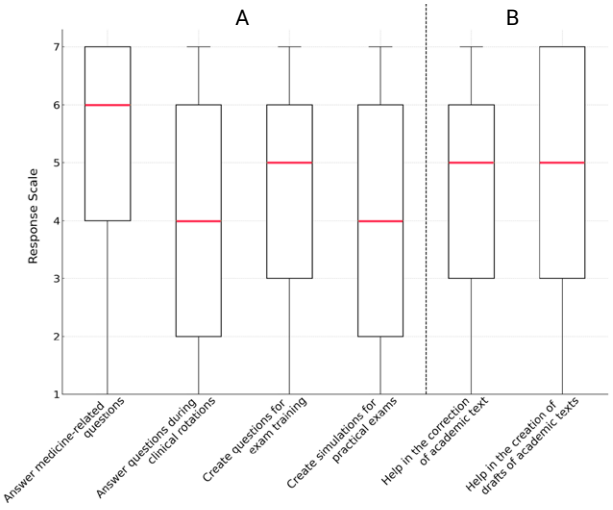

FIGURE 3. Perceived usefulness of AI in medical education.

Tasks related to doubt resolution and exam preparation (A) and academic writing, including assignments (B) are separated by a dashed grey line. Red lines indicate medians, boxes represent interquartile ranges (IQR), whiskers extend to 1.5 × IQR from Q1 and Q3, and outliers are shown as dots.

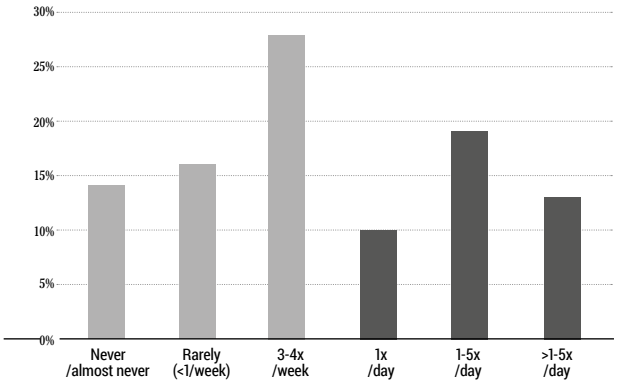

FIGURE 4. ChatGPT-3.5 (free Version) Usage Frequency.

Usage categories include less frequent users (grey bars) and more frequent users (black bars).

Addressing these concerns requires AI literacy programs that provide training on how AI generates outputs, its limitations, and strategies for critically evaluating its recommendations. Educational initiatives should incorporate data on AI error rates, reliability, and comparative performance against human decision-making to facilitate a more evidence-based approach to AI use in medical practice.

The monthly subscription fee (~€20) could also be a barrier for some. Others might see subscription services as binding or costly commitments. Unsurprisingly, 58% of respondents favoured institution-sponsored access, mirroring broader findings that they perceive cost is a major hurdle[23,24]. For instance, at Harvard, 40% of students use AI daily, but only 30% pay for subscriptions; those receiving financial aid are half as likely to do so[25]. Some institutions address this barrier through curricular integration or subsidized partnerships. At FMUL, universal premium access for around 2,300 students at €20 per month each would total €552,000 annually, roughly 2.5% of the university's €22.7 million budget and 13% of its goods-and-services allocation[26]. However, while significant, such an expense could be justified if it may yield equitable access, improved learning outcomes, and alignment with evolving global trends in medical education.

Academic misconduct involving generative AI has raised significant concerns in higher education[27,28]. However, our data suggest that this does not seem to be a major issue at the moment. Students are aware of AI for text creation, and it was rated as moderate, yet considerably lower than the use of traditional peer-created study materials such as *Sebentas* (see Supplementary Data). Interestingly, the exceptionally high rating for *Sebentas* seem to suggest a certain level of honesty in responses, given that they lack formal faculty endorsement. Limited experience with AI and restricted access to advanced models (like GPT-4) may also contribute to these findings. As AI writing tools become more sophisticated and widely available, usage patterns might likely shift, perhaps increasing the risk of misconduct. While institutions may consider AI-detection technologies, these appear to be more unreliable than often assumed[29,30] and risk falsely flagging legitimate student work. A balanced approach, integrating technological solutions with clear guidelines, may ultimately prove more effective in preserving academic integrity.

The low response rate (13.4%) with a ±5.2% margin of error may affect generalizability, particularly given the underrepresentation of certain academic years. However, studies in higher education suggest that response rates as low as 10–20% can still provide reliable estimates if nonresponse bias is minimal[31]. Furthermore, the proportion of students who are nev-

er/almost never users and heavy users (>5x/day) was relatively high and similar, making it less likely that our conclusions are driven by a non-representative sample favoring one site. While broader participation would strengthen representativeness, our findings align with international trends, supporting their relevance. It is possible that students less interested in AI may have been less inclined to participate. Additionally, self-reported data can be influenced by recall bias or social desirability. Despite these constraints, our findings mirror international trends of AI use in medical training[15,16].

The relatively high proportion of students who are either very low/low or high/very high users highlights the need to discuss the benefits of AI and increase foundational knowledge competencies for the very low/low users, as well as to call students' attention to the limitations of AI, potential overreliance, and ethical concerns for the high/very high users. Going forward, structured AI competencies—emphasizing not only technical skills and responsible use but also limitations—could help foster more informed integration of AI into clinical education. Coupled with institutional investments that mitigate cost barriers, such curricula could accelerate the safe and ethical adoption of AI tools among future physicians.

While AI is poised to enhance access to information and support critical thinking and medical decision-making, it is crucial to recognize that medicine inherently involves complexities that technology cannot simplify. As highlighted by Elder, delivering high-quality, patient-centered care requires medical training that is long enough, broad enough, and deep enough[32]. Therefore, it should not be expected that AI tools will make medical education and practice less challenging.

CONCLUSION

In summary, student interest in AI appears high scepticism about clinical reliability, concerns over cost, and limited exposure to more advanced models constrain broader acceptance. Addressing these issues, through dedicated AI-focused curricula, institutional support for premium tools, and ongoing investigations into AI's accuracy and ethical implications, will be pivotal in shaping a medical education landscape where AI enhances rather than undermines clinical expertise.

SUPPLEMENTARY FILE 1

Complete questionnaire (GPT4 - translation to English)

USE OF ARTIFICIAL INTELLIGENCE (AI) BY MEDICAL STUDENTS

My name is Sara Pereira, and I am a 6th-year medical student at FMUL.

Artificial Intelligence, despite its long history, has recently emerged as the leading technology of the moment, with countless applications in everyday life, clinical practice, and medical education.

As part of my Master's Final Project in the field of Medical Education, I am conducting a study on the use of Artificial Intelligence by medical students. Through this study, I aim to gather relevant data on students' perceptions of Artificial Intelligence, with the goal of positively influencing the medical school curriculum at FMUL.

The questionnaire, intended for FMUL medical students from the 1st to the 6th year, should take approximately 5 to 10 minutes to complete. This questionnaire has been approved by the President of the Pedagogical Council and the President of the Department of Medical Education (DEM).

Your participation in this study is completely anonymous and voluntary, and you may withdraw at any time. The confidentiality of your data is guaranteed in accordance with the legislation in force and the guidelines of the National Data Protection Commission (CNPD) (*Deliberação n.º 1704/2015, de 22 de outubro,* and *Decreto-Lei n.º 67/1998, de 18 de março*), as well as the terms required by the General Data Protection Regulation (GDPR; Regulation (EU) 2016/679 of the European Parliament and of the Council, of April 27, 2016).

INFORMED CONSENT – By proceeding with the completion of the questionnaire, I declare that I have been informed of its objectives and authorize the processing of my data exclusively for research purposes.

SECTION 1 – STUDY METHODS

The following questions will address the techniques and resources you use to study throughout the semester and prepare for evaluations.

1. What is your year of study? *(Mandatory question; Select only one option)*

- 1st year
- 2nd year
- 3rd year
- 4th year
- 5th year
- 6th year

2. On a scale of 1 to 7, where 1 means "Not relevant at all" and 7 means "Extremely relevant", rate how you would classify the following resources in terms of their relevance for learning during the semester:

- Attending theoretical classes in person.
- Watching recorded video lectures provided by the university.
- Attending practical and theoretical-practical classes.
- Studying using recommended bibliography textbooks (paper or PDF).
- Using study materials (notes, transcripts, summaries, etc.) prepared by students from previous years.
- Using Amboss for studying.
- Using other online study platforms (e.g., Sketchy, Osmosis, Lecturio, etc.) for studying.
- Using AI platforms (e.g., ChatGPT) for studying.
- Watching online videos (e.g., YouTube) for studying.
- Using search engines (e.g., Google) or online encyclopedias (e.g., Wikipedia) to clarify doubts.
- Using AI platforms (e.g., ChatGPT) to clarify doubts.

3. On a scale of 1 to 7, where 1 means "Not relevant at all" and 7

means "Extremely relevant", rate how you would classify the following resources in terms of their relevance to your evaluations (e.g., written exams, oral exams, TEM, OSCE, tests, quizzes):

- Watching recorded video lectures provided by the university.
- Consulting recommended bibliography textbooks.
- Using study materials prepared by students from previous years.
- Using online study platforms (e.g., Amboss, Sketchy, etc.).
- Watching online videos (e.g., YouTube).
- Using search engines (e.g., Google) or online encyclopedias (e.g., Wikipedia).
- Using AI platforms (e.g., ChatGPT).

4. If you use another method not mentioned or have any comments, please write them here: *(Open-ended, long answer question)*

SECTION 2 – USE OF ARTIFICIAL INTELLIGENCE

In this section, the goal is to better understand how you integrate Artificial Intelligence (AI) into your daily life and how you envision its utility in the future.

5. How often do you use the following AI platforms? *(>5x/day; 1-5x/day; 1x/day; a few times per week; rarely; never/almost never)*

- ChatGPT 3.5 (free version)
- ChatGPT 4 and 4o (paid version)
- Google Gemini
- Microsoft Copilot
- Other AI platform

6. If you selected "Other AI platform," please indicate which one. *(Open-ended short answer question).*

7. On a scale of 1 to 7, where 1 means "Not useful at all" and 7 means "Extremely useful," indicate how relevant you find the use of AI platforms (e.g., ChatGPT) in the following areas:

- Searching for general knowledge information.
- Learning practical skills unrelated to medicine (e.g., new languages, recipes, etc.).
- Clarifying medicine-related doubts.
- Clarifying doubts during clinical rotations.
- Assisting with the correction of academic text (e.g., assignments, medical histories, theses).
- Assisting with drafting academic texts (e.g., assignments, medical histories, theses).
- Creating exam training questions (multiple choice, open-ended, oral).
- Simulating clinical scenarios (e.g., OSCE training).

8. The following scenario aims to investigate how you would use information provided by GPT-4 and assess the extent to which you trust its accuracy in a clinical context:

A 35-year-old patient returns from Thailand with severe malaria. Immediate intravenous treatment must be urgently initiated. After consulting GPT-4 for guidance on treatment, it suggests that both intravenous artesunate and quinine are valid options for treating severe malaria, stating that artesunate is generally superior to quinine. However, GPT-4 also mentions that "artemisinin resistance" has been widely reported in Southeast Asia, citing a recent publication ("Time to contain artemisinin resistance," The Lancet, link). Despite this, GPT-4 recommends starting treatment with intravenous artesunate as the first-line therapy.

Based on the scenario above, and considering GPT-4's response, how would you proceed with the patient's treatment?

- Given the urgency of the situation and the information about artemisinin resistance, you base your decision on GPT-4's response and therefore initiate intravenous quinine as an alternative.
- You find GPT-4's response about artesunate being clinically superior interesting but consider starting quinine because "artemisinin resistance" has been reported in the region.
- Despite the urgency, you verify the information about "artemisinin resistance" in the region using other sources, and if confirmed, switch to quinine.
- Given the urgency of the treatment and the fact that GPT-4 mentions the superiority of artesunate, you follow the recommendation to initiate treatment with intravenous artesunate despite the mention of "artemisinin resistance," as it states that artesunate remains the recommended and effective first-line option.
- You ignore GPT-4's information about the continued usefulness of artesunate when "artemisinin resistance" is reported and consider it a "confabulation" or "hallucination" by the AI, deeming it unreliable.

9. On a scale of 1 to 7, where 1 means "Strongly disagree" and 7 means "Strongly agree," indicate how much you agree with the following statements about the use of Artificial Intelligence in Medicine:

- AI can be helpful in complex clinical situations (e.g., absence of clear clinical signs and symptoms, multimorbidity, deprescription of medications, etc.).
- AI can be helpful in non-clinical specialties (e.g., radiology, neuroradiology, clinical pathology, etc.).
- AI can assist with administrative tasks (e.g., writing clinical records, discharge summaries, etc.).
- AI can serve as a tool to reduce healthcare errors.
- AI could make some non-clinical specialties irrelevant.
- AI can substantially change clinical practice.
- AI can increase healthcare errors.
- AI raises ethical concerns regarding data protection and patient privacy.

10. To what extent do the following statements about AI knowledge apply to you? *(Select only one option)*

- I am interested in AI and am learning about it independently.
- I am interested in AI and am taking a course on the subject outside of university.
- I am interested in AI but do not have much knowledge.
- I am not interested in the subject and have no knowledge about it.

11. Would you like FMUL to promote the integration of Artificial Intelligence into the curriculum? *(Select only one option)*

- I do not believe AI knowledge is relevant for a medical student.
- Yes, I would like FMUL to promote the integration of AI into the curriculum.

12. If you answered yes to the previous question, how would you like FMUL to promote the integration of AI into the medical curriculum? *(Select all that apply)*

- I would like FMUL to integrate knowledge about AI into various subjects in the mandatory curriculum.
- I would like FMUL to create a mandatory subject about AI.
- I would like FMUL to teach about AI in the optional curriculum.
- I would like FMUL to include questions about AI in medical ethics subjects.
- I would like FMUL to promote the frequent use of AI in learning (e.g., through funding subscriptions to ChatGPT Plus for all students).

13. If you have another opinion not mentioned or any comments, please write them here *(open-ended long answer question).*

SUPPLEMENTARY FILE 2

Description of dual-purpose question

Question 8 served two main purposes. Firstly, it was designed to assess whether students understood the concept of partial resistance to a drug, rather than complete resistance. The correct answer (option d) would only be selected by students who were aware that "artemisinin resistance" refers

to delayed parasite clearance, not complete resistance, as described in the literature. Because most students did not choose the correct answer, this revealed a widespread misunderstanding of the term. These findings were the basis for a separate publication (Pereira SM, Grobusch MP, Hänscheid T. How a GPT-aided survey reveals a medical student's misunderstanding of the term 'artemisinin resistance'. New Microbes New Infect. 2024 Dec 5;63:101552. doi: 10.1016/j.nmni.2024.101552. PMID: 39759404; PMCID: PMC11699336.).

At the same time, the question also tested how students trusted information provided by GPT-4. The scenario described a clinical case of severe malaria, with the GPT-4 chatbot indicating that "artemisinin resistance" had been documented yet still maintaining that intravenous artesunate was the recommended first-line therapy. Only 16% of students selected the correct answer by following the chatbot's guidance (figure), while most of the remaining participants chose to switch to quinine or verify the suggested resistance through external sources before deciding on a treatment. Some found the chatbot's recommendation merely "interesting" but did not adhere to it, and a small subset dismissed the AI-based guidance entirely. These patterns show the challenges in establishing trust in AI systems for clinical decision-making, especially when the concepts involved—in this case, partial versus complete drug resistance—are subject to misunderstanding.

Original questions with *[comments in square brackets]***:**

The following scenario aims to investigate how you would use information provided by GPT-4 and assess the extent to which you trust its accuracy in a clinical context:

A 35-year-old patient returns from Thailand with severe malaria. Immediate intravenous treatment must be urgently initiated. After consulting GPT-4 for guidance on treatment, it suggests that both intravenous artesunate and quinine are valid options for treating severe malaria, stating that artesunate is generally superior to quinine. However, GPT-4 also mentions that "artemisinin resistance" has been widely reported in Southeast Asia, citing a recent publication ("Time to contain artemisinin resistance," The Lancet, link). Despite this, GPT-4 recommends starting treatment with intravenous artesunate as the first-line therapy.

Based on the scenario above, and considering GPT-4's response, how would you proceed with the patient's treatment?

a) Given the urgency of the situation and the information about artemisinin resistance, you base your decision on GPT-4's response and therefore initiate intravenous quinine as an alternative.
[3% – This indicates partial trust in GPT-4 (acknowledging its mention of resistance) but ultimately opting against its recommended first-line therapy.]

b) You find GPT-4's response about artesunate being clinically superior interesting but consider starting quinine because "artemisinin resistance" has been reported in the region.
[15% – These respondents acknowledge GPT-4's superiority claim for artesunate but choose quinine, reflecting uncertainty or scepticism about the AI's recommendation.]

c) Despite the urgency, you verify the information about "artemisinin resistance" in the region using other sources, and if confirmed, switch to quinine.
[62% – This represents the majority, who prefer caution by verifying GPT-4's statement externally before potentially changing the recommended treatment.]

d) Given the urgency of the treatment and the fact that GPT-4 mentions the superiority of artesunate, you follow the recommendation to initiate treatment with intravenous artesunate despite the mention of "artemisinin resistance," as it states that artesunate remains the recommended and effective first-line option.
[16% – This is the correct option, indicating trust in GPT-4's guidance despite the mention of resistance.]

e) You ignore GPT-4's information about the continued usefulness of artesunate when "artemisinin resistance" is reported and consider it a "confabulation" or "hallucination" by the AI, deeming it unreliable.
[4% – These respondents fully reject GPT-4's assertion that artesunate remains effective, dismissing the AI's input as untrustworthy.]

TABLE S1 Number of responses per question

Question number	Responses (n)	Responses (%)
1 *(mandatory)*	*306*	*100%*
2	*306*	*100%*
3	*305*	*99.6%*
4 *(open)*	*10*	*3.2%*
5	*306*	*100%*
6 *(open)*	*22*	*7.1%*
7	*305*	*99.6%*
8	*285*	*93.1%*
9	*301*	*98.3%*
10	*303*	*99%*
11	*297*	*97%*
12	*250*	*81.6%*
13 *(open)*	*16*	*5.2%*

The table summarizes the number of responses per survey question. Question 1 was the only mandatory question, ensuring a response from all participants (306), while questions 4, 6 and 13 were open questions where participants could enter text.

TABLE S2 Comparison of Learning Methods Between Preclinical and Clinical Students

Learning Method	p-value	Effect Size (d)	Effect Size Interpretation	Preference
Classes	< 0.001	0.242	Small to Medium	Preclinical
Sebentas	<0.01	0.180	Small	Preclinical
Online Videos	< 0.001	0.315	Small to Medium	Preclinical
Video Lectures	< 0.001	-0.274	Small to Medium*	Clinical
Amboss	< 0.001	-0.517	Medium to Large*	Clinical

"Classes" refer to practical and theoretical-practical classes, and "Video Lectures" to faculty-recorded lectures. Effect sizes (Cliff's δ) reflect differences in ratings: positive values favour preclinical students; negative values favour clinical students. The strongest effect was for Amboss showing a marked increase in its relevance among clinical students. *Sebentas:* study materials prepared by students from previous years.

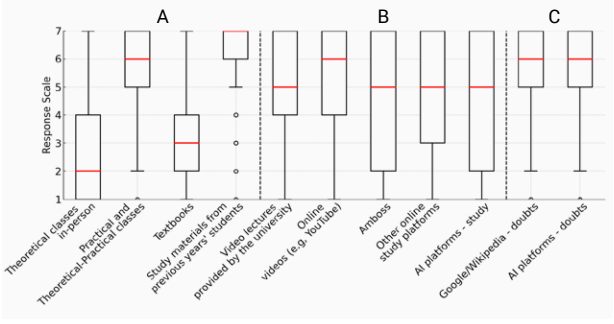

FIGURE S1 Methods used for learning during the semester

Study methods used by students to learn throughout the semester, rated on a scale from 1 ("Not relevant at all") to 7 ("Extremely relevant"). Red horizontal lines indicate the medians. The first vertical bar separates traditional methods (A: theoretical classes, practical/theoretical-practical classes, textbooks, and *"Sebentas"*: study materials prepared by students from previous years) from online resources. The second bar divides study-focused platforms (B: video lectures, Amboss, and others) from doubt-resolution tools (C: Google/Wikipedia and AI platforms).

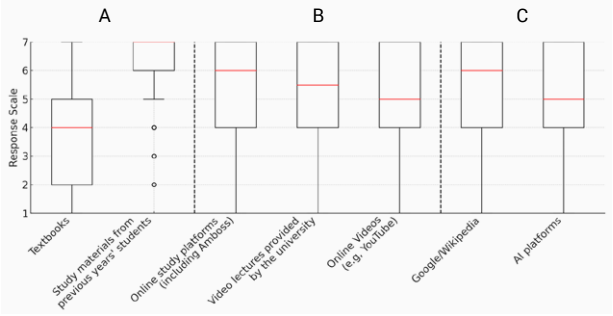

FIGURE S2 Methods used for exam preparation

Boxplot showing students' relevance ratings (1 = "Not relevant at all" to 7 = "Extremely relevant"). Red lines indicate medians. The first vertical bar separates traditional resources (A: textbooks and *"Sebentas"*: study materials prepared by students from previous years) from online resources. The second bar divides general online platforms (B: Amboss, video lectures, and YouTube) from tools primarily used for resolving doubts (C: Google/Wikipedia and AI platforms). Theoretical and practical classes were excluded, as they are unavailable during exams. Amboss was categorized under "Online study platforms," and AI-related options were combined into a single category, "AI platforms."

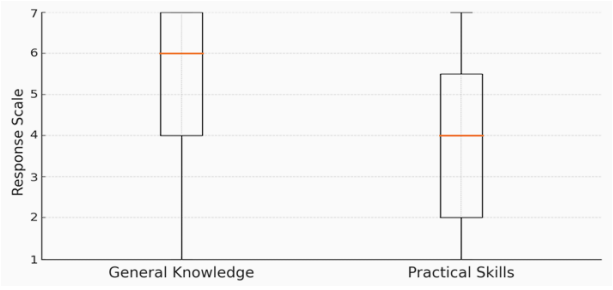

FIGURE S3 AI's utility for general knowledge vs. practical skills

Comparison of AI's usefulness in acquiring general knowledge and developing practical skills (scale: 1 = "Not useful at all" to 7 = "Extremely useful"). Red lines indicate median values.

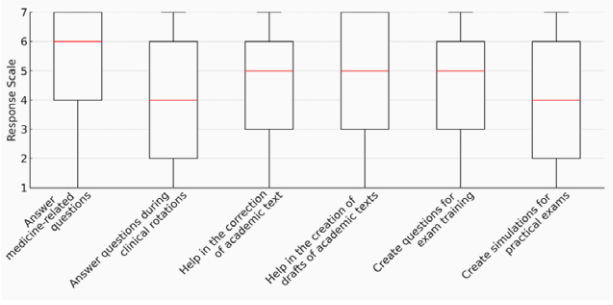

FIGURE S4 Perceived AI utility in medical education

Boxplot showing ratings for AI's usefulness in medical education (scale: 1 = "Not useful at all" to 7 = "Extremely useful"). Red lines indicate median values.

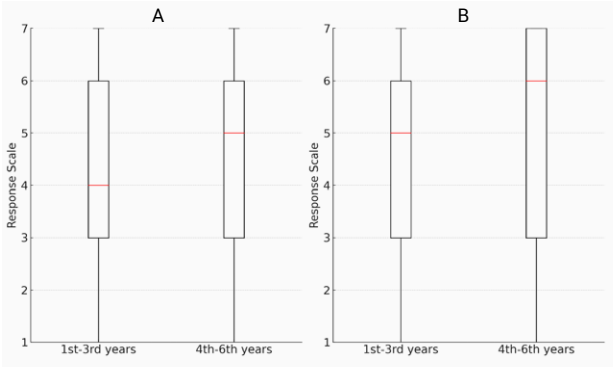

FIGURE S5 Students' perceived utility of AI in academic text tasks

(a) AI was rated moderately to highly useful for correcting academic texts, with slightly higher median ratings among clinical (4th–6th years) compared to preclinical (1st–3rd years) students.
(b) Clinical students also rated AI higher for drafting texts, though their responses showed greater variability than those of preclinical students.

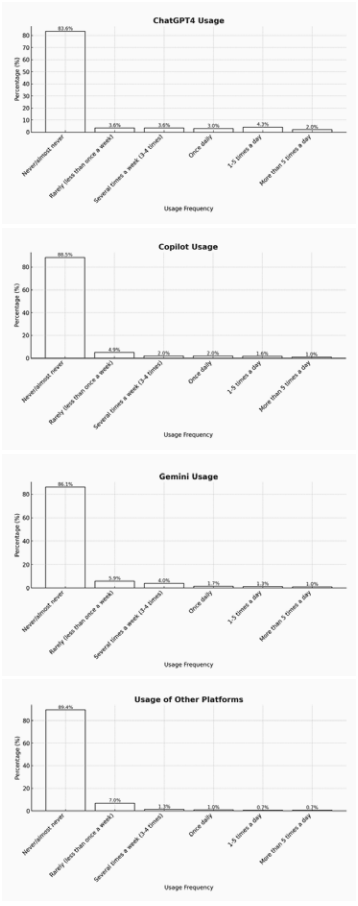

FIGURE S6 Frequency of AI use of different platforms

This figure presents the reported usage frequency of four AI platforms among students: ChatGPT 4 and 4o, Google Gemini, Microsoft Copilot, and Other AI platforms. These platforms typically require a paid subscription or have restricted access. The x-axis categorizes usage into six levels: 1-5 times per day, 1 time per day, more than 5 times per day, several times per week (3-4 times per week), rarely (less than once per week), and never/almost never. The y-axis represents the percentage of respondents for each category. The data indicate that almost 90% of students either never or very rarely use these platforms, highlighting their limited adoption among the surveyed population.

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

The Role of Palliative Care Interventions in Improving End-of-Life Quality: Insights from a Systematic Review

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ABSTRACT: **Introduction:** Palliative care (PALC) interventions are pivotal in enhancing the quality of life and quality of care (QOC) for patients with terminal illnesses. Assessing their impact across diverse settings is essential for improving patient outcomes and family satisfaction with care (SWC). This systematic review examined the impact of PALC interventions on end-of-life care outcomes. **Methods:** Articles from PubMed, Web of Science, and Scopus published between 2013-2023 were reviewed. Participants included staff and/or family members of adult individuals who have recently died. Interventions encompassed any PALC intervention in end-of-life care compared to usual care. Outcomes assessed included symptom management/burden, comfort around dying, QOC, and SWC. The risk of bias was evaluated using Cochrane tools. **Results:** Five studies (n=1905 patients) were included reporting deaths occurring either in nursing homes (n=3) or hospital wards (n=2). Some studies showed improved symptom management, particularly for discomfort and anxiety, while others found no significant differences between groups. Variability was noted in comfort around dying, with improvements reported by healthcare professionals but inconsistent support from family assessments. QOC outcomes varied, with some studies indicating improvements while others did not. SWC outcomes were heterogeneous, influenced by acute comorbidities. **Conclusions:** PALC interventions demonstrate potential in enhancing aspects of end-of-life care, though findings are varied. Further research is essential to address methodological limitations and standardize intervention protocols to optimize PALC's impact on patient and family outcomes.

KEY WORDS: Hospital Units; Nursing Home; Palliative Care; Patient Comfort; Patient Satisfaction; Quality of Health Care; Symptom burden; Systematic Review.

KEY SUMMARY POINTS: **Aim:** This systematic review investigated the impact of palliative care interventions on symptom management, comfort around dying, quality of care, and satisfaction with care in patients receiving end-of-life care. **Findings:** The review found that palliative care interventions improved symptom management for discomfort and anxiety in some studies, while others showed no significant differences. Additionally, comfort around dying was reported to improve by healthcare professionals, but family support remained inconsistent. Quality of care outcomes varied, with satisfaction influenced by acute comorbidities. **Message:** These findings highlight the need for consistent support from family members and healthcare providers to optimize the effectiveness of palliative care interventions at the end of life.

INTRODUCTION

Rationale – Death is an inevitable part of human life. While medicine aims to maintain and improve quality of life, ensuring a dignified and comfortable dying process is equally crucial. Delivering optimal end-of-life care (EOLC) presents significant challenges due to the complex needs of dying individuals, requiring a comprehensive approach.[1,2]

In developed countries, many cancer patients and individuals with life-threatening illnesses die in hospitals,[3,4] with studies indicating that 25% to 85% of those who could benefit from palliative care (PALC) pass away in these settings, often without adequate relief from their suffering.[5] This trend is also seen in nursing homes, where over a quarter of residents die, frequently experiencing unrecognized and untreated symptoms,[6,7] which impacts the quality of care (QOC). Many endure burdensome treatments that compromise the quality of the dying process.[1,8-13] Despite the importance of quality healthcare in the final stages of life, services often fail to meet patients' needs, highlighting the necessity for new strategies to improve the quality of dying.

Evidence shows that specialized PALC enhances both quality of life and quality of death,[14] reduces hospital admissions,[15,16] and promotes advance care planning.[17] Tailored advanced care preferences are associated with better quality of death by minimizing futile interventions and improving medical teams' skills in discussing EOLC.[18,19] This comprehensive approach should consider patients' social, cultural, and spiritual contexts.[20,21]

Studies suggest that specialist PALC services are linked to higher satisfaction with care (SWC) among patients' family members,[15,17] emphasizing the importance of involving surrogates in symptom management and decision-making.[22-24]

This study aimed to examine the evidence on PALC and its impact on end-of-life quality.

Objectives – This study addressed the primary question: "What is the impact of PALC strategies compared to usual care on the quality of the end-of-life process?

We aimed to systematically review the literature assessing the impact of PALC on the quality of the end-of-life, focusing on four key outcomes: symptom management/burden; comfort around dying; QOC; and SWC.

METHODS

This systematic review adhered to the recommendations outlined in the "Cochrane Handbook for Systematic Reviews of Interventions",[25] and was reported in accordance with the guidelines provided by the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses".[26]

Eligibility Criteria – ***Participants:*** Staff members and/or Family members of adult individuals who have recently died; ***Interventions:*** Any form of PALC intervention implemented in the end-of-life; ***Comparators:*** Usual care; ***Outcomes:*** Symptom management/burden, comfort around dying, QOC, and SWC; ***Study Design:*** Clinical studies/trials and randomized controlled trials (RCT). Studies were required to provide a detailed description of the PALC intervention employed.

Information Sources – The PubMed, Web of Science and Scopus databases were searched for articles published between January 1, 2013, and December 31, 2023. Each source was last searched on January 07, 2024.

Search Strategy – The search encompassed free-text terms in the title, abstract, and keyword fields, and utilized specific database headings: ("palliative care" or "hospice care" or "terminal care" or "end-of-life care") AND (death or dying) AND (comfort* or symptom* or "quality of care" or "satisfaction with care"). Filters for age (adults), language (English), and article type (RCT, clinical studies and trials) were applied during the search process.

Selection Process – During the initial screening phase, articles were chosen based on the examination and analysis of their titles and abstracts, with both authors independently participating in this process. Subsequently, a list of potentially relevant articles was compiled, leading to a full-text analysis conducted independently by two reviewers. Any discrepancies regarding study selection and data extraction were resolved through discussion between the authors. No automation tools were employed in this selection process.

Data Charting Process – Two independent reviewers extracted data from each report using a data extraction form created with Excel 16.0® spreadsheet software (Microsoft Corporation, 2023). The original authors

were not contacted to obtain or verify the data, and no automation tools were utilized throughout the process.

Data Items – Data were sought for four outcomes: comfort, symptom management/burden, QOC, and SWC. These outcomes were examined individually or in combination. All results relevant to each outcome domain in each study were extracted.

Additionally, data were gathered for other variables, including: article characteristics (e.g., authors, country of origin, year of publication); study design; objectives; population; setting; outcomes; intervention; comparator or control; relevant results; and observations (any additional pertinent information identified by the reviewers).

Study Risk of Bias Assessment – The risk of bias assessment was conducted using the RoB 2 Cochrane tool for RCT,[27] and the ROBINS-I Cochrane tool for non-RCT.[28] Each study underwent an independent assessment by two reviewers, followed by subsequent discussion and consensus between them. No automation tools were employed in the assessment process.

Effect Measures – For each outcome, we accepted the effect measures as declared by the authors of each study. These measures were utilized both in the synthesis and in the presentation of the results.

Synthesis Methods – A meta-analysis was not conducted due to the limited number of studies and the lack of homogeneity in the subjects, interventions, and outcomes. Therefore, the evidence was presented in a narrative format.

Tables 1, 2, and 3 summarize the study characteristics, PALC interventions, and key results, respectively.

RESULTS

Study Selection – A total of 529 articles were identified through searches in the PubMed, Web of Science, and Scopus databases. Additionally, seven more articles were found through the references of the initially retrieved articles, bringing the total to 536 references. After removing duplicates, 477 unique publications remained for eligibility assessment. During the title screening, 435 articles were excluded, and an additional 22 were excluded during abstract screening. The full texts of the remaining 20 articles were thoroughly reviewed, leading to the exclusion of 15 additional articles. Specifically, four were excluded due to the absence of any relevant outcomes, five for not focusing on a relevant or specific population, two due to limited access to the study protocol, two for not being relevant interventions for the review, and two for having a different study design.

Five articles were deemed eligible for review and qualitative synthesis. The flow diagram of the search process can be found in Figure 1.

Study Characteristics – Four randomized studies,[29-32] and one uncontrolled before-after trial33 were considered eligible for inclusion in this review. Two studies were conducted in Australia,[29,31] and the remaining in European countries: one in Italy,[33] one in Belgium,[30] and one across seven countries: Belgium, England, Finland, Italy, the Netherlands, Poland, and Switzerland.[32] The total number of patients across the

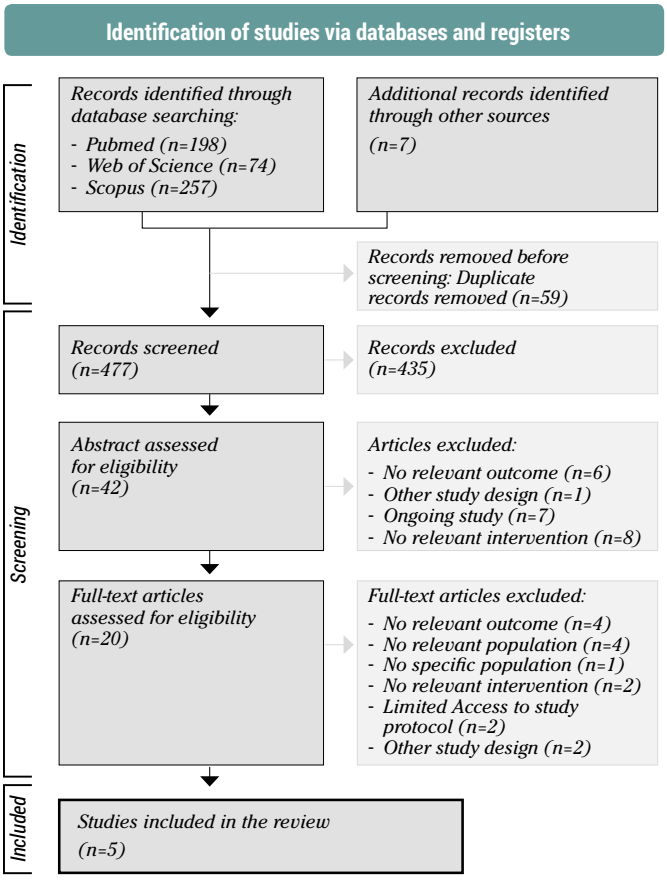

Fig 1. Flow diagram of the study selection process.

TABLE 1. Characteristics of individual studies (n=5)

Study; Country; Year	Type of Study	Participants (Staff and/or Family members) & Setting	Characteristics of the patients	Intervention & Control groups	Main Outcomes	Comments
AGAR et al.; Australia; 2017 [29]	Parallel cluster randomized controlled trial	**Participants:** Nurses and family members of deceased patients with advanced dementia from 20 nursing homes in two major cities in Australia.	**Intervention group** (n=67): Mean age 84.7±7.9 years; 61.0% female. Length of stay in nursing home – 29.0 months. FAST: level 7 (72.0%), level 6 (28.0%). **Control group** (n=64): Mean age 85.8±8.2 years; 58.0% female. Length of stay in nursing home – 20.5 months. FAST: level 7 (80.0%), level 6 (20.0%).	**Intervention:** Facilitated Family Case Conferencing. **Control:** Usual care.	**Primary outcomes:** CAD-EOLD in the last 7 days of life, family-rated; SM-EOLD in the last 90 days of life, family-rated; SWC-EOLD during the last 90 days of life, family/caregiver-rated. **Secondary outcomes:** Nurse-rated CAD-EOLD; nurse-rated SM-EOLD; nurse-rated QUALID monthly; Symptoms and care in the last month of life pharmacological/non-pharmacological strategies, symptom assessment frequency, acute care episodes, and potentially inappropriate non-palliative interventions.	- The study lasted for 18 months. - It was reported a lower than estimated mortality rate, causing the study's primary endpoint on EOLC quality to be underpowered. - Higher reporting by nurses of pain and other symptoms in the intervention arm suggests that case conferences foster greater proactivity and awareness in symptom identification.
BEERNAERT et al.; Belgium; 2017 [30]	Multicentre cluster randomized control trial	**Participants:** nurses and family carers of deceased elderly patients in acute geriatric wards of ten hospitals in the Flemish region, Belgium. **Intervention group** (n=164): 80% nurses; 29% family carers: 76.6% son/daughter, 8.5% partner, 2.1% brother/sister. **Control group** (n=118): 92% nurses; 19% family carers: 65.2% son/daughter, 21.7% partner, 4.3% brother/sister.	**Intervention group:** Mean age 85.8±6.8 years; 53.0% male. Length of hospital stay 28.0 days; Cause of death: 27.0% pneumonia, 19.0% other infections, 13.0% cancer. **Control group:** Mean age 84.0±7.5 years; 56.0% male. Length of hospital stay 23.3 days. Cause of death: 20.0% pneumonia, 13.0% other infections, 21.0% cancer	**Intervention:** The Care Programme of the Last Days of Life – CAREFuL. **Control:** Usual care.	**Primary outcomes:** CAD-EOLC in the last 48h of life and SM-EOLD, nurse- and family-rated. **Secondary outcomes:** Symptoms and Care Needs in the last three days of life (PCOS); SWC-EOLD in the last 48 hours of life, family-rated; nurses-reported symptomatic burden in the last 48 hours of life (self-developed items) **Additional secondary outcomes:** medical and nursing interventions in the last 48 hours of life, medication use, communication between clinical staff and patients/relatives and among clinical staff, and level of grief of the relatives.	- This study included elderly patients who were hospitalized for more than 48 hours and died in the ward. - Due to the low participation of family caregivers, the negative effect of the intervention on satisfaction with care is difficult to interpret. - The standardization of care, resulting from the implementation of the care guide, may have occurred, with some nurses believing that distributing leaflets to patients' families would be sufficient, neglecting communication in response to their questions and needs.
LIU et al.; Australia; 2020 [31]	Prospective stepped wedge randomized control trial	**Participants:** staff members of 12 care homes for elderly individuals in Canberra, Australia. **Staff self-reported capability assessment:** 161 responses post-intervention; 84 responses pre-intervention.	**Intervention group** (n=263): Mean age 86.0±8.6 years; 33% male. Primary diagnosis: 31% dementia, 17% cardiovascular disease, 7% frailed aged, 22% other (hypertension, anxiety, depression, schizophrenia, macular degeneration and blindness). **Control group** (n=208): Mean age 88.0±8.2 years; 42% male. Primary diagnosis: 34% dementia, 13% cardiovascular disease, 10% frailed aged, 26% other (hypertension, anxiety, depression, schizophrenia, macular degeneration and blindness).	**Intervention:** Palliative Care Needs of Rounds. **Control:** Usual care.	"Quality of Death and Dying", based on the domains of symptom control, preparation, connectedness, and transcendence; staff self-reported capability and confidence in caring for people at the end of life (CAPA); completion of advance care plans and appointment of medical power of attorney.	- All facilities crossed over bi-monthly from the control group to the intervention group in clusters of 2 or 3, with monthly follow-up on all sites. - The investigation concluded six months following the implementation of the intervention at the last sites.

TABLE 1. *(continue)*

Study; Country; Year	Type of Study	Participants (Staff and/or Family members) & Setting	Characteristics of the patients	Intervention & Control groups	Main Outcomes	Comments
VAN DEN BLOCK et al.; Belgium, England, Finland, Italy, the Netherlands, Poland, and Switzerland; 2020 [32]	Multi facility cluster - randomized clinical trial	**Participants:** nursing staff and family members of deceased elderly patients in 78 nursing homes from seven European countries. **Intervention group** (staff) (n=1159): mean age 44.1±11.7 years; 85.5% female. Staff: nurses 51.9%, care assistants 48.1%. Experience working in direct resident care 14.9±10.7 years. **Control group** (staff) (n=1278): mean age 42.3±12.1 years; 89.0% female. Staff: nurses 49.8%, care assistants 50.2%. Experience working in direct resident care 14.9±11.0 years. Relatives responses: 221 in intervention group; 273 in control group. Staff responses: 425 in intervention group; 558 in control group.	**Intervention group** (n=425): Mean age 85.9±8.6 years; 64.0% female. Functional status 1-month before death: 18.75±5.14 **Control group** (n=558): Mean age 85.6±8.8 years; 64.7% female. Functional status 1-month before death: 18.9±4.9	**Intervention:** The Pace Steps to Success Program. **Control:** Usual care.	**Primary outcomes:** CAD-EOLD during the last week of life, staff-rated; staff knowledge of Palliative Care (Knowledge Construct of the Palliative Care Survey). **Secondary outcomes:** Staff-reported quality of EOLC during the last month of life (QOD-LTC); Staff's self-efficacy in end-of-life communication with residents and their families (Self-Efficacy in EOLC Survey); Staff's self-perceived educational requirements concerning communication, cultural, and ethical values (End-of-Life Professional Caregiver Survey); Staff's opinions on palliative care (Rotterdam Move2PC). **Other secondary outcomes:** CAD-EOLD in the last week of life, family-rated; SWC-EOLD, family-rated; Physician-family communication (FPPFC), family-rated.	Randomization was performed by a median number of beds independently for each country in a ratio of 1:1.
COSTANTINI et al.; Italy; 2014 [33]	Uncontrolled before-after intervention cluster trial	**Participants:** Family members of patients who died of cancer in three general medicine wards and one respiratory diseases ward at the Villa Scassi Hospital in Genoa, Italy. **Intervention group** (n=33): face to face interviews 63.6%; telephone interviews 36.4%; mean interval death-interview 130.0±33.0 days. **Control group** (n=46): face to face interviews 93.5%; telephone interviews 6.5%; mean interval death-interview 145.7±22.0 days.	**Intervention group** (n=33): Mean age 73.0±9.8 years; 54.5% male. Length of hospital stay (median) 14.0 days. Primary tumor (system): respiratory 48.6%, digestive 12.1%, genitourinary 12.1%. **Control group** (n=46): Mean age 75.3±9.1 years; 65.2% male. Length of hospital stay (median) 10.0 days. Primary tumor (system): respiratory 45.7%, digestive 26.1%, genitourinary 13.0%.	**Intervention:** The Liverpool Care Pathway (Italian version). **Control:** Usual care.	Quality of EOLC in the last week of life (Toolkit After-Death Family Interview) which involves: Shared decision-making among patients, family, and medical team; Respect, dignity, and kindness; Symptom control (pain, dyspnea, and nausea-vomiting); Emotional support for the family and self-efficacy of the family; Spiritual/religious support; Care coordination.	- Experimental phase- 6 months. Subsequently, patients who died of cancer 4 months before/after the intervention were assigned to the control/intervention group, respectively. - Quality of EOLC was measured using the 'Toolkit After-Death Family Interview', conducted with the closest family member two months after the patient's death, preferably in person or by telephone if necessary.

LEGEND – **CAD-EOLD:** Comfort Assessment in Dying – End-of-Life in Dementia scale; **CAPA:** Capacity to Adopt a Palliative Approach tool; EOLC: End-Of-Life Care; **FAST:** Functional Assessment Staging Tool; **FPPFC:** Family Perception of Physician-Family Communication; PCOS: Palliative Care Outcome Scale; QOD-LTC: Quality of Dying in Long Term Care; **QUALID:** Quality of life in Late-Stage Dementia scale; **SM-EOLD:** Symptom management- End-of-Life in Dementia scale; **SWC-EOLD:** Satisfaction with Care-End-of-Life in Dementia scale.

TABLE 2. Palliative Care Interventions Used in the Included Studies (n=5)

Study; Country; Year of publication	Palliative care interventions
AGAR et al.; Australia; 2017 [29]	"Facilitated Family Case Conferencing" – involved meetings with family members, nursing home staff, and external health professionals to discuss current and future care plans and treatment strategies, and to share information about each patient's preferences and needs.
BEERNAERT et al.; Belgium; 2017 [30]	"Care Programme for the Last Days of Life" (CAREFuL) – provided care guidelines and tools for the last days of life, as well as support documents such as leaflets for the patient's family about the dying phase and grieving.
LIU et al.; Australia; 2020 [31]	"Palliative Care Needs Rounds" – involved monthly meetings between a PALC specialist and healthcare professionals at care homes to discuss care strategies and management for residents with higher symptom burdens and risk of dying.
VAN DEN BLOCK et al.; Belgium, England, Finland, Italy, the Netherlands, Poland, and Switzerland; 2020 [32]	"Palliative Care for Older People Steps to Success Program" – included six steps: advance care planning discussions with residents and families, regular assessments, multidisciplinary palliative review meetings, pain and depression management, and family support after death.
COSTANTINI et al.; Italy; 2014 [33]	"Liverpool Care Pathway" – a structured 10-step program designed to enhance the QOC across all relevant dimensions at the end of life, including symptom control, comfort, psychological-insight measures, religious-spiritual support, and communication with the patient, family, and care team.

TABLE 3. Results of individual studies (n=5)

Study; Year	Statistically significant differences favoring the Intervention group	No statistically significant differences between Intervention and Control groups
AGAR et al.; 2017 [29]	• MORE DOCUMENTATION in the intervention arm of **pain/discomfort** (p=0.04), **restlessness** (p=0.02), **constipation** (p=0.002), **skin tears** (p=0.005), and **other symptoms** (p<0.001). • MORE CHANGES in the intervention arm of both **pharmacological** (p<0.01) and **non-pharmacological** (p<0.05) strategies during the last month of life. • MEDICATION INITIATIONS in the intervention arm were more frequently **symptom-oriented** than diagnosis-oriented (83% vs 9%).	• **Comfort during the last 7 days of life;** family-rated & nurse-rated (CAD-EOLD; p>0.05). • **Symptom management in the last 90 days of life;** family-rated & nurse-rated (SM-EOLD; p>0.05). • **Satisfaction with care during the last 90 days of life;** family-rated (SWC-EOLD; p>0.05).• DOCUMENTATION of **breathlessness, coughing, difficulty swallowing/eating/drinking, choking/gurgling, vomiting, fear or anxiety, diarrhea and depression** (all p>0.05).
BEERNAERT et al.; 2017 [30]	• **Comfort in the last 48h of life;** nurse-rated (CAD-EOL; BAMD=4.30, 95%CI 2.07–6.53, p<0.0001, ICC=0.025, ES=0.78). • SYMPTOMS RELATED TO COMFORT; nurse-rated (CAD-EOL): **discomfort** (BAMD=0.57, 95%CI 0.32–0.82, p<0.001, ICC=0.023, ES=0.85); **pain** (BAMD=0.29, 95%CI 0.04–0.54, p=0.02, ICC<0.001, ES=0.42); **restlessness** (BAMD=0.45, 95%CI 0.19–0.72, p=0.001, ICC=0.017, ES=0.64); **shortness of breath** (BAMD=0.31, 95%CI 0.02–0.60, p=0.04, ICC=0.05, ES=0.40); **choking** (BAMD=0.28, 95%CI 0.03–0.53, p=0.03, ICC=0.010, ES=0.43); **difficulty swallowing** (BAMD=0.39, 95%CI 0.09–0.68, p=0.01, ICC=0.011, ES=0.50); **fear** (BAMD=0.34, 95%CI 0.07–0.60, p=0.01, ICC=0.040, ES=0.47); **serenity** (reversed) (BAMD=0.3, 95%CI 0.05–0.56, p=0.02, ICC=0.037, ES=0.46); **peace** (reversed) (BAMD=0.29, 95%CI 0.04–0.54, p=0.02, ICC=0.037, ES=0.45); **calm** (reversed) (BAMD=0.28, 95%CI 0.05–0.52, p=0.02, ICC=0.036, ES=0.46). • **Symptoms and care needs in the last 3 days of life,** nurse-rated (PCOS, BAMD=-2.62, 95%CI -4.96–0.71, p=0.009, ICC<0.0001, ES=-0.51). • **Symptom burden during the last 48h of life** (self-developed items assessed by nurses): **bothersome mucus** (BAMD=0.27, 95%CI 0.00–0.54, p=0.047, ICC<0.0001, ES=0.38), **vomiting** (BAMD=0.23, 95%CI 0.05–0.41, p=0.014, ICC=0.016, ES=0.47).	• **Comfort in the last 48h of life,** family-rated (CAD-EOL; BAMD=-0.62, 95%CI -6.07–4.82, p=0.82, ICC<0.0001, ES=-0.10). • **Symptom management in the last 48h of life,** both nurse-rated (SM-EOLD, BAMD=-0.41, 95%CI -1.86–1.05, p=0.58, ICC=0.037, ES=-0.12) and family-rated (SM-EOLD, BAMD=-0.59, 95%CI -3.75–2.57, p=0.71, ICC=0.078, ES=-0.17). • SYMPTOMS RELATED TO COMFORT; nurse-rated (CAD-EOL): **gurgling** (BAMD=0.17, 95% CI -0.11–0.44, p=0.24, ICC=0.002, ES=0.22); **anxiety** (BAMD=0.22, 95%CI -0.04–0.49, p=0.10, ICC=0.015, ES=0.32); **crying** (BAMD=0.09, 95%CI -0.05–0.23, p=0.19, ICC<0.001, ES=0.24); **moaning** (BAMD=0.11, 95%CI -0.13–0.35, p=0.37, ICC=0.003, ES=0.17). • **Satisfaction with care in the last 48h of life,** family-rated (SWC-EOLD; BAMD=-4.00, 95%CI -7.87– -0.12, p=0.04, ICC<0.0001, ES=-0.74). • **Symptom burden in the last 48h of life** (self-developed items assessed by nurses): **nausea** (BAMD=0.15, 95%CI -0.03–0.35, p=0.11, ICC=0.026, ES=0.31), **reduced appetite** (BAMD=0.04, 95%CI -0.27–0.35, p=0.81, ICC=0.080, ES=0.05), **fatigue** (BAMD=0.19, 95%CI -0.09–0.48, p=0.19, ICC=0.003, ES=0.25)
LIU et al.; 2020 [31]	• **Quality of Death and Dying** (treatment effect=8.07, 95%CI 3.8–12.4, p<0.01).• **Staff capability in looking after people at end of life** (CAPA; difference of average scores between groups= 4.7, 95% CI 2.7–6.7, p<0.01)	N/A
VAN DEN BLOCK et al.; 2020 [32]	• **Quality of care in the last month of life,** staff-rated (QOD-LTC; BAMD=3.40, 95%CI 2.01–4.80, p<0.001, ICC=0.05).• **Staff knowledge of palliative care** (Palliative Care Survey; BAMD=0.02, 95%CI 0.001–0.03, p=0.03, ICC=0.02).	• **Comfort in the last week of life,** both staff-rated (CAD-EOLD; BAMD=-0.55, 95%CI -1.71–0.61, p=0.35, ICC=0.08) and family-rated (CAD-EOLD; BAMD=0.91, 95%CI -1.03–2.85, p=0.36). • **Perception of the quality of end-of-life care,** family-rated (SWC-EOLD; BAMD=1.72, 95%CI -0.15–3.59, p=0.07). • **Family perception of physician-family communication** (BAMD=-0.02, 95%CI -0.29–0.25, p=0.90).
COSTANTINI et al.; 2014 [33]	• **Respect, dignity and kindness in the last week of life** (TADS; MDBG=16.8, 95%CI 3.6–30.0, p=0.015, ES=0.53). • **Family emotional support in the last week of life** (TADS; MDBG=20.9, 95%CI 9.6–32.3, p<0.001, ES=0.77).	• **Overall control of pain in the last week of life** (VOICES; OR=1.4, 95%CI 0.5–4.0, p=0.514). • **Overall control of breathlessness in the last week of life** (VOICES; OR=1.5, 95%CI 0.6–4.2, p=0.408). • **Overall control of nausea/vomiting in the last week of life** (VOICES; OR=2.3, 95%CI 0.6–9.5, p=0.261).

LEGEND – **BAMD:** baseline-adjusted mean difference; **CAD-EOLD:** Comfort Assessment in Dying – End-of-Life in Dementia scale; **CAPA:** Capacity to Adopt a Palliative Approach tool; **CI:** Confidence Interval; **ES:** Effect size; **ICC:** unconditional Intraclass Correlation coefficient; **MDBG:** mean difference between groups; **N/A:** not available/not found; **OR:** odds ratio; **PCOS:** Palliative Care Outcome Scale; **PCS:** Palliative Care Survey; **QOD-LTC:** Quality of Dying in Long Term Care; **SM-EOLD:** Symptom management- End-of-Life in Dementia scale; **SWC-EOLD:** Satisfaction with Care-End-of-Life in Dementia scale; **TADS:** Toolkit After-Death scales; **VOICES:** Views Of Informal Carers - Evaluation of Services.

included studies was 1905, comprising 146 with cancer, 294 with advanced dementia, and the remainder were elderly patients in hospital acute wards or nursing homes. Three studies reported that patients died in nursing homes,[29,31,32] while two studies indicated that deaths occurred in hospital wards.[30,33]

The key characteristics of the studies and the PALC interventions in each study are summarized in Table 1 and Table 2, respectively.

Risk of Bias in Studies – Concerning the randomized reviewed articles, the overall risk of bias in all of them is assessed as having some concerns. As shown in Figure 2, there is a low risk of bias in the randomization process domain in all studies since appropriate methods, such as computerized random sequence generators, were used to generate random allocation sequences. Additionally, measures were taken to ensure that researchers involved in the investigations were unaware of the allocations of the various randomization units. Furthermore, all studies demonstrate a low risk of bias in the selection of the reported results and deviations from the intended intervention domains.

However, all studies present some concerns in the measurement of outcomes domain. Despite patients and their families being blinded to the outcomes and aims of the research, it was not possible to ensure the blinding of the staff and healthcare professionals who provided care to the patients and subsequently assessed the outcomes through surveys. Their training and expertise in implementing intervention strategies were necessary for the study.

Regarding Agar et al., in addition to concerns about the measurement of outcomes, this study also presents issues related to missing outcome data.[29] Due to a lower-than-expected participant mortality rate, the study was underpowered, which introduced some risk of bias. Furthermore, the study does not elaborate on the techniques used to manage missing data, raising concerns about its impact on the results. Additionally, Beernaert et al. revealed a lower response rate in the intervention group compared to the control group, and an appropriate statistical method for handling missing outcome data was not used, resulting in a risk of bias in the missing outcome data domain.[30]

Recall bias of unknown direction should also be considered in all studies, as the outcomes were assessed after the events occurred, potentially influencing respondents' responses and, consequently, the results. Despite this, due to the nature of the intervention and the necessity of data collection after patients died in each arm, mitigating this risk was not feasible.

Finally, in a nonrandomized study by Constantini et al., the overall risk was deemed serious (Figure 3) due to serious risks of bias in the Selection of Participants and Measurement of Outcomes domains.[33] In this before-after study, differences in the characteristics of participants in both groups and the modality of data acquisition were observed. Some risk was also identified regarding the distinct distribution of researchers who assessed the data in both groups,[34] as well as a Hawthorne Effect given that patients' families were aware of the aims of the study.

Due to the scarcity of studies on this topic, and despite the high risk of bias identified in the appraisal, we decided to include the study by Constantini et al. in our systematic review.[33]

The rationale for the risk of bias assessment is detailed for all articles in the "Supplementary File 1".

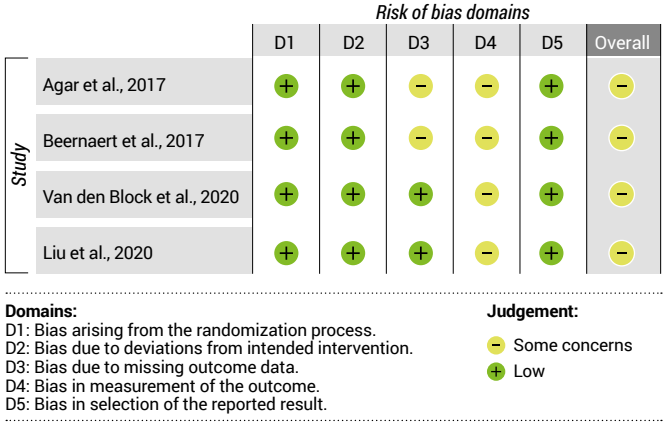

Fig 2. Risk of bias summary for randomized studies (n=4).

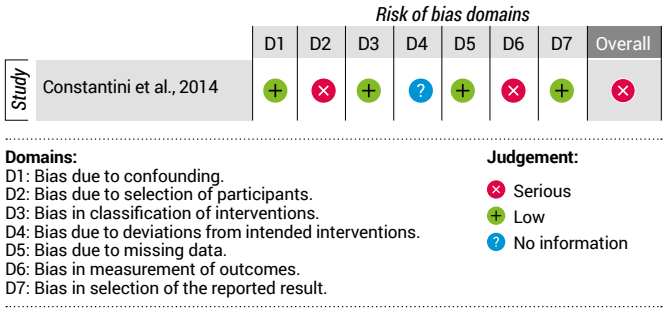

Fig 3. Risk of bias in non-randomized studies (n=1).

SUPPLEMENTARY FILE 1 Rationale for Risk of Bias Assessment

Assessment of Risk of Bias in **AGAR** et al., 2017[29]

Domain	Support for Judgment	Review authors' judgment
Randomization process	In this study, randomization was performed in blocks using a computer-generated allocation sequence. Although blocked randomization can pose a risk of bias regarding allocation concealment, there is no evidence that it was possible to predict future assignments based on previous assignments. Thus, the allocation was adequately concealed and was implemented at the nursing home level after the collection of baseline data, without baseline imbalances that are incompatible with chance.	**Low risk**
Deviations from intended intervention	Although the staff, residents, and their families in each of the nursing homes were blinded to the study's objective, researchers, project managers, and nursing home managers could not be blinded. Additionally, healthcare professionals in the institutions allocated to the intervention arm were aware of differences in care delivery implementation. There were deviations from the intended interventions, but since researchers implemented analyses that excluded nursing homes that did not implement the intervention to any planned degree, these deviations were not likely to have affected the outcome.	**Low risk**
Missing outcome data	In this domain, it is important to consider that, due to the observed mortality rate among study participants being lower than expected, " (…) the study's primary endpoint of quality of end-of-life (EOL) care was underpowered and did not show evidence of effect." Furthermore, the study does not elaborate on the techniques used to manage missing data, which raises concerns about the impact of this missing data on the results. However, it is unlikely that the missing outcome data depended on its true value.	**Some concerns**
Measurement of the outcomes	The outcome assessments in the study were conducted by both staff of the nursing home, who provided nurse-rated assessments, and research staff who collected both nurse-rated and family-rated outcome measures through face-to-face or telephone interviews. The authors of the study emphasize that the research staff were blinded regarding the aim of the study and collected data from only one of the study arms in an attempt to minimize the risk of bias. However, as mentioned in the article, "(...) those in the nursing homes in the intervention arm were aware of the introduction of the PCP role and changes in case conferencing and staff education and so may have been more inclined to report favorably on the quality of palliative care offered as a result". However, it is not likely that knowledge of the intervention status influenced the outcome assessment.	**Some concerns**
Selection of the reported results	The study protocol is made available, and all predefined outcomes are analyzed and reported in the results section, along with the respective scales and analytical methodologies used, in accordance with the predefined plan. The results are unlikely to have been selected based on the outcomes.	**Low risk**
Overall risk of bias	The study raises some concerns in several domains, including deviations from intended interventions, missing outcome data, and measurement of the outcome. Although the intention-to-treat analysis was conducted, it did not adequately manage the missing outcome data as no detailed imputation methods were used. Additionally, the potential for performance and detection bias due to lack of blinding and variability in outcome measurement timing introduces further concerns. However, the study does not exhibit a high risk of bias in any single domain.	**Some concerns**

Assessment of Risk of Bias in **BEERNAERT** et al., 2017[30]

Domain	Support for Judgment	Review authors' judgment
Randomization process	"A statistician outside the research group allocated the hospitals to the CAREFuL or control group using a random number generator", and the researchers considered the number of beds and the proportion of patients who had given consent during the baseline period in the stratification of each hospital. Thus, the allocation sequence was random and adequately concealed, and there is no evidence that baseline differences between groups suggest a problem with the randomization process.	**Low risk**
Deviations from intended intervention	Although patients and their families were unaware of the arm to which they had been allocated, due to the nature of the intervention, hospital staff could not be masked regarding the allocation. However, there is no evidence that deviations from the intended intervention arose because of the trial context. In fact, as the authors of the study point out, "The fidelity measures done during the study showed that CAREFuL was implemented according to the protocol in most of the wards." On the other hand, intention-to-treat analysis was used to estimate the effect of assignment to intervention, which is considered appropriate.	**Low risk**
Missing outcome data	A lower response rate was observed among nurses in the intervention group compared to the control group, while among family members, the intervention group had a higher response rate compared to the control group and an appropriate statistical method for handling missing outcome data was not used ("All results in the main text were analyzed without a technique for missing data"). However, it is unlikely that the missing outcome data depended on its true value.	**Some concerns**
Measurement of the outcomes	Primary outcomes were assessed by nurses and family members using validated scales and as previously mentioned, nurses were not blinded to the intervention, which could have influenced the assessment of the outcomes. However, it is not likely that knowledge of the intervention status influenced the outcome assessment, which is a condition for evaluating the risk of bias as high risk. Therefore, this domain presents some concerns regarding the risk of bias.	**Some concerns**
Selection of the reported results	The study protocol is made available, and all predefined outcomes are analyzed and reported in the results section, along with the respective scales and analytical methodologies used, in accordance with the predefined plan. The results are unlikely to have been selected based on the outcomes.	**Low risk**
Overall risk of bias	The trial is judged to raise some concerns in at least one domain for this result, but not to be at high risk of bias for any domain.	**Some concerns**

Assessment of Risk of Bias in **LIU** et al., 2020 [31]

Domain	Support for Judgment	Review authors' judgment
Randomization process	Randomization was conducted by an independent researcher at the institutional level, not at the individual level, to avoid contamination of staff exposure to the intervention. Simple randomization was performed using an internet program that randomly selected sites for each step. After this stage was completed, the various institutions were informed of the date of their transition from the control group to the intervention group under study. For those reasons, the allocation sequence wase random and concealed. Furthermore, any baseline differences observed between intervention groups appear to be compatible with chance.	**Low risk**
Deviations from intended intervention	Considering the intervention under study, blinding participants and care home staff at the sites involved in the study was not possible. The variability in intervention fidelity across care homes could be a potential bias. However, the authors monitored the level of adherence and fidelity of the institutions to the intervention and the previously mentioned protocol, and they analyzed the different outcomes taking this factor into account. Additionally, intention-to-treat analysis was used to estimate the effect of assignment to intervention, which is considered appropriate.	**Low risk**
Missing outcome data	There is insufficient information in the study regarding how missing data were addressed, as well as the reasons that led one of the institutions to withdraw from the study shortly after its initiation. However, it is not likely that the missing outcome data depended on its true value.	**Low risk**
Measurement of the outcomes	The method of measuring the outcomes was appropriate and did not differ between both phases. However, the assessment of the outcome could have been influenced by the fact that the staff was not blinded to the intervention, but there is no reason to believe that it did, which would be a condition for classifying this domain as having a high risk of bias.	**Some concerns**
Selection of the reported results	The study protocol is made available, and all predefined outcomes are analyzed and reported in the results section, along with the respective scales and analytical methodologies used, in accordance with the predefined plan. The results are unlikely to have been selected based on the outcomes.	**Low risk**
Overall risk of bias	The trial raises some concerns in the domain "measurement of the outcome". However, it is not judged to be at high risk of bias in any single domain.	**Some concerns**

Assessment of Risk of Bias in **VAN DEN BLOCK** et al., 2020 [32]

Domain	Support for Judgment	Review authors' judgment
Randomization process	The randomization process was conducted by independent individuals, stratified by country and number of beds, in a 1:1 ratio using a computer-generated random sequence. Furthermore, there is no information about baseline imbalances.	**Low risk**
Deviations from intended intervention	Owing to the nature of the study, blind treatment was not possible for participants or researchers. However, despite the authors admitting that the implementation of the intervention might have been suboptimal in some nursing homes, there is no evidence that deviations from the intended intervention arose because of the trial context. Intention-to-treat analysis was used to estimate the effect of assignment to intervention, which is considered appropriate.	**Low risk**
Missing outcome data	The article mentions nonresponse rates for some outcome measures, with discrepancies in response rates between the intervention and control groups, as well as varying timing of data collection, indicating incomplete outcome data. However, it is unlikely that the missing outcome data depended on its true value. All data were analyzed using intention-to-treat and complete-case analysis methods, reducing the risk of bias.	**Low risk**
Measurement of the outcomes	Staff members who were asked to complete questionnaires about resident outcomes were not blinded to the group allocation of their nursing home, and their awareness of the intervention status could potentially bias their responses. However, it is unlikely that knowledge of the intervention status influenced the outcome assessment, which is a condition for evaluating the risk of bias as high risk. Furthermore, the outcomes were measured using validated tools (like EOLD-CAD). Therefore, this domain presents some concerns regarding the risk of bias.	**Some concerns**
Selection of the reported results	The study protocol is made available, and all pre-defined outcomes are analyzed and reported in the results section, along with the respective scales and the analytical methodologies used. The results are unlikely to have been selected based on the outcomes.	**Low risk**
Overall risk of bias	The trial raises some concerns in the domain "measurement of the outcome". However, it is not judged to be at high risk of bias in any single domain.	**Some concerns**

SUPPLEMENTARY FILE 1 *(continue)*

Assessment of Risk of Bias in **CONSTANTINI** et al., 2014 [33]

Target randomized trial specific to the study

Design: *Cluster Randomized Trial*
Participants: *Clusters would include hospital wards participating in the pilot implementation of the Italian version of the Liverpool Care Pathway (LCP) program. Patients admitted to these wards who are identified as being in the last weeks of life will be included.*
Experimental intervention: *In the experimental arm, hospital wards will implement the Italian version of the Liverpool Care Pathway (LCP) program for end-of-life care. This intervention includes structured pathways, staff training, documentation procedures, and support from a specialized palliative care team.*
Comparator: *In the control arm, hospital wards will continue providing usual end-of-life care without implementing the LCP program.*
Preliminary considerations of confounders and co-interventions: *The review's authors did not identify confounders and co-interventions in this study.*

This study's effect of interest is the assessment of the effectiveness of starting and adhering to the interventions as specified in the protocol, as it seeks to understand how these interventions impact the quality of end-of-life care for cancer patients.

Domain	Support for Judgment	Review authors' judgment
	Pre-intervention	
Confounding	Outcomes are unlikely to be influenced by factors affecting treatment decisions, so the study can be considered at low risk of bias due to confounding, similar to a fully randomized trial.	**Low risk**
Selection of participants	The study notes differences in characteristics between the before and after groups, such as gender, time spent in hospital and ward, and the interview modality of families. On the other hand, ward staff and caregivers who witnessed improvements in the study outcomes in the after group may have shown greater availability and motivation to participate in the study, demonstrating the association between participant selection in the study and the intervention and outcomes. The study does not provide information on whether adjustments for this selection bias were made in the analyses.	**Serious risk**
	At intervention	
Classifications of interventions	The intervention status is well defined, as are the intervention groups. The timing of the intervention implementation provides a clear delineation between the before and after groups, indicating that the intervention status is based on the information collected at the time of the intervention implementation. For these reasons, the risk of bias in this domain is low.	**Low risk**
	Post-intervention	
Deviations from intended interventions	*In this study, it would be important to consider the fidelity of implementation and adherence to the protocol by healthcare professionals from different wards. However, information on whether there is deviation from the intended intervention and its influence on the outcomes is not provided.*	**No information**
Missing data	*In this study, 73% of the families from the "before intervention" group and 68.8% from the "after intervention" group were interviewed, with similar reasons for the inability to collect data in these cases. Moreover, it is significant to note that an intention-to-treat approach was used, helping to mitigate the impact of missing data and thereby reducing the risk of bias in this domain.*	**Low risk**
Measurement of outcomes	*In this domain, it is important to consider that the interviewed families were aware of the study's aims, which may have led to some degree of the "Hawthorne effect". Additionally, the fact that the all of the interviewers conducted data collection in both groups means they were not blinded to the allocation of the groups in the intervention. On the other hand, "the distribution of the interviewers is substantially different before and after for at least two interviewers" (Costantini et al., 2011), and it is important to note that some interviewers tend to measure systematically higher or lower values than the other interviewers, thereby increasing the risk of bias.* *Finally, there were differences in obtaining data through interviews with patients' family members regarding modality (face-to-face versus telephone), timing and duration, and the proportion of these differences varied between both groups.*	**Serious risk**
Selection of the reported results	The measurements and analyses of outcomes are consistent to a pre-established plan. There is no evidence suggesting the selection of specific analyses among multiple options, and there is no indication of selecting cohorts or subgroups for analysis based on the results.	**Low risk**
Overall RoB judgement		**Serious risk**

Results of Individual Studies – Table 3 presents the key results of each study included in this review.

Results of Synthesis

1) Symptom management/burden

Symptom management and burden were analyzed in three articles.

Agar et al.,[29] in a moderate risk-of-bias study, found no statistically significant differences between groups in symptom management during the last 90 days of life, as assessed by both nurses and families. However, they noted that medication initiations in the intervention arm were symptom-oriented rather than diagnosis-oriented ($p<0.05$), with more frequent pharmacological ($p<0.01$) and non-pharmacological ($p<0.05$) changes during the last month of life. Moreover, formal assessments of various symptoms were conducted more frequently in the intervention group. There were no statistically significant differences between groups in the assessment of several symptoms, including difficulty swallowing/eating/drinking, breathlessness, coughing, choking/gurgling, vomiting, fear or anxiety, diarrhea, and depression.[29]

In the study by Beernaert et al.,[30] which was assessed as having a moderate risk of bias, when analyzing separate items from the "Comfort Assessment in Dying – End-of-Life in Dementia" (CAD-EOLD), statistically significant improvements in favor of the intervention group were observed in several symptoms assessed by nurses, including discomfort, pain, restlessness, shortness of breath, choking, difficulty swallowing, and fear. However, no differences were found between groups in other symptoms of the CAD-EOLD, namely gurgling, anxiety, crying, or moaning. Regarding "Symptom Management", both nurse-assessed and family-assessed, there were no statistically significant differences between groups. There were statistically significant improvements in "Symptoms and Care Needs" in the intervention. Likewise, in the "Symptom Burden", in the intervention group, there were statistically significant improvements in troublesome mucus and vomiting, but no differences were found for nausea, reduced appetite, and fatigue between groups.[30]

Costantini et al.,[33] in a high-risk-of-bias study, demonstrated statistically significant improvements favoring the intervention group during the last week of life in family spiritual support and self-efficacy. No significant differences were found between groups in overall pain, dyspnea, nausea, and vomiting control.[33]

2) Comfort around dying

Comfort around dying was evaluated in three studies.

Beernaert et al.[30] reported a statistically significant improvement in comfort during the last 48 hours of life in favor of the intervention group, as assessed by nurses. Analysis of individual items from the CAD-EOLD revealed statistically significant improvements favoring the intervention group, namely in peace, serenity, and calmness. However, no statistically significant differences were found in comfort as rated by family members.[30]

Agar et al.[29] found no statistically significant differences in comfort ratings by both nurses and family members during the last seven days of life. They also observed that higher levels of acute intercurrent comorbidities were associated with lower comfort assessments by staff during the dying process.[29] Similarly, in the study by Van den Block et al.,[32] which had a moderate risk of bias, no statistically significant differences in comfort were found between groups in the last week of life, as rated by both staff and family members.

3) Quality of Care

QOC was assessed in three studies.

Van den Block et al.[32] showed statistically significant differences favoring the intervention group in the QOC in the last month of life, as assessed by staff members and from families' perceptions. Similarly, Liu et al.,[31] in a moderate risk-of-bias study, found a statistically significant improvement in quality of EOLC, demonstrating that professionals in the care homes felt more capable and confident during the implementation phase of the PALC intervention. Conversely, Costantini et al. found no statistically significant differences between groups in the QOC in the last week of life.[33]

4) Satisfaction with care

SWC was assessed in three studies.

Beernaert et al.,[30] and Van den Block et al.[32] found a statistically significant difference in family SWC, favoring the control group in the former and the intervention group in the latter study. Conversely, Agar et al.[29] found no significant differences between groups in family-assessed SWC during the last days of life. Additionally, Agar et al. observed that the more patients faced acute intercurrent comorbidities, the lower the family-rated SWC.[29]

DISCUSSION

Summary of Evidence – This systematic review encompasses five articles (n=1905 patients) investigating PALC interventions targeting improvements in EOLC within hospital wards and nursing homes. The review included four randomized studies and one uncontrolled before-after trial, with three studies conducted in Europe and two in Australia. While some studies showed improved symptom management, particularly for discomfort and anxiety, others found no significant differences between groups. Variability was noted in comfort around dying, with improvements reported by healthcare professionals but inconsistent support from family assessments. QOC outcomes varied, with some studies indicating improvements while others did not. SWC outcomes were heterogeneous, influenced by acute comorbidities.

Symptom management and burden – Evidence gathered from our review suggests mixed findings regarding the impact of PALC interventions on symptom management and burden across various settings, including cancer patients in hospital wards,[33] elderly patients with dementia in nursing homes,[29] and older patients with multiple conditions in acute care facilities.[30]

Some physical symptoms improved with PALC interventions, consistent with findings from Quinn et al.'s systematic review and meta-analysis of 28 randomized clinical trials involving 13664 patients (mean age 74 years) with chronic non-cancer illnesses.[35] This study showed that PALC interventions were statistically significantly associated with a modestly lower symptom burden compared to usual care.[35] Similarly, in a population-based study of 11242 patients who died from gastrointestinal cancers, Merchant et al.[36] found that while 50% experienced moderate-severe scores in tiredness, lack of well-being, and lack of appetite earlier (weeks 18 to 12 before death), and 50% experienced moderate-severe scores in drowsiness, pain, and shortness of breath later (weeks 5 to 2 before death), the initiation of outpatient PALC was associated with a 1- to 3-point decrease in subsequent scores, with the greatest reductions in pain [odds ratio (OR) -1.91, 95% confidence interval (CI) -2.11 to -1.70] and nausea (OR -3.01, 95% CI -3.31 to -2.71).[35] Conversely, a systematic review incorporating data from four studies involving 525 participants on pain management in hospital-based specialist PALC found no evidence of a difference compared to usual care.[37] Our systematic review findings did not consistently show improvements in the management of pain, dyspnea, and vomiting with PALC interventions compared to usual care.[30,33]

Kavalieratos et al.'s meta-analysis of 43 RCT revealed that PALC interventions were associated with statistically and clinically significant improvements in symptom burden at the 1- to 3-month follow-up,[38] although this association was not statistically significant in trials at low risk of bias (n=5).

Health-related suffering is serious when it cannot be relieved without professional intervention and when it compromises physical, social, spiritual, and/or emotional functioning.[39]

In a cross-sectional study involving 1549 terminally ill patients (mean age 77.4 years),[40] the top five distressing symptoms identified through Relative Importance Index analysis were poor mobility (64.4%), family anxiety (63.5%), difficulty sharing feelings with family/friends (61.4%), weakness/lack of energy (58.1%), and hardly feeling at peace (50.7%). Among patients with dementia, the most distressing symptom was poor mobility (67.8%), while cancer patients rated perceived family anxiety (66.1%) as the most distressing symptom.[40]

In a study with bereaved respondents (n=2796), the largest gaps in symptom management were found in settings such as home without hospice and acute care.[41] The adjusted marginal difference for unmet need for pain was 25.6 percentage points higher (95% CI 16.7 to 34.6) at home without hospice, while in acute care settings, the unmet need for dyspnea was 20.7 percentage points higher (95% CI 10.1 to 31.3), and the unmet need for emotional support was 20.5 percentage points higher (95% CI 11.5 to 29.5), compared to dying at home with hospice.[41]

Makaroun et al.[42] showed that bereaved respondents (n=1653) reported that decedents experiencing late transitions at the end-of-life were more likely to be treated without respect [21.3% vs. 15.6%; adjusted odds ratio (AOR) 1.59, 95% CI 1.09 to 2.33) and had more unmet needs for spiritual support (67.4% vs. 55.2%; AOR 1.48, 95% CI 1.03 to 2.13).[42]

Miyashita et al.,[43] in a study involving 885 bereaved relatives, found that during the last three months before death, symptom severity was moderate to overwhelming in over 30% of cases for all causes of death. The absence of a reliable key health professional was consistently associated with higher symptom burden (p=0.002) and more practical problems (p=0.001).[43]

Agar et al.[29] did not find the PALC intervention effective in improving symptom outcomes, but they did observe that nurses performed formal assessments of patients' pain more frequently during the implementation of the PALC strategy. These results are noteworthy, considering that all included studies suggest that healthcare professionals expressed greater confidence in pain control and felt more capable of providing EOLC after the PALC intervention. When interpreting these results, it is important to consider that the emphasis on symptom assessment and the necessity to fill out questionnaires may make staff more attentive and efficient at recognizing symptoms, potentially resulting in enhanced reporting and masking the potential effects of the PALC intervention. Additionally, life-threatening diseases in their terminal phase tend to present a greater symptomatic burden, which is inherently more difficult to control.

Comfort around dying – Evidence from our systematic review suggests mixed findings regarding the impact of PALC interventions on comfort around dying. Only one study indicated improvements with the intervention, as assessed by nurses, in elderly patients admitted to acute hospital settings.[30] Similarly, a study that introduced a bundle of care to enhance palliative and EOLC in an acute tertiary hospital demonstrated increased use of comfort care, improved recognition of dying patients, higher referral rates to PALC nurses and physicians, and a reduction in the number of medical emergency team calls.[44] Conversely, Miranda et al.[45] analyzed results from two retrospective epidemiological studies in Flanders and found that, between 2010 and 2015, there was a 15% increase in dementia prevalence ($p<0.01$) and an 11% decrease in cognitive impairment ($p=0.04$) among 381 nursing home residents with dementia. However, when controlling for residents' characteristics, there was no significant change in overall comfort, although a 20% increase in the use of pain assessment was verified in the last week of life ($p<0.03$).[45]

Although the study by Costantini et al.[33] did not focus specifically on comfort, there was a statistically significant difference in favor of the intervention group in terms of respect, dignity, and kindness. These factors could contribute to a "sense" of comfort around dying.

Tappen et al.,[46] in a qualitative study involving 16 nursing home residents, 10 family members, and 20 staff members, identified three main themes from the content analysis: promoting comfort; the centrality of comfort; and what matters most at the end of life. All participant groups overwhelmingly endorsed comfort as a priority. Some participants would accept aggressive treatment to alleviate suffering and promote comfort. Residents were concerned about the well-being of their families, whereas family members emphasized the importance of their presence and ensuring their dying relatives were not suffering. Staff sometimes filled this role on their behalf. Ancillary staff emphasized bathing, dressing, and grooming the residents to preserve their dignity.[46]

Given that comfort is a priority for EOLC, there is a need for more discussion to enhance and promote comfort for patients and families. In our systematic review, three studies found no statistically significant differences between PALC intervention and usual care in comfort, as rated by family members.[29,30,32] These data demonstrate that there is room for improvement, not only in optimizing patient comfort but also in addressing the concerns and doubts of family members to better serve them. The World Health Organization emphasizes the value of PALC as a holistic approach to care, recognizing both the patient and family members as the unit of care, and advocating for its early application in the course of the illness.[47]

The non-inclusion of the family in EOLC decision-making processes, limited communication from healthcare professionals regarding the inherent symptoms of the dying process, and the emotional and psychological stress and grief can often lead to anxiety among bereaved family members. This can exacerbate their perception that their loved one experienced discomfort and that their needs were unmet in the last moments of life.[48]

Quality of care – Findings from our systematic review suggest that PALC interventions enhance the QOC compared to usual care. Two studies with low to moderate bias demonstrated improvements in QOC as assessed by staff members,[31,32] and families' perceptions.[32]

The positive relation between PALC interventions and QOC was observed in several settings of care. In a study with 2796 bereaved family members or close friends examining the episode of care in the last month of life, hospice at home was associated with a higher rating of the QOC, with 60.2% (95% CI 40.2 to 69.0) stating the care was excellent.[41] In contrast, inpatient PALC services in hospital, hospice residence, or hospice inpatient unit settings received lower ratings.[41]

A national Danish survey assessing the quality of EOLC for cancer patients who received specialized PALC received responses from 787 bereaved spouses.[49] The study revealed that in the last three months of life, 83% of respondents rated the overall quality of all services as good, excellent, or outstanding, with a significant association with the place of death (p=0.0051), indicating that fewer respondents rated the care as "fair" or "poor" if the patient died at home. Additionally, 93% of respondents reported that the patient died at the right place, although only 74% of patients died at their preferred place.[49]

A qualitative study involving 24 caregivers (12 from PALC units and 12 from non-PALC units) identified two main themes related to QOC: perception of person-centered care and perception of the scientific and technical appropriateness of care.[50] The latter was further subdivided into diagnostic tests and treatment, and symptom control. Caregivers of patients in PALC units described their EOLC experiences positively, while those in non-PALC units reported negative experiences.[50]

Vandenbogaerde et al.[51] analyzed 208 questionnaires from bereaved relatives of nursing home residents with dementia regarding the quality of EOLC. The study found that the quality of EOLC was positively associated with relatives receiving information on PALC and medical care from care providers.[51]

In an observational study involving 329 bereaved caregivers of decedents with advanced cancer who received hospice care it was found that, controlling for covariates, better symptom control was independently associated with an improved overall quality of dying.[52] Likewise, in an international prospective cohort study involving PALC units across Japan, South Korea, and Taiwan, among 998 patients, improved symptom control, particularly for dyspnea and delirium was significantly associated with better QOC.[53]

In a study on perceptions of EOLC quality, bereaved respondents (n=1653) were less likely to rate the QOC as excellent when there was a late transition at the end-of-life (43.6% vs. 48.2%; AOR 0.79, 95% CI 0.58 to 1.06).[42] Subgroup analyses showed that transitions between a nursing home and hospital (13% of all late transitions) were associated with even worse QOC.[42]

In a Canadian study, 100 caregivers of patients who passed away in a residential hospice were interviewed four to six months after the patient's death to assess the quality of dying and death.[54] The study revealed an overall intermediate quality rating ("neither good nor bad"), with significantly higher ratings observed for patients who had a hospice length of stay exceeding one week compared to those with a shorter stay (p<0.001).[54] This could be explained by the importance of a strong professional-patient relationship in effective healthcare. As this relationship develops, patients feel more engaged, understood, and supported. Trust forms the foundation, enabling open communication, shared decision-making, and better health outcomes. The perception of person-centered care encompasses effective communication, emotional support, and facilitating the farewell process.[50]

Satisfaction with care – Evidence from our systematic review indicates mixed findings regarding the impact of PALC interventions on SWC. One study favored the PALC intervention,[32] another favored the control group,[30] and a third found no differences between groups.[29]

In a pooled analysis of mortality follow-back surveys (n=885), across all causes of death, 28%-38% of bereaved relatives reported some level of dissatisfaction with care during the last three months before death.[43] Patients with cardiovascular disease and dementia experienced lower symptom burden and dissatisfaction compared to those with cancer. Higher dissatisfaction with care was associated with the absence of a reliable key health professional (p=0.001).[43]

A longitudinal, descriptive correlational design study involving 101 caregivers of deceased patients with advanced cancer revealed, through multiple linear regression analyses, that caregiver SWC was negatively impacted by patient admissions to the intensive care unit and by having more than one hospitalization before death.[55] Similarly, our review indicated that Agar et al. observed a decrease in family-rated SWC as patients faced more acute intercurrent comorbidities.[29]

In a study involving 202 bereaved caregivers of cancer decedents who received hospice care in Uganda, it was found that family SWC was independently associated with better preparation for death.[52]

In a prospective pre- and post-intervention study utilizing a PALC pathway in a hospital setting, questionnaires were distributed to relatives of deceased cancer patients to assess perceptions of communication and satisfaction with EOLC.[56] The study found no significant overall effect of the PALC pathway on the communication process or SWC reported by bereaved relatives.[56] Findings from very low- to low-quality evidence sug-

gested that hospital-based specialist PALC, compared to usual care, may provide modest benefits for person-centered outcomes such as patient QOL, symptom burden, and patient SWC.[37]

In a prospective pre- and post-loss study involving 114 family caregivers of terminal cancer patients, SWC provided at the end of life was found to be associated with the quality of life of bereaved family caregivers six months post-loss.[57]

Findings from very low- to low-quality evidence suggested that hospital-based specialist PALC, compared to usual care, may provide modest benefits for person-centered outcomes such as patient QOL, symptom burden, and patient SWC.[37]

Limitations

This systematic review has several limitations. Firstly, the review was not registered with any specific registration platform. Additionally, we only consulted three databases for our review. Another limitation of this study is that the governance structures and care standards in nursing homes and hospital wards are not equivalent, which likely contributed to the heterogeneity of our findings.

The studies included in the review exhibit considerable heterogeneity in participant characteristics, methodologies, employed measures including assessment tools, follow-up and evaluation periods, types of analyses, and reported statistics. This variability impeded direct comparisons and affected the results. The components, format, and duration of the programs provided by the PALC intervention teams varied across studies. Additionally, the control or comparator groups, mostly designated as usual care, differed between studies according to local practices.

It is also important to emphasize the quality of the included publications, with all of them revealing a moderate to high overall risk of bias. This included recall bias and the Hawthorne effect due to the methodological characteristics of the studies. Considering the research question, other strategies were not feasible. The diversity of the studies restricted our capacity to conduct meaningful comparisons and meta-analyses.

These limitations may have influenced the results presented in this review.

CONCLUSIONS

This systematic review synthesizes findings from five studies involving 1905 patients to assess the impact of PALC interventions on EOLC within hospital wards and nursing homes.

Symptom management outcomes were mixed, with some studies showing no significant differences between PALC interventions and usual care, while others noted improvements in specific symptoms like discomfort and anxiety.

Similarly, comfort around dying showed variability, with improvements reported by nurses in one study but no consistent differences found by family members in others.

Regarding QOC, PALC interventions showed improvements in two studies, though one study found no significant differences.

SWC also varied, with some studies showing benefits from PALC interventions while others did not, especially in cases involving acute comorbidities.

In conclusion, while PALC interventions hold promise in enhancing aspects of EOLC, the evidence is mixed and highlights the need for further research. Addressing methodological limitations, standardizing intervention components, and ensuring comprehensive evaluation methods are crucial to better understand and optimize the impact of PALC on patient and family outcomes at the end of life.

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LIST OF ACRONYMS AND ABBREVIATIONS

CAD-EOLD – *Comfort Assessment in Dying - End-of-Life in Dementia*
CI – *Confidence Interval*
EOLC – *End-of-Life Care*
OR – *Odds Ratio*
PALC – *Palliative Care*
QOC – *Quality of Care*
RCT – *Randomized Controlled Trial(s)*
SWC – *Satisfaction With Care*

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Induced Pluripotent Stem Cell Lines as a Model for Studying the Cellular Phase of Parkinson's Disease

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ABSTRACT: Parkinson's disease (PD) is a complex neurodegenerative disorder, characterized by the progressive loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) as a result of intraneural deposition of aggregated alpha-synuclein (aSyn) in Lewy bodies (LB). aSyn is an intrinsically-disordered protein, encoded by the SNCA gene, and is implicated in both familial and sporadic forms of PD. However, we still do not fully understand if and how aSyn causes cell dysfunction and death. Therefore, it is essential to develop and explore robust models for bridging the gap between preclinical research and clinical applications, creating platforms for testing hypotheses and assessing potential interventions. The emergence of patient-derived induced pluripotent stem cells (iPSCs) offers unique opportunities for investigating the cellular phase of PD and related synucleinopathies by enabling the systematic assessment of phenotypes in various cell types of relevance for disease. Moreover, advances in PD-derived iPSC technology also hold promise for cell replacement therapy and drug discovery efforts using pharmacological or genetic screening approaches. In this review, we focus on the application of aSyn iPSC models in PD research, summarizing their anticipated merits, challenges and present-day implementations. .

KEY WORDS: Parkinson's disease; alpha-synuclein; induced pluripotent stem cells; midbrain dopaminergic neurons; disease modeling

I. INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder afflicting growing number of people worldwide due to the ageing of the human population. PD is a complex, multisystem disorder characterized both by motor and non-motor clinical features. The four cardinal motor abnormalities (bradykinesia, rigidity, resting tremor, and postural instability) have routinely defined PD clinical diagnosis. However, PD is also associated with non-motor symptoms (e.g. REM sleep behavior disorder, autonomic dysfunctions) that often precede motor symptoms and significantly contribute to overall disease morbidity[1–6]. The hallmark pathological features of PD are the progressive loss

and degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), and the intraneural accumulation of alpha-synuclein (aSyn), a small protein encoded by the SNCA gene, in the form of Lewy bodies (LB) and Lewy neurites[1,7,8]. While the exact physiological function of aSyn is still unclear, different studies suggest that it may act as a molecular chaperone[9], play a role in both Ca^{+2} and dopamine homeostasis[10,11], and play a role in synaptic vesicles trafficking[11–13]. Under physiological conditions, aSyn is a natively unfolded protein which, under pathological conditions, can aggregate and form insoluble intracellular inclusions causing neurodegeneration[14–16]. In general, ~5-10% of PD cases are classified as familial, while the vast majority of cases represent sporadic PD. Most forms of PD share similar clinical and pathological features, although differences have been noted. The SNCA gene is associated with both familial and sporadic forms as point mutations and multiplications of the gene cause familial PD forms, while single nucleotide polymorphisms have been identified in idiopathic PD forms as susceptibility factors[17].

Given the increased prevalence of PD and our inability to effectively prevent onset or to modify disease progression, we urgently need to decipher the complex etiology of the disease in order to enable the development of novel therapeutic strategies[12,18,19].

II.

From biology to pathology: aSyn as a marker and target for intervention

aSyn is a 140 amino acid protein whose primary sequence can be divided into three main domains: (i) an N-terminal domain[1–60], which includes a multi-repeated hexameric motif (KTKEGV) and has alpha-helical propensity enabling lipid membrane-binding; (ii) a central domain[61–95], which is highly hydrophobic and represents the most aggregation-prone part due to conformational changes; and (iii) a C-terminal domain[96–140], which is characterized by a non-defined structure and enriched in negative charged and proline residues that plays a role in preventing aSyn self-aggregation[20]. aSyn is an intrinsically-disordered protein with a conformational plasticity that can adopt different conformations depending on the environmental context[21]. Physiologically, two forms of aSyn seem to coexist in a dynamic balance: the intrinsically disordered cytosolic monomer and (in large part) a membrane-bound and aggregation-resistant, helically folded tetramer[22,23]. However, tetramer destabilization and imbalances in the ratio folded tetramer:unfolded monomer may result in accumulation of pro-aggregating forms, as in LB pathology, where aSyn adopts a β-sheet conformation that further recruits monomers to form oligomers and amyloid fibrils[12,24].

aSyn is predominantly located at presynaptic nerve terminals, although initial studies pointed out its presence within the nucleus[25]. The occurrence of aSyn in the nucleus has been a matter of debate and its roles in the nucleus are still underappreciated. However, accumulating evidence, including from our own work, confirms the presence of aSyn in the nucleus of neuronal cells in human brain tissue as well as in animal models[25–32]. Consensus has not been reached regarding the role of nuclear aSyn (aSynNuc). Some studies demonstrated a protective role against stress, in maintaining genomic integrity as well as in DNA repair processes[33–36]. Other studies suggest a putative function in nucleocytoplasmic transport via the interaction with Ras-related nuclear protein (RAN), which is impaired by aSyn mutations[37]. On the other hand, evidence from both in vitro and in vivo suggests a detrimental role of aSynNuc highlighting its contribution to the pathogenesis of PD and other synucleinopathies[30,38,39]. Moreover, accumulation of aSynNuc can induce significant transcriptional dysregulation and epigenetic modifications which are linked to gliosis, increased inflammation, oxidative stress and mitochondrial dysfunction, DNA damage and cell cycle disruption as well as altered ribosomal RNA processing, ultimately accelerating cell senescence and neurodegeneration[27,31,40–43].

aSyn has emerged both as a biomarker and therapeutic target based on its central role in PD pathogenesis[44,45]. Ongoing efforts using aSyn seed amplification assays (aSyn-SAAs) were recently reported to detect seed-competent aSyn species in the CSF and to distinguish, with high sensitivity and specificity, healthy controls from prodromal and non-manifesting carriers, and from sporadic PD patients[46–48]. Given such biomarker advances and the fact that aSyn pathology is the gold standard for establishing the ultimate diagnosis, two recent studies proposed a major shift from a clinical to a biological definition of PD using different levels of 'biological' information[49,50]. The SynNeurGe classification system and the neuronal aSyn disease integrated staging system (NSD-ISS) were the two initial attempts to propose research criteria that may prove instrumental for guiding future clinical trials[51,52]. Nevertheless, it

is now necessary to harmonize such classification systems, and to refine them so that, one day, they may be used in the clinical practice[53].

aSyn PTMs, including phosphorylation, nitration, acetylation, O-GlcNAcylation, glycation, SUMOylation, ubiquitination, and C-terminal cleavage, may act as modifiers of both aSyn biology as well as pathological processes. As such, there is growing interest in assessing their potential as biomarkers of disease[54].

A substantial body of evidence suggests that alteration/reduction of aSyn aggregation might constitute a promising avenue for therapeutic intervention in PD. As a result, a plethora of anti-aggregation compounds, encompassing both small molecular entities, antibodies, and other macromolecular modalities, have been investigated for their potential to mitigate aSyn aggregation and its associated neurotoxicity[55,56]. Moreover, passive immunization is one of the major therapeutic approaches that have been attempted recently to target aberrant aSyn. Two monoclonal antibodies, Prasinezumab[57,58] (PRX002) and Cinpanemab[59,60] (BIIB054) that target C-terminal and N-terminal of aSyn respectively, have successfully passed phase I clinical trials. However, the outcomes of the phase II trials were negative. Currently, further studies with Prasinezumab are ongoing, and there is hope that some of the strategies being currently tested may prove beneficial[12,61].

Numerous cellular and animal models[62], with own strengths and limitations, have been established for modeling various aspects of PD. However, due to our limited understanding of the molecular underpinnings of PD, and to inherent limitations of model systems which fail to recapitulate important features of PD, we still need to continue to develop alternative models. In this context, induced pluripotent stem cell (iPSC) models hold a great promise due to their potential to provide a far greater supply of disease-relevant cells[63,64].

III.

Developing aSyn iPSC models

The emergence of induced pluripotent stem cells (iPSCs) technology led to a scientific breakthrough in PD modeling, affording the possibility of establishing cellular models of neurons from live PD patients. IPSCs refer to pluripotent stem cells that can be generated by introducing the four transcription factors OCT4, Sox2, Klf4, and c-Myc (Yamanaka factors) into adult somatic cells[65,66]. The delivery of Yamanaka factors is performed using both viral and non-viral based key techniques. For instance, viral vectors such as Sendai virus (SeV), measles virus and RNA virus-based episomal vector (REVec) system represent host genome integration-free options, showing superior differentiation potential and enhanced safety and genetic modification versatility, respectively[67–71]. Additionally, non-viral based approaches including episomal vectors, self-replicating RNA "srRNA" and nanoparticles delivery are gaining attention recently due to their potential to avert viral integration associated risks e.g. insertional mutagenesis[72–79].

Once reprogrammed, iPSCs can be differentiated into any cell type (e.g. dopaminergic neurons "DANs"); while maintaining the patient's complete genomic background and the capability of self-renewal. After the first successful establishment of PD iPSC models[80], as well as the first differentiation of iPSCs into DANs[81], revolutionary advances occurred in PD models derived from iPSCs, holding a great promise as a valuable tool not only in PD disease modeling, but also cell replacement therapy, and drug discovery[82–87].

Given the valuable contribution and the promising application of iPSCs in PD research as well as the pivotal role of aSyn in PD pathology, this review will highlight the use of aSyn iPSC models in PD encapsulating their potential, limitations and up-to-date applications.

Applying advanced viral and non-viral based approaches, researchers have established various aSyn iPSC lines (derived from patients with sporadic PD, SNCA duplication or triplication, point mutations (e.g. A30P, A53T, E46K, G51D)[63,88–93] which have been utilized in revealing underpinning PD molecular and cellular mechanisms, potential leveraging therapeutics and the role of aSyn in disease progression. Interestingly, episomal vectors and mRNA-based non-viral approaches were employed to generate iPSCs from patients with sporadic PD and missense G51D mutation, respectively[72,94]. Furthermore, genome-editing techniques such as CRISPR/Cas9 and zinc-finger nucleases (ZFNs) have been used to generate isogenic iPSC lines that varies in SNCA gene copies and aSyn expression levels, as well as point mutations' introduction or correction[92,95–102].

Finally yet importantly, iPSCs derived from healthy individuals have a therapeutic potential as well[103]. They serve as a baseline for comparison besides being crucial research models for decreasing experimental variability and improving reproducibility,

investigating non-pathological cellular processes and differentiation potential[87,99,104].

It is noteworthy that the generated iPSC lines must be validated whether they maintain pluripotency and differentiation potential to ensure their use in further downstream applications[72,99,101]. High-content screening and fluorescence-activated cell sorting (FACS) are examples of advanced screening approaches employed to not only select and validate iPSC clones' genetic correction, but to confirm the high quality of generated isogenic iPSCs[96]. (Table 1)

IV.
Applications of aSyn iPSC models in PD research

aSyn iPSC lines have been primarily differentiated into midbrain dopaminergic neurons (mDANs); the most affected neuronal subtype in PD. Studies demonstrate that either SNCA multiplication iPSCs or those from patients with SNCA mutations can still differentiate into DA neurons and their differentiation efficiency are not influenced by elevated SNCA dosage or aSyn overexpression. However, in some models with

TABLE 1. Examples of aSyn induced pluripotent stem cell models

iPSC model	Differentiated cells	Differentiation protocol	Phenotypic manifestations	Methods to induce/detect aSyn aggregation	Main research applications	Ref.
Triplication SNCA	mDANs	Dual SMAD inhibition	↑aSyn mRNA levels;↑aSyn protein expression and release	Not demonstrated	Disease modeling; Addressing epigenetic challenges	[63]
A53T SNCA; isogenic counterparts	(A9) mDANs	Dual SMAD inhibition	LB/neurite-like pathology; ↑Vulnerability to mitochondrial toxins; Mitochondrial dysfunction; ↑NS, OS & resultant apoptosis	Basal aSyn pathology; Mitochondrial toxins; NS, OS/ThT staining; Phosphorylation AB-based detection; WB	Disease modeling; studying gene-environment interactions; Identifying (MEF2C-PGC1α) pathway as a potential therapeutic target; HTS for drug discovery	[95]
Triplication SNCA; Triplication SNCA KD	DANs; NPCs	Neural induction from EBs & Dual SMAD inhibition	↑aSyn expression; Neurite outgrowth deficits; Delayed maturation; ↑Autophagic flux; Electrophysiological impairments	Not demonstrated	Disease modeling; Understanding neuronal differentiation; investigating bioenergetics dysfunctions; Genetic and epigenetic research	[105]
Triplication SNCA; A53T SNCA	pMac	pMacpre	↑Both intracellular & extracellular aSyn levels; Phagocytosis impairment; Cytokine dysregulation	Fibrils assembling from monomeric aSyn/ Fluorescent labeling and microscopy; Flow cytometry	Disease modeling; Exploring non-neuronal contributions to PD	[89]
A53T SNCA	DANs	Dual SMAD inhibition	aSyn & Tau aggregation; Compromised neuritic outgrowth; Axonal neuropathology; Defective synaptic connectivity; Dysregulated synaptic signaling genes' expression; Pathological phenotypes linked to PD-associated dementia	Basal aSyn pathology/ Proteinase K Treatment; ThS Staining; Fluorescence-based assay; WB	Disease modeling; Identifying SMs (NPT100-18A, NPT100-14A, ELN484228) targeting aSyn; Understanding synaptic connectivity; Investigating cellular stress responses	[110]
A53T SNCA; isogenic counterparts; E46K SNCA (hESCs)	(A9) mDANs	Dual SMAD inhibition	↑Accumulation of soluble aSyn in mitochondrial fractions; Impaired mitochondrial dynamics; Fragmented mitochondria; Pathology transmission	Basal aSyn pathology; Cardiolipin interaction & prolonged exposure/ WB analysis; FRET analysis; PLA; SR imaging	Disease modeling; Advancing aSyn immunotherapy; Exploring mitophagy; modeling disease transmission	[102]
Triplication SNCA; A53T SNCA	mDANs	Dual SMAD inhibition	↑Intracellular aSyn accumulation and extracellular release; Oligomeric aSyn pathology; mitochondrial dysfunction and aberrant morphology; ↑ER stress; Lipid metabolism disruption; Lysosomal dysfunction	PLA; MSD; Image acquisition	Disease modeling; Exploring bioenergetic and metabolic Pathways	[90]
YOPD	mDANs	Modified dual SMAD inhibition protocol	Accumulation of soluble aSyn; ↑p-PKCα levels; Lysosomal & mitochondrial dysfunction; ↓Na^+ current	Not demonstrated	Developing a new diagnostic tool; Highlighting the potential of Phorbol Ester drugs as potential therapeutics; Inclusion criteria for mechanistic studies and clinical trials; Suggesting further animal model studies	[118]

TABLE 1. *(continue)*

iPSC model	Differentiated cells	Differentiation protocol	Phenotypic manifestations	Methods to induce/detect aSyn aggregation	Main research applications	Ref.
A30P SNCA; isogenic counterparts	vmDANs	Neural induction from EBs & Dual SMAD inhibition	Neuritic pathology; Mitochondrial dysfunction; ↓DAT gene expression; sporadic presence of astrocytes & ↑expression of radial GPCs; Impaired electrical activity	Not demonstrated	Disease modeling; Understanding energy deficits and vulnerability in PD; Application of high-throughput approaches (MEAs)	*[96]*
Duplication SNCA	mDANs; CPNs; NPCs	FGF8- and small molecule-based midbrain protocol; FGF2-based cortical protocol	↑aSyn pathology; HMW aSyn oligomers' formation; ↑ROS & protein nitration; ↑cell death; Metabolic dysfunction; Mitochondrial impairment	Basal aSyn pathology; ↑ROS/ Sequential protein extraction; WB; Denaturing SDS-PAGE; Phosphorylation AB-based detection	Disease modeling; Understanding neuronal vulnerability; Exploring genetic contributions	[107]
Triplication SNCA; A53T SNCA	mDANs	Modified midbrain FP-based protocol	LB-like pathology; Mitochondrial abnormalities; Vulnerability to mitochondrial damage	Combining seeding with PFFs and extended culture duration/Immunostaining; Phosphorylation AB-based detection	Disease modeling; Understanding genetic influences; Investigating mitochondrial dysfunction	*[88]*
Triplication SNCA	DANs	Dual SMAD inhibition	↑aSyn expression; Altered firing pattern & DA release; ↑Burst activity; ↓D2 receptor functionality	Not demonstrated	Disease modeling; Targeted therapies development (D2 receptor agonists); Gene expression analysis	*[111]*
Triplication SNCA; A53T SNCA; DJ-1 KO	DANs	Modified FP protocol	Nuclear fragmentation; pSYN-positive aggregates; Neuronal death	Treatment with exogenous de novo-generated polymorphs (fibrils or ribbons) or brain-amplified fibrils/Cell Fractionation & WB; FRET assay; BioID2; Confocal microscopy; Mass spectrometry	Disease modeling; Genetic studies & gene therapy; Biomarker discovery through understanding the specific proteins interacting with aSyn aggregates; Identifying potential drug targets (DJ-1)	*[112]*
Isogenic iPSCs panel from Triplication SNCA	mDANs	Neural induction from EBs & Dual SMAD inhibition	aSyn aggregation; mitochondrial dysfunctions & fragmented morphology; ↑OS; Ca^{+2} mishandling; Vulnerability to aSyn aggregation	Basal aSyn pathology; seeding with synthetic fibrils/Nanobody-based biosensor (FluoReSyn); Immunostaining; PLA; Near-IR fluorescence	Disease modeling; Investigating aSyn aggregation; Studying genetic modifiers; Identifying modulators of aSyn aggregates clearance (TAX1BP1)	*[98]*
Triplication SNCA; A53T SNCA; isogenic counterparts	mDANs	SMs-based	↑aSyn expression; Small β-sheet-rich oligomeric aggregates formation; Mitochondrial damage (↓ PTP opening threshold) & fragmentation; Lysosomal pathology; Ca^{+2} desregulation; ↑cell death	Basal aSyn pathology; Endogenous aggregate formation/SML & SR microscopy; ELISA	Disease modeling; Investigating protein aggregation; Exploring Ca^{+2} Dysregulation; Dissecting the temporal sequence of pathological events	*[7]*
A53T SNCA	DANs; NPCs	Neural induction from EBs & Dual SMAD inhibition	↑aSyn mRNA & protein levels; Synaptic defects & early synaptic dysfunction; Poor neuronal networks formation; Overlap with ND disorders	Not demonstrated	Disease modeling; Comparative transcriptomics analysis; Exploring ND components	*[140]*
Triplication SNCA (opto-a-syn); SNCA KO	mDANs; MOs	Neural induction from EBs & Dual SMAD inhibition	Pathological aSyn aggregates; ↓ TH^+ mDANs; ↑PD–related cytokines & chemokines (e.g. MIF)	OASIS; Using optogenetic proteins/Immunostaining & automated image analysis; AIS	Optogenetic control of protein aggregation; HTS & HCS; Exploring autophagy-dependent mechanisms & autophagic clearance promoting cpds (BAG956); Development of drug screening platforms	*[106]*
G51D SNCA	Ectodermal, mesodermal, & endodermal cells	Spontaneous (EBs) & Direct (STEMdiff™ Trilineage Differentiation Kit)	pathogenic c.G152A mutation in Exon 3 of SNCA gene	Not demonstrated	Disease modeling; Genetic and pathological hallmarks studies	*[94]*

TABLE 1. *(continue)*

iPSC model	Differentiated cells	Differentiation protocol	Phenotypic manifestations	Methods to induce/detect aSyn aggregation	Main research applications	Ref.
KOLF2 hiPSC line; Triplication SNCA; isogenic SNCA-2KO; SNCA-4KO	mDAOs; Chimeric mDAOs	Neural induction from EBs & Dual SMAD inhibition	↑aSyn expression; Rotenone sensitivity; DANs vulnerability associated with genes involved in synaptic signaling and cholesterol biosynthesis; Molecular dysfunctions; Translation & OS tolerance	Not demonstrated	Disease modeling; Single-cell transcriptomics; Understanding synaptic signaling and cholesterol biosynthesis; Investigating non-cell autonomous effects and idiopathic PD through chimera organoids	*[104]*
Sporadic PD	vmDANs	Modified FP protocol; Dual SMAD inhibition	↑Pathological aSyn expression with somatic localization; ↓Viability; ↑ROS; Mitochondrial abnormalities; Dysregulated autophagy; Altered neuronal electrophysiology	Immunocytochemistry & WB	Disease modeling; Understanding PD as a generalized disorder rather than a neuron-centric condition.; Providing a platform for biomarker discovery	*[141]*
Triplication SNCA	*mDAOs*	*Neural induction from EBs & Dual SMAD inhibition*	*LB-like inclusions; DANs loss; ↑Apoptosis; Neurite deterioration*	*Basal aSyn pathology; 3D culture system/IF staining & microscopy; Quantification of immunoreactive areas*	*Disease modeling; Exploring spatiotemporal LB-related events; Studying genetic contributions*	*[108]*
Triplication SNCA; isogenic SNCA-4KO	*DANs; FPPs*	*Modified protocol using Dopaminergic Neuron Differentiation Kit*	*↑aSyn expression; Maturation variability; Dysregulated DA release & firing activity; ↓TH neuronal expression*	*Not demonstrated*	*Disease modeling; Development of cell replacement & stem cell-based therapies*	*[97]*
A30P SNCA; A30P_ChR2	*DANs*	*Modified dual SMAD inhibition*	*↑aSyn release with increasing neuronal activity (pharmacologically or by optogenetic stimulation) & vice versa*	*Not demonstrated*	*Disease modeling; Pharmacological and optogenetic Modulation (bicuculline, CNQX, Ch-R2); Exploring a-syn propagation*	*[142]*

LEGEND – **mDANs**: Midbrain dopaminergic neurons; **LB**: Lewy body; NS: Nitrosative stress; **OS**: Oxidative stress; **ThT**: Thioflavin T; **AB**: Antibody; **DANs**: Dopaminergic neurons; **NPCs**: Neural progenitor cells; **EBs**: Embryoid bodies; **pMac**: Macrophages; **pMacpre**: Non-adherent macrophage precursors; **WB**: Western blotting; **HTS**: High-throughput screening; **ThS**: Thioflavin S; **SMs**: Small molecules; **FRET**: Fluorescence Resonance Energy Transfer; **PLA**: Proximity Ligation Assay; **SR**: Super- resolution; **ER**: Endoplasmic Reticulum; **MSD**: Meso Scale Diagnostic Human aSyn Kit; **p-PKCα**: Phosphorylated protein kinase Cα; **vmDANs**: Ventral midbrain dopaminergic neurons; **DAT**: Dopamine Active Transporter; **GPCs**: Glia progenitor cells; **MEAs**: Multielectrode arrays; **CPNs**: Cortical projection neurons; **HMW**: High molecular weight; **ROS**: Reactive oxygen species; **FP**: Floor plate; **PFFs**: Preformed fibrils; **DA**: Dopamine; **BioID2**: Proximity-dependent Biotin identification; **IR**: Infrared; **PTP**: Permeability Transition Pore; **SML**: Single-molecule localization; **ND**: Neurodevelopmental; **TH**: Tyrosine hydroxylase; **MIF**: Macrophage migration inhibitory factor; **OASIS**: Optogenetic Alpha-Synuclein Induction System; **AIS**: Aggregates Induction Score; **HCS**: High-content screening; **cpds**: Compounds; **mDAOs**: Midbrain dopaminergic organoids; **IF**: Immunofluorescence; **FPPs**: Floorplate progenitors; **CNQX**: Cyanquixaline; **ChR2**: Channelrhodopsin-2; ↑: Increased; ↓: Decreased

elevated aSyn expression as well as SNCA mutations, delayed neuronal maturation, compromised neurite growth, poor neuronal activity and increased neuronal death are observed[89,92,97,101,105–107]. Furthermore, increased aSyn expression and SNCA mutations in differentiated neurons are thought to be associated with aSyn aggregation and LB-like pathology; which attributes to mDANs distinct vulnerability resulting in various phenotypic alterations and cellular dysfunctions. These deficits include perturbed synaptic connectivity, mitochondrial dysfunction, calcium dysregulation, elevated endoplasmic reticulum (ER) stress, firing activity and dopamine release dysregulation[88,90,92,98,107–111]. Moreover, upon exposure of differentiated neurons to de novo or brain-amplified fibrils, accelerated aSyn aggregation (in a time and dose-dependent manner) as well as LB-like deposits exist[98,112]. Thereby, aSyn iPSCs and/or derived neurons have been considered valuable and essential models for advancing our understanding of aSyn pathology and propagation in PD through their capability in recapitulating disease-relevant phenotypes, elucidating related cellular dysfunctions and investigating the underlying molecular mechanisms and pathways involved[64,113–115]. Besides facilitating the study of aSyn toxicity, these models have been instrumental, in conjunction with other systems, for the development of personalized medicine and cell therapy strategies[104,116,117], and testing of therapeutic compounds[106,111,118,119] that help mitigate pathology and restore neuronal function.

Recent studies highlighted the prominent implications of midbrain organoids (MOs) as they can mimic

the spatial cellular interactions, hence offering a novel platform for studying the spatiotemporal dynamics of LB pathology and exploring therapeutic interventions in a more physiologically-relevant context using non-invasive approaches[82,93,104,106,108,114,120–122].

Other viable strategies include consolidating stem cell and gene therapy (e.g. knocking down mutant aSyn in iPSCs using shRNA)[123], OASIS (Optogenetic Alpha-Synuclein Induction System) platform for compound screening[106], the promising utilization of extracellular single-chain variable fragments (scFvs) in mitigating aSyn spread[124], rapid aSyn inclusionopathy iPSC models[125], and switching the focus of the therapeutic pipeline on lowering insoluble aSyn to be more on restoring its soluble levels[126]. (Table 1)

V.
Challenges and future directions

Despite the significant insights that iPSC models provide into PD research and treatment, they face challenges associated with recapitulating the multifactorial and heterogeneous nature of the disease[64,127–131]. These limitations reduce their effectiveness in capturing the full-spectrum of PD pathology thus hamper findings' applicability. As demonstrated in (Figure 1), obstacles include confined replication of disease complexity, reproducibility issues, limited predictive value, challenges in modeling aSyn pathology, technical and methodological challenges, genetic and cellular limitations, and ethical concerns[2,64,82,84,93,101,113,114,132–136].

Ongoing efforts have been crucial to refine aSyn iPSC models further in order to enhance their applicability in PD research. A recent body of research has proposed strategies to enhance models' fidelity and complexity, and alleviate temporal limitations and lack of consistency. Several procedures have been promising such as MOs, 3D bioprinting and scaffolding approaches, using multi-modal approaches including mechanistic and artificial intelligence models, rapid induction of aSyn inclusions, and seeding techniques[2,75,82,98,119,134,135,137–139]. Nevertheless, 3D organoid models are more complex and challenging to interpret. A recent study(108) discussed various obstacles that arise for an A53T SNCA-derived MO model, such as inconsistent LB-like pathology with incomplete morphogenesis, difficulties in detection, and limited maturity. They therefore suggested that additional maturation and prolonged culture may be necessary to develop a more complete LB-like pathology.

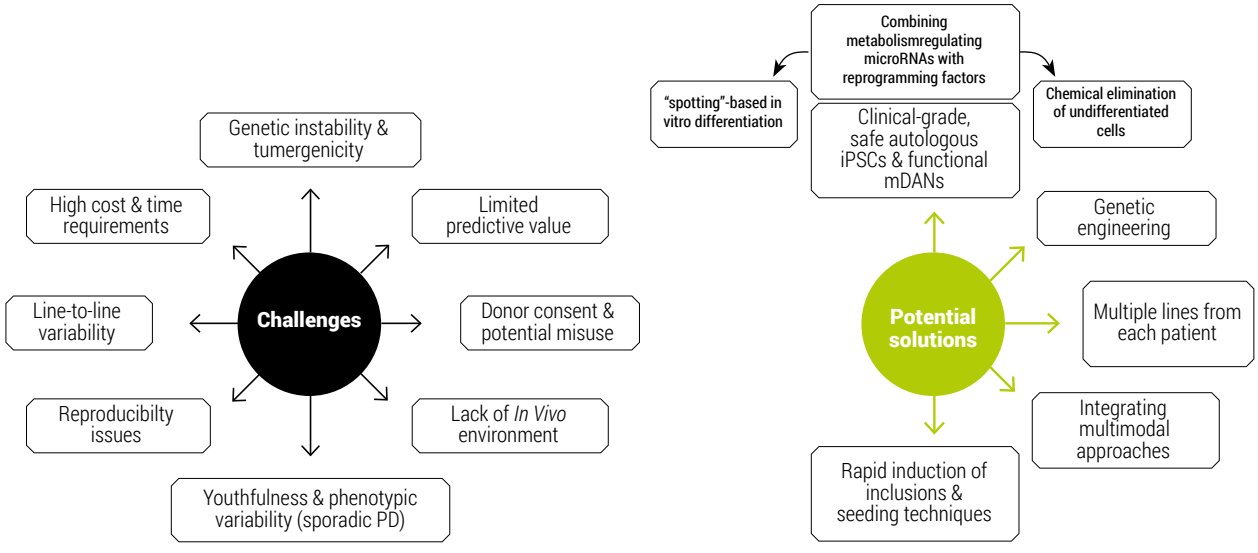

Fig 1. Main limitations of current iPSC models in PD and potential tackling approaches. The use of iPSC models in PD research offers significant potential but also comes with several technical, ethical, and genetic obstacles. Recent studies have proposed a range of methodologies e.g. genetic engineering, and multimodal integration to address substantial challenges.

VI. CONCLUSIONS AND OUTLOOK

Unlike the artificially-derived counterparts, iPSC systems maintain the native cell machinery and transcription feedback mechanisms. Moreover, genetic correction of iPSC lines followed by back transplantation into the same patient is opening up new routes in the development of personalized medicine and PD-directed therapies. In particular, the aSyn

iPSC models provide an exceptional platform for studying the crucial PD pathological features (such as LB formation and neuronal degeneration), discovering novel therapeutic targets, and testing various compounds that might contribute to mitigating the pathology.

To conclude, there is an urgent need for reliable models in PD context to promote further understanding of the disease mechanisms and early diagnosis, leading to the discovery of effective treatment options. The broad contribution made so far by iPSCs technology is recognized, and it surely represents a promising avenue into the future for managing PD. Ongoing refinements and technological developments will be constantly needed in order to take full advantage of iPSCs potentials.

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Diplexil R

Valproato Semisódico

CONFIANÇA NUMA VIDA COM QUALIDADE[1,2]

INFORMAÇÕES ESSENCIAIS COMPATÍVEIS COM O RESUMO DAS CARACTERÍSTICAS DO MEDICAMENTO

▼Este medicamento está sujeito a monitorização adicional. Isto irá permitir a rápida identificação de nova informação de segurança. Pede-se aos profissionais de saúde que notifiquem quaisquer suspeitas de reações adversas. Para saber como notificar reações adversas, ver secção 4.8 do RCM.

NOME, COMPOSIÇÃO QUALITATIVA E QUANTITATIVA e FORMA FARMACÊUTICA DO MEDICAMENTO: Diplexil-R 250 mg (269,1 mg de valproato semisódico) comprimidos gastrorresistentes cor de pêssego. Excipiente(s) com efeito conhecido: Amarelo sunset (E110) – 0,2 mg, Sódio: 18,5 mg por comprimido. Diplexil-R 500 mg (538,2 mg de valproato semisódico) comprimidos gastrorresistentes cor-de-rosa. Excipiente(s) com efeito conhecido: Carmoisina (E122) - 0,104 mg, Vermelho de ponceau 4R (E124) - 0,091 mg, Sódio: 37,0 mg por comprimido. **INDICAÇÕES TERAPÊUTICAS:** Epilepsias generalizadas e parciais: Generalizadas primárias: Pequeno e grande Mal, epilepsias mioclónicas; Parciais: simples e complexas. Generalizadas secundárias: síndroma de Lennox-Gastraut, síndroma de West; Formas mistas. Epilepsias especiais: Convulsões febris na criança. Privação do sono. Alterações do comportamento associados à epilepsia. Tratamento de episódios maníacos na doença bipolar quando o lítio está contraindicado/ não é tolerado. Considerar a continuação do tratamento após a ocorrência de episódios maníacos em doentes que responderam ao valproato semisódico na mania aguda. Em: Doentes com ciclos rápidos; bipolares difíceis (idosos e doentes em situações de comorbilidade com abuso de substâncias). - Tratamento profilático dos seguintes tipos de cefaleias: Enxaqueca, Cefaleia Crónica Diária (Enxaqueca Transformada e enxaqueca Persistente e Resistente) e Cefaleia em salvas. **POSOLOGIA E MODO DE ADMINISTRAÇÃO:** Via oral. Se tratamento prévio com Ácido valpróico, iniciar a terapêutica com a mesma dose diária e esquema posológico. Após estabilização do doente poderá instituir-se um esquema posológico de 2 a 3 tomas diárias. A frequência de efeitos adversos (particularmente enzimas hepáticas elevadas) pode estar relacionada com a dose. Avaliar benefício-risco de doses mais elevadas. Concentrações de Fenitoína no sangue podem ser afetadas com aumento de dosagem. Para os doentes que se queixam de irritação gastrointestinal, recomenda-se a administração do fármaco durante as refeições, e aumento gradual da dose a partir de um nível inicial baixo. Epilepsia: dose inicial recomendada:15 mg/kg/dia 3 x dia, com aumentos de 5 a 10 mg/kg/dia em intervalos de uma semana até controlo das crises ou até dose tolerável. A dose máxima recomendada é de 60 mg/kg/dia. Se exceder os 2500 mg, esta deverá ser administrada em doses repartidas. Para a maior parte dos doentes, as concentrações séricas terapêuticas de Valproato situam-se no intervalo de 50-100 mcg/ml. Caso a posologia diária seja igual ou superior a 50 mg/Kg/dia, recomenda-se monitorizar níveis sanguíneos. Episódios maníacos na doença bipolar: Em adultos: A dose diária deve ser estabelecida e controlada pelo médico. Dose diária inicial recomendada é de 750 mg. Em ensaios clínicos, a dose inicial de 20 mg de valproato/kg de peso corporal também demonstrou um perfil de segurança aceitável. A dose deve ser aumentada tão rapidamente quanto possível, de forma a atingir a dose terapêutica mais baixa que produza o efeito clínico desejado. Ajustar a dose à resposta clínica. A dose média diária varia, habitualmente, entre 1000 a 2000 mg de valproato. Se doses superiores a 45 mg/kg de peso corporal, monitorizar. A continuação do tratamento deve ser adaptada individualmente usando a dose mínima eficaz. Em crianças e adolescentes: A segurança e a eficácia de Diplexil-R para o tratamento de episódios maníacos na doença bipolar não foram avaliadas em doentes com idade inferior a 18 anos. Crianças do sexo feminino e mulheres em idade fértil: O valproato deve ser iniciado e supervisionado por um especialista com experiência no tratamento da epilepsia, perturbação bipolar ou enxaqueca. O valproato não deve ser utilizado em crianças do sexo feminino e mulheres em idade fértil a não ser que outros tratamentos sejam ineficazes ou não tolerados. O valproato é prescrito e dispensado de acordo com o Programa de Prevenção do valproato na Gravidez. O valproato deve ser prescrito preferencialmente em monoterapia e na dose eficaz mais baixa, se possível numa formulação de libertação prolongada. A dose diária deve ser dividida pelo menos em duas tomas únicas. Homens: Recomenda-se que Diplexil seja iniciado e supervisionado por um especialista com experiência no tratamento da epilepsia ou perturbação bipolar ou enxaqueca. Cefaleias: A dose mínima eficaz é de 250 mg 2x dia e o tratamento deverá ter a duração mínima de 3 meses. A dose média é de 1000 a 1500 mg/dia. Em doentes com insuficiência renal: pode ser necessário diminuir a dosagem, ou aumentar a dosagem em doentes em hemodiálise. Valproato é dialisável. A dosagem deve ser modificada de acordo com a monitorização clínica do doente. Diplexil-R só deve ser iniciado e supervisionado por um especialista com experiência no tratamento da enxaqueca. O tratamento só deve ser iniciado se outros não forem eficazes ou tolerados e os benefícios e riscos devem ser cuidadosamente reavaliados em revisões regulares do tratamento. **CONTRAINDICAÇÕES:** Diplexil-R está contraindicado nas seguintes situações: - Hipersensibilidade às SA ou excipientes. - Doença hepática ou disfunção significativa. - Antecedentes pessoais ou familiares de hepatite grave, nomeadamente medicamentosa. - Porfiria hepática. - Doentes que tenham doenças mitocondriais causadas por mutações no gene nuclear que codifica a enzima mitocondrial polimerase gama (POLG), por exemplo a síndrome de Alpers-Huttenlocher, e em crianças com menos de 2 anos de idade em que se suspeita de terem doenças relacionadas com a POLG. - Doentes com distúrbios do ciclo da ureia. Tratamento da epilepsia: - Na gravidez, a não ser que não exista um tratamento alternativo adequado. - Em mulheres em idade fértil, a não ser que as condições do programa de prevenção da gravidez sejam cumpridas. Tratamento da perturbação bipolar e profilaxia de crises de enxaqueca: - Na gravidez. - Em mulheres em idade fértil, a não ser que as condições do programa de prevenção da gravidez sejam cumpridas. **ADVERTÊNCIAS E PRECAUÇÕES ESPECIAIS DE UTILIZAÇÃO:** trombocitopénia, alterações da hemostase/coagulação, hiperamonemia com ou sem letargia, alterações nos testes de função da tiroide, insuficiência hepática, muito raramente pancreatites graves, reações adversas cutâneas graves e angioedema. **Programa de Prevenção de Gravidez:** O valproato tem um elevado potencial teratogénico e as crianças expostas ao valproato in utero têm um elevado risco de malformações congénitas e perturbações do desenvolvimento do sistema nervoso. Ver RCM. Foram relatados casos de ideação e comportamentos suicidas em doentes tratados, com medicamentos antiepiléticos, para várias indicações terapêuticas. Não é recomendado o uso concomitante de ácido valpróico/ valproato de sódio com os antibióticos do grupo dos cabapenemos. Doentes com doença mitocondrial conhecida ou presumida: O valproato pode desencadear ou agravar sinais clínicos de doenças mitocondriais subjacentes causadas por mutações do ADN mitocondrial bem como do gene nuclear que codifica a POLG. Se há suspeita de uma deficiência no ciclo enzimático da ureia, devem ser feitos estudos metabólicos antes do tratamento, devido ao risco de hiperamoniemia com o valproato. Poderá ocorrer aumento de peso no início do tratamento. Doentes com uma deficiência tipo II em carnitina palmitoiltransferase subjacente (CPT) devem ser advertidos do risco aumentado de rabdomiólise. Nos insuficientes renais pode ser necessário proceder-se a uma diminuição da dosagem. Excipientes. **INTERAÇÕES MEDICAMENTOSAS E OUTRAS FORMAS DE INTERAÇÃO:** Efeitos do valproato semisódico nos outros medicamentos: Neurolépticos, antidepressivos, benzodiazepinas e barbitúricos; Fenobarbital e Primidona; Fenitoína; Carbamazepina; Etossuximida; Lamotrigina; Zidovudina; Felbamato; Olanzapina; Rufinamida; Propofol; Nimodipina. Efeito de outros medicamentos sobre o valproato de sódio: Antiepiléticos; Mefloquina; Fármacos com ligação elevada às proteínas plasmáticas; Anticoagulantes; Cimetidina ou eritromicina; Fluoxetina; Carbapenemos; Rifampicina; Inibidores de protéase; Colestiramina; Medicamentos que contêm estrogénio; Metamizol; Outras interações: Topiramato ou acetazolamida; Quetiapina; Álcool; Lítio; Clonazepam; Clozapina. Ver RCM. **EFEITOS INDESEJÁVEIS:** Os efeitos indesejáveis notificados mais comuns para o valproato são perturbações gastrointestinais, as quais ocorrem em aproximadamente 20% dos doentes. Têm sido observados casos de lesões hepáticas graves (ou mesmo fatais), especialmente em crianças tratadas com doses elevadas ou em combinação com outros antiepilépticos. Os efeitos indesejáveis foram classificados por ordem de frequência segundo a seguinte convenção: Neoplasias benignas, malignas e não especificadas (incl. quistos e polipos): Raros: Síndrome mielodisplásico. Doenças do sangue e do sistema linfático: Frequentes: Trombocitopenia, leucopenia. Pouco frequentes: Hemorragia. Raros: Anemia macrocítica, macrocitose. Muito raros: Perturbações da medula óssea, concentração reduzida de fibrinogénio e/ou de fator de coagulação VIII, alteração da agregação plaquetária, tempo de coagulação prolongado, linfocitopénia, neutropénia, pancitopénia, anemia ou aplasia da linhagem de células vermelhas. Desconhecido: Agranulocitose. Doenças do sistema imunitário: Pouco frequentes: Angioedema. Raros: Lúpus eritematoso, erupção medicamentosa com eosinofilia e sintomas sistémicos (síndrome DRESS). Desconhecido: Reações alérgicas (ver também "pele e perturbações dos tecidos subcutâneos"), síndrome da resposta inflamatória sistémica. Doenças endócrinas: Raros: Hiperandrogenismo (hirsutismo, virilismo, acne, alopécia com aparência típica masculina e/ ou aumento dos níveis de androgéneos), hipotiroidismo. Doenças do metabolismo e da nutrição: Frequentes: Hiperamonemia, aumento do peso (fator de risco para a síndrome do ovário poliquístico, requer monitorização cuidadosa) ou diminuição de peso; aumento ou diminuição de apetite. Pouco frequentes: Síndrome de secreção inapropriada de hormona antidiurética (SIADH). Raros: Hiperinsulinemia, baixos níveis da IGFBP-1 (insulin-like growth factor binding protein 1), obesidade. Muito raros: Foram relatadas anomalias das provas da função tiroideia, com relevância clínica duvidosa. Hiponatremia. Vasculopatias: Raros: Vasculites. Perturbações do foro psiquiátrico: Frequentes: Agressividade*, agitação*, atenção alterada*. Raros: Irritabilidade, alucinações, confusão, comportamento anormal*, hiperatividade psicomotora*, perturbação da aprendizagem*. * Estas reações adversas são observadas principalmente na população pediátrica. Doenças do sistema nervoso: Frequentes: Sonolência, tremores, parestesias, defeito de memória, nistagmo, tonturas. Pouco frequentes: Coma transitório, em alguns casos associado a aumento da frequência das crises, ataxia. Raros: Cefaleias, hiperatividade, espasticidade, estupor, perturbação cognitiva, diplopia. Muito raros: Encefalopatia, demência associada a atrofia cerebral (reversível após a descontinuação do tratamento), distúrbios extrapiramidais p. ex. síndrome parkinsónica (reversível). Desconhecido: Agravamento das crises, sedação, letargia. Afeções do ouvido e do labirinto: Muito raros: Perda de audição (reversível ou irreversível), acufenos. Doenças respiratórias, torácicas e do mediastino: Pouco frequentes: Derrame pleural (eosinofílico). Doenças gastrointestinais: Muito frequentes: Dores, náuseas, vómitos. Frequentes: Diarreia, perturbação gengival (principalmente hiperplasia gengival), estomatite. Raros: Pancreatite (por vezes fatal), hipersalivação, ileus, obstrução intestinal. Afeções hepatobiliares: Frequentes: Alterações nas provas da função hepática. Raros: Lesão hepática grave que inclui insuficiência hepática. Afeções dos tecidos cutâneos e subcutâneos: Frequentes: alopecia, enfraquecimento do cabelo e aparecimento de cabelo encaracolado, alterações nas unhas e leito ungueal. Raros: Exantema, eritema multiforme. Muito raros: Síndrome de Stevens-Johnson, Síndrome de Lyell. Desconhecido: Hirsutismo (por ex. resultante da síndrome do ovário poliquístico), hiperpigmentação. Afeções musculosqueléticas e dos tecidos conjuntivos: Raros: Rabdomiólise. Desconhecido: Foram notificados casos de densidade mineral óssea diminuída, osteopenia, osteoporose e fraturas ósseas em doentes sob tratamento prolongado com valproato. Ainda não se conhece o mecanismo pelo qual o valproato afeta o metabolismo ósseo. Doenças renais e urinárias: Frequentes: incontinência urinária. Muito raros: Síndrome de Fanconi (com acidose metabólica, fosfatúria, aminoacidúria, glicosúria, reversíveis após a descontinuação do tratamento), enurese nas crianças. Desconhecido: Insuficiência renal, nefrite intersticial, deterioração da função renal. Doenças dos órgãos genitais e da mama: Frequentes: Amenorreia, dismenorreia. Raros: Síndrome de ovário poliquístico, infertilidade masculina. Desconhecido: Espermatogénese anormal (com contagem reduzida de espermatozóides e/ou motilidade). Afeções congénitas familiares e genéticas: Malformações congénitas e alterações no desenvolvimento. Perturbações gerais e alterações no local de administração: Raros: Hipotermia, edema. Exames complementares de diagnóstico: Raros: Redução dos fatores de coagulação (pelo menos um), testes de coagulação anormais (tais como prolongamento do tempo de protrombina, prolongamento do tempo parcial de tromboplastina ativada, prolongamento do tempo de trombina, valor de INR aumentado), carência de biotina/biotinidas. População pediátrica: Existe risco particular de lesão hepática grave em bebés e crianças pequenas, especialmente com idade inferior a 3 anos, e de pancreatite em crianças pequenas. Estes riscos diminuem com o aumento da idade. Transtornos psiquiátricos como agressão, agitação, perturbação da atenção, comportamento anormal, hiperatividade psicomotora e transtorno de aprendizagem são observados principalmente na população pediátrica. **Para mais informações deverá contactar o Titular da AIM:** TECNIFAR - Indústria Técnica Farmacêutica, S.A. Rua José Da Costa Pedreira, Nº 11 – B - Torre Sul - 1750-130 Lisboa. **Medicamento sujeito a receita médica.** Regime de comparticipação: Escalão A. **RCM aprovado a 05-02-2025 (versão 24.0)**

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

Swallowed Acupuncture Needle in an Aphagic Woman with Bulbar Amyotrophic Lateral Sclerosis

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KEY WORDS: acupuncture, pseudobulbar palsy, amyotrophic lateral sclerosis, dysphagia.

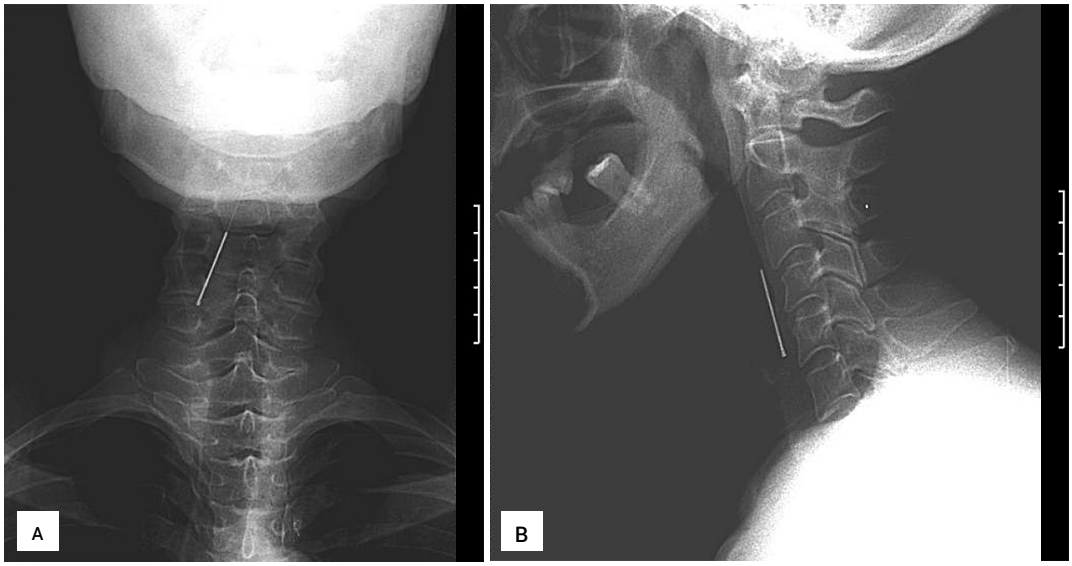

Fig 1. X-ray shows the needle located in the hypopharynx with its bulbous end facing downwards (frontal and lateral views, A and B, respectively). Written informed consent was obtained from the patient for X-ray publication.

A 56-year-old woman had been followed in our Unit for 4 years due to progressive pseudobulbar palsy that started in 2008. The patient was anarthric and severely dysphagic with excessive drooling. Feeding was only possible through gastrostomy. Limb strength was normal. On clinical examination, there was spasticity affecting the tongue, the upper and lower limbs; deep tendon reflexes were markedly increased, with very brisk jaw jerk; and bilateral extensor plantar responses. Fasciculations were only observed in the tongue. Brain magnetic resonance imaging was normal. Needle electromyography confirmed fasciculations and chronic neurogenic changes in tongue, but did not show signs of lower motor neuron loss in any limb muscle (biceps, first dorsal interosseous, vastus medialis, tibialis anterior, on both sides). Nerve conduction studies were unremarkable. The diagnosis of motor neuron disease was made. A progressive clinical deterioration occurred. In order to relieve sialorrhea the patient decided to try acupuncture, which included the insertion of a needle close to the mouth corner. As a consequence of her severe drooling, whilst wiping the mouth, the needle was displaced and ended up being swallowed (Figure 1A-B). Needle removal was achieved by endoscopic procedure with sedation. No complications were noticed.

We speculate that the bulbar spasticity has facilitated a rapid reflex swallowing of the needle once inside the patien' s mouth. This case illustrates that extra caution is necessary to avoid dangerous objects nearby the mouth of these patients.

DISCLOSURE

The authors have no conflict to disclose.

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

Carolina Beatriz Ângelo and Adelaide Cabete – Pioneer Female Medical Doctors

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ABSTRACT: Carolina Beatriz Ângelo (1878-1911) and Adelaide Cabete (1876-1935) were two of the earliest Portuguese female doctors. Carolina devoted herself to Gynecology and political activity. She was the first Portuguese female surgeon and the first woman who voted in parliament elections in Portugal and also in Europe. While Adelaide Cabete, another female doctor, fought for adequate medical assistance to women and children in maternities/hospitals.

KEY WORDS: Carolina Beatriz Ângelo; Adelaide Cabete; women doctors; human rights.

CAROLINA BEATRIZ ÂNGELO

Carolina Beatriz Ângelo was one of the first women in Portugal to complete a degree in Medicine and being the first woman to practice surgery.

Carolina was born in April 16, 1878, in the Portuguese town of Guarda. Her father, Viriato Ângelo, owned a printing press where the newspaper *O Districto da Guarda* was printed. As a journalist, Carolina's father was an open-minded and supported her daughter when, as a brilliant student, she wanted to be a physician.

In the year of 1895, she moved to Lisbon, and in 1902, she graduated from the Lisbon Medical-Surgical School[1] (Fig. 1). She was one of the first Portuguese female doctors and the first woman to practice surgery. Being a woman among men, she felt no constraints as proudly appears in a photograph among her colleagues. (Fig. 2)

In the same year, she married Januário Gonçalves Duarte Barreto (1877-1910), a physician and republican activist. The couple shared the same commitment both to medicine and politics[2].

In that year, feminist and progressive magazines began to be published. Female doctors were part of the movement and a few societies were created, such as "Sociedade Futura" (The Society of the Future) (1902-1904), directed by Adelaide Cabete (1867-1965), also a female physician.

The first article, entitled: "O Movimento Feminista em Portugal" (The Feminist Movement in Portugal) was published by Carolina Michaëlis de Vasconcelos (1851 – 1925), a female German-Portuguese Romanist[3].

In 1903, Carolina presented her dissertation *Genital Prolapses – Clinical Notes*[2], expressing her commitment to improving women´s health since she considered it placed at the background of society. In its prologue, she defined uterine and vaginal prolaps as the object of investigation based on cases she cared. It is worth highlighting her commitment with care during surgery and follow-up in order to reduce complications[3].

Carolina Beatriz Ângelo's activity was not confined to medicine, which she practiced in her private office at Rua do Almada, 64 in Lisbon. Her struggle for the dignity of women extended to institutional and political grounds.

In 1907, Ana de Castro Osório created the Portuguese Feminist Studies Group. Carolina and two other female doctors were part of the team. That year, she was initiated into Free-masonry, at the Humanity lodge and Adelaide Cabete (1867-1935), another female physician and companion of Carolina, was also initiated at the same lodge. They fought for a fairer and more open society in which women should have an active role.

In 1908, the "Liga Republicana das Mulheres Portuguesas" (Republican League of Portuguese Women) was created, supported by the Portuguese Republican Party. Leading writers, educators, journalists, and feminists were co-founders (Fig.3). The vote, the right of education, the rights of the workers, the combat against prostitution and child begging, were aims that guided their action.

On June 23, 1910, Carolina's husband died, aged 33. As a widow and house-holder, she applied on April 4, 1911 for inclusion in the electoral roll. On May 28, 1911, she voted in the elections for the national constituent assembly taking advantage of a "hole" in legislation aimed for males, that guaranteed the right of voting to house-holders, which she was because she was a widow bearing two children.

Her dids were reported not only in Portugal but also in foreign newspapers. Carolina Beatriz Ângelo was the first woman to vote in Portugal and in Europe.

On October 3, 1911, she died suddenly, aged 33, while returning from a political meeting[4].

On July 13 1913, the electoral laws were cleared excluding women, since such right was exclusively for "Portuguese male citizens over 21 years of age". Only in 1931 the right of voting was allowed in Portugal for women.

According to a biographer "*the life of Carolina Beatriz Ângelo is reason enough to place Portugal in the front of the women's emancipatory movements in the world*"[5].

In 2012, one hundred and one years later, a new hospital near Lisbon was open bearing her name: Hospital Beatriz Ângelo.

ADELAIDE CABETE

Adelaide de Jesus Damas Brazão was born in 1867, in the small village of Alcáçova, near Elvas, Portugal, from a family of rural workers (Fig.4). In 1886, at age of 18, she married to Manuel Ramos Fernandes Cabete (1849-1916), an army sergeant who encouraged her to continue studying. At 22 y, she completed her high school with high rankings being the only woman in her class. In order to support his wife, Manuel Cabete decided to move to Lisbon so that Adelaide, enter the Lisbon Medical-Surgical School and graduated in 1900, aged 33[6].

Cabete and her husband, were a perfect match, sharing the same ideals of education and political ac-

Fig 1. Carolina Beatriz Ângelo.
(Photo restored by João Pena Fonseca for the Guarda Museum for an exhibition organized in 2010. Public domain)

Fig 2. Carolina Beatriz Angelo and colleagues: the Medical Course 1899-1902.

Fig 3. The suffragettes of the Republican League of Portuguese Women (LRMP), published on May 12, 1910. [**5:** Ana de Castro Osório; **6:** Maria Veleda; **7:** Beatriz Paes Pinheiro de Lemos; **8:** Maria Clara Correia Alves; **13:** Sofia Quintino; **14:** Adelaide Cabete; **15:** Carolina Beatriz Ângelo; **16:** Maria do Carmo Joaquina Lopes.] Supplement of the newspaper: O Século

Fig 4. Adelaide Cabete. *in* Ramos. J. e Derouet, Luis. 1908. Álbum Republicano, Lisboa: Typ. Adolpho de Mendonça.

tivities. Her medical thesis shows the concerns with the health of poor women during pregnancy and childbearing: *"Protection of poor pregnant women as a way for promoting the physical welfare of the new generations"* [7].

Unlike Carolina, whose short life seemed like a meteor, Adelaide Cabete lived long enough to defend consistently the improvement of women and children's health and, at the same time, fighting for human and specially women's rights.

She practiced gynecology and obstetrics and proposed a period of rest in the last two months of pregnancy defending that puerperal women should stay in maternity wards for post-partum follow-up, ensuring that hygienic conditions were necessary for the development of healthy newborns. She advised maternity leave, which did not exist. Always bringing together medical science, social and political activities.

Alfredo da Costa (1869-1910), the Professor of gynaecology and obstetrics at the Lisbon Medical-Surgical School, was her partner in the claim for the construction of a maternity in Lisbon for poor women. She always stood in defence of life, against abortion, but also denounced the terrible conditions in which poor women succumbed to the unprepared hands of midwives. Both advocated a better medical care in neonatology, pediatrics and general social welfare.

She stood out also in the fight against tuberculosis, alcoholism, venereal diseases, prostitution and in the support of single mothers.

She gave speeches at various educational institutions and left numerous publications in these areas being "Alma Feminina" (Female Soul) one of them.

She advised healthier women's clothing, focusing on old fashion standards from a medical point of view. She disapproved the use of long and tail-back skirts, which could make women stumble and fall. Tight corsets, causing internal injuries by compression of the organs and high heels, causing instability and postural complains[8]. Cabete stood against beauty contests, who placed too much emphasis on physical appearance in the detriment of their female qualities: "*A little better would be perhaps to put in a contest: Which will be the best daughter, the best wife or the best mother*"[9].

In her medical writings and civic interventions, Cabete always promoted the dignity of women.

Cabete's husband died in 1916. She never forgot his kindness and love. In 1929, she moved to Portuguese territory Angola. In 1932, the Maternity Dr. Alfredo da Costa was finally inaugurated in Lisbon. In 1931, the vote was finally available for women and she voted in Luanda (Angola). She was the only woman voting at the plebiscite for a new Portuguese Political Constitution (1933). In 1934, she returned to Lisbon and died the next year at the age of 68.

Her name and political interventions are well known in the history of Portuguese of the XX century.

CONCLUSION

These female physicians still stand as two burning candles in the long night of the fight for women's rights. One must not forget their work and the support by their families namely their husbands. As for their aspirations and struggle for women's rights, they still inspire us with their determination. They never gave up, dreaming for a fairer and more harmonious world.

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PFIZER RESEARCH AWARDS 2024

CLINICAL RESEARCH

Syndecan-4 is a key maestro of stomach cancer progression with patient's prognosis association

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Scientific Background

Stomach cancer is often a silent disease, frequently diagnosed at advanced disease stage, and with still limited therapeutic options. Our research group and several others have shown that gastric cancer cells display aberrant cell surface glycosylation signatures, which hold promise not only as diagnosis biomarkers but also as novel clinical targets.

In recent years, a growing amount of scientific evidence has highlighted that protein post-translation modifications, such as glycosylation, harbour significant relevance and functional impact in determining cancer cell biological behaviour. The glycoconjugates (molecules modified with covalently linked carbohydrate chains) expressed at every cell surface glycocalyx are essential players within the tumour microenvironment. These glycosylated molecules display pivotal roles in defining extracellular matrix (ECM) biochemical and biophysical properties and shaping cancer cell communication in the tumour but also at distant sites. Among these molecular regulators, heparan sulfate proteoglycans (HSPGs), particularly Syndecan-4 (SDC4), are emerging as critical players in tumour development, cell migration, invasion, and extracellular communication [1-3].

Syndecans (SDCs) comprise a family of four type-I transmembrane HSPGs. SDCs are highly abundant at the cell surface and act in cooperation with different transmembrane receptors and ECM molecules with multiple functions in cell signalling, adhesion, proliferation, migration, apoptosis, and differentiation. The SDCs expression profile, and their glycosylation features, have been frequently described as aberrantly altered in various cancers, including gastrointestinal tumours. More-

over, we have recently reported that the heparan sulfate (HS) cellular balance shapes cancer cell motility features and contributes to gastric cancer cells aggressiveness [2].

Lately, proteoglycans have been pinpointed as important partners in extracellular vesicle (EVs) mediated communication in the tumour microenvironment. Particularly, SDCs have been implied in EVs biogenesis, cargo selection and secretion, as well as for defining cancer cell-derived EVs uptake by recipient cells, ultimately fine-tuning tumour dissemination.

Research Project

In this study we evaluated syndecan-4 (SDC4) expression in a retrospective series of 152 gastric carcinomas from Instituto Português de Oncologia do Porto (IPO-Porto). We showed that SDC4 is highly expressed in intestinal subtype gastric tumours and its expression significantly associates with lower patient overall survival. Noteworthy, a similar SDC4 expression profile was also observed in lymph node metastasis from the same patients. The independent prognostic value of SDC4 in the intestinal subtype gastric cancer was demonstrated by multivariate analysis.

To determine the cellular functional impact of SDC4, its expression was knock-out (KO) in the MKN74 intestinal subtype gastric cancer cell line using gene editing by CRISPR/Cas9. We evaluated the impact of SDC4 KO in cellular features by performing migration and invasion assays. We demonstrated that SDC4 promoted an aggressive cancer phenotype, characterized by higher cellular motility in different ECM contexts and increased invasion capacity.

We further addressed the impact of SDC4 KO on gastric cancer cells' EV secretion and their biological activity using *in vitro* assays and mice models. We provided evidence, for the first time, that fully glycosylated-SDC4 is packed on gastric cancer cells secreted EVs and impacts EV protein cargo, uptake by recipient cells and, importantly, defines the tropism of cancer EVs. Interestingly, lack of SDC4 led to the production of a higher amount of EVs, but with smaller size and a distinct protein cargo. On the other hand, the SDC4 on EVs associated with the presence of cancer-associated molecules, such as Transforming growth factor beta-1 proteins (TGFβ1) and growth arrest specific protein 6 (GAS6). We demonstrated that SDC4 is EVs is critical for EV uptake by recipient cells and that SDC4 and HS on EVs modulates their functional effects on the recipient cells, namely the invasion capacity. Noteworthy, mass spectrometry analysis of SDC4 KO cells treated with SDC4-positive EVs showed that these cells, with restored invasion capacity, presented de novo incorporation of SDC4, further supporting the SDC4-associated aggressive phenotype.

By organ distribution analysis of fluorescently labelled EVs in NOD SCID mice, we disclosed that SDC4 dictates the tropism of EVs for common gastric cancer metastatic sites, like the liver and lungs. Moreover, loss of SDC4 alters the EV protein cargo and reduces EV uptake by recipient cells, disrupting cancer cell communication.

Collectively, our findings highlight SDC4 as an independent prognostic factor for intestinal subtype gastric cancer, emphasizing its potential as a biomarker for aggressive disease. Furthermore, we disclose previously unappreciated roles of HS glycosaminoglycans in gastric cancer biology and unveil their potential as tumour biomarkers but also as therapeutic targets to block cancer cell signalling and communication [4].

Implications for Future Research and Clinical Practice

SDC4 emerges as a central regulator in gastric cancer progression and therefore as a promising target for therapeutic intervention. Our study suggests further investigation into the molecular mechanisms through which SDC4 influences EV-mediated communication and metastatic site tropism. Moreover, the molecular interaction of SDC4 with molecules like TGFβ1 and GAS6 in immune regulation within the tumour microenvironment remains to be further elucidated.

The association of SDC4 expression with patients' prognosis, supports that measuring SDC4 levels may help to identify patients at risk of aggressive disease progression. Moreover, proteoglycans in EVs have already been identified as relevant biomarkers for minimally invasive cancer diagnosis.

The understanding of SDC4 role in EV-mediated cell communication offers new insights into cancer metastasis and highlights its potential for advancing personalized oncology strategies targeting cancer dissemination. From a therapeutic perspective, blocking SDC4 or specific glycosylation features of SDC4 has potential to inhibit cancer cell invasion and prevent the establishment of metastatic niches.

Finally, our results support investment in the designing of specific inhibitors targeting HS biosynthetic enzymes and HS-protein interactions, including those mediated by SDC4, due to their potential as novel, still under explored, therapeutic approaches in cancer clinical settings.

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PFIZER RESEARCH AWARDS 2024

BASIC RESEARCH

Retinal Study Reveals Non-Canonical Neuronal Migration Modes that Orchestrate Concomitant Growth and Differentiation

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Scientific Background

This coordination between growth and differentiation is a hallmark of organogenesis in diverse settings, including the pancreas, liver, heart, and many others. Disruptions in these processes can lead to developmental disorders and structural abnormalities of organs.

This coordination is particularly evident during brain formation, where neuronal migration is a key event, ensuring that newly born neurons reach their correct positions to establish functional circuits. This is necessary, as most neurons are born away from the place where they ultimately function. Neuronal migration in all areas of the brain must be tightly coordinated with concurrent tissue growth and differentiation. Extensive research has been conducted on the role of neuronal migration for cell positioning and circuit formation, mainly in the developing neocortex. Furthermore, many of the cytoskeletal drivers and molecular cascades that guide neuronal migration have been revealed. However, how neuronal migration might contribute to tissue-wide morphogenesis during periods of rapid growth and differentiation is not as well understood. Specifically, it was unknown how migrating neurons and proliferative progenitors avoid spatial competition, ensuring both the establishment of functional architecture and the continued expansion of the tissue.

We used the vertebrate retina, with its conserved architecture across species, as a powerful model to study neuronal migration in the context of overall tissue development. We focused on cone photoreceptor cell migration, as these cells are

born relatively early in development, when the tissue is still undergoing substantial proliferation. At the same time, these cells are instrumental for light perception in all vertebrates, including humans. Our model organism of choice was the zebrafish, as this model allows for studying neuronal migration *in vivo,* while it happens, where it happens. Zebrafish have small, transparent embryos that can be generated in large numbers daily and feature many transgenic lines to mark different cell populations and intracellular components. Another significant advantage of this system is their rapid development, chemical and genetic manipulability. Findings can be generated in a depth hardly possible for any other vertebrate and, in turn, be compared to the human system, either in the form of donated human tissue or newly arising human brain and retinal organoids. We took full advantage of this cross-species comparison in our study, the main findings of which are outlined below.

Research Project

Our study investigated photoreceptor (PR) migration during retinal development in zebrafish, human fetal retinas, and retinal organoids derived from induced pluripotent stem cells (iPSCs). We used advanced imaging techniques, mainly light-sheet microscopy, to track PR movements and analyze the cytoskeletal mechanisms underlying their migration, which, to our initial surprise, was bidirectional.

Overall, this comprehensive approach provided critical insights into how neuronal migration ensures the coexistence of growth and differentiation, offering potential implications for understanding neurodevelopmental disorders and retinal repair strategies. The following key findings were made:

1. As photoreceptors are born at the same location where they later function, we were surprised to find that these cells actually do not remain stationary after their birth but instead migrate basally before returning apically to their final position. We noted that these movements occur with different kinetics, basal movement being much faster and more directed than apical movement.
2. The finding that basal and apical movements show different kinetics made us explore the underlying cytoskeletal elements responsible. We found that basal movements depend on stabilized microtubules, while apical movement relies on actomyosin contractility driven by Rho-ROCK signaling. This led us to realize that distinct cytoskeletal systems coordinate these directional movements in a highly specialized manner, which is interesting from a cell biology perspective, as this has not been shown within one particular neuronal cell type.
3. Using zebrafish, human fetal retinas, and retinal organoids, we realized that this bidirectional photoreceptor migration pattern is conserved across species. This led us to propose that this bidirectional movement is a fundamental mechanism for correct retinal development.
4. It is important to note that during the time photoreceptors emerge, the retinal tissue is still highly proliferative. From diverse previous studies, we know that progenitors need to divide at apical positions; otherwise, tissue integrity is not ensured. Interestingly, when we disrupted photoreceptor migration in zebrafish using genetic interventions targeting the microtubule cytoskeleton, we found that apical congestion by photoreceptors occurs. This, in turn, leads to improper progenitor cell divisions and lamination defects. This led us to conclude that neuronal migration is not only about positioning cells but also about orchestrating the delicate balance of growth and differentiation in developing tissues.

Together, this study shows that photoreceptor migration is a conserved and essential mechanism for orchestrating retinal tissue growth and differentiation, relying on distinct cytoskeletal systems to prevent spatial congestion, safeguard progenitor divisions, and ensure the coordinated formation of retinal architecture. Importantly, this phenomenon is conserved across species, from fish to humans, showing its overall impact and significance

Future Implications for Research

The findings revealed in our study have significant implications for future research on neuronal migration and its role in tissue morphogenesis in the retina and beyond. The discovery that photoreceptor migration prevents spatial competition and orchestrates growth and differentiation highlights a previously underappreciated function of neuronal movements in ensuring correct and timely morphogenesis of neural tissues. This phenomenon most likely extends beyond the retina. This means that, with the basic knowledge generated here, the investigation of similar phenomena in other regions of the central nervous system and across different organ systems can start.

From a cell biological point of view, the identification of distinct cytoskeletal mechanisms that drive basal and apical migration opens avenues for targeted research into how microtubules and actomyosin interact to coordinate complex cellular movements during neuronal migration and other cell migration phenomena. As most cells in our body are born at different locations than where they later function, this is of outstanding developmental relevance but also crucial for understanding homeostasis. In the retinal context, future studies can now aim to dissect the upstream molecular signals that initiate and regulate these directional migrations, focusing on how the change in directionality is sensed and achieved by the cell.

Another critical direction for future research is the investigation of how disruptions of neuronal migration processes, in the retina and other areas of the brain, could contribute to neurodevelopmental disorders. By understanding the consequences of apical congestion and defective lamination at a mechanistic level, it might be possible to identify potential intervention points to mitigate the impact of such disruptions on neural tissue architecture and function.

The conservation of photoreceptor migration across zebrafish, human fetal retinas, and human organoids also underscores the utility of retinal organoids as models for studying human neurodevelopment when the work is based on strong in vivo findings. Future work should leverage organoid systems to explore how migration dynamics are altered under pathological conditions, such as in inherited retinal diseases or neurodevelopmental disorders, to identify therapeutic strategies using the extensive knowledge that can be gathered in the zebrafish system.

In conclusion, our study highlights the dual roles of neuronal migration in cell positioning and, new and non-canonically, tissue morphogenesis. This work sets the stage for research aimed at understanding and potentially manipulating these processes to address developmental anomalies and support regenerative approaches in neurobiology.

PAPER DISCUSSED

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PFIZER RESEARCH AWARDS 2024

BASIC RESEARCH

Restoring tumor immunogenicity with dendritic cell reprogramming

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Scientific Background

Cancer cells develop mechanisms to evade the immune system, including immunosuppression, intratumor heterogeneity, exclusion of immune cells from the tumor microenvironment, and downregulation of antigen presentation. Immunotherapies, such as immune checkpoint blockade (ICB) and adoptive T cell therapies, changed paradigms in cancer treatment. However, existing immunotherapies show limited efficacy in some patients. Notably, studies report that the success of ICB depends on tumor immunogenicity and antigen presentation to promote efficient CD8+ T cell priming.

Cellular reprogramming is the process of changing cell fate through epigenetic rewiring of a somatic cell type and the imposition of another desired cell identity, usually mediated by transcription factors (TFs). In cancer, cell fate reprogramming has been shown to lead to the disruption of oncogenic pathways and decreased tumorigenicity, but this strategy is limited by the requirement of reprogramming most cancer cells in the tumor. Ideally, a reprogramming-based strategy would be combined with an immunotherapeutic approach that has the potential to target immune activation while decreasing tumorigenicity. Immune activation greatly depends on type 1 conventional dendritic cells (cDC1), which cross-present tumor antigens to CD8+ T cells, prompting antitumor immunity. In fact, the presence of cDC1 in tumors correlates with better survival and response to immunotherapy. Overexpressing the three TFs PU.1, IRF8, and BATF3 (referred to as PIB) promotes direct reprogramming of mouse and human fibroblasts into immunogenic cDC1 (Rosa et al., 2018; 2022, Science Immunology). The current study (Zimmermannova et al., 2023,

Science Immunology) aimed at reprogramming cancer cells into professional, cDC1-like antigen-presenting cells (APCs) to counteract tumor immune evasion and induce tumor immunogenicity.

Research Project

This project aimed at testing the hypothesis that applying cDC1 reprogramming to cancer cells would efficiently reinstate immunogenicity. A lentiviral polycistronic vector encoding mouse or human PIB was used to evaluate whether direct cDC1 reprogramming could elicit antigen presentation in cancer cells. PIB overexpression induced surface expression of the pan-hematopoietic marker CD45 and the APC marker major histocompatibility complex (MHC)-II in mouse cells. Similarly, reprogramming progressed in human cells transduced with PIB, resulting in the expression of CD45 and the APC marker HLA-DR. The induced murine CD45+ MHC-II+ and human CD45+ HLA-DR+ populations were named tumor-APCs. CD45+ MHC-II+ cells showed surface expression of the cDC1-specific marker CLEC9A, and reprogrammed CD45+ HLA-DR+ cells showed CLEC9A, CD226, and CD11c expression, as well as cDC1-like morphological features. Transcriptional changes during reprogramming were evaluated by analyzing reprogrammed (CD45+ MHC-II+/CD45+ HLA-DR+) and partially reprogrammed (expressing either CD45 or MHC-II/HLA-DR) cells after 9 days of reprogramming. The transcriptome of reprogrammed cells mapped close to natural cDC1, irrespective of the species of origin. Particularly, tumor-APCs showed upregulation of genes related to antigen processing and presentation and immune interactions. Hence, the reprogramming process promoted transcriptional and phenotypic changes attributed to a cDC1 fate.

Further focusing on the transcriptional and epigenetic remodeling of cells undergoing cDC1 reprogramming, reprogrammed and partially reprogrammed populations were profiled along the reprogramming time course (days 3, 5, 7, and 9). Day 7 and day 9 populations mapped closer to peripheral blood cDC1, pointing to a gradual acquisition of a cDC1 transcriptional program. Analyzing differentially open chromatin regions revealed that epigenetic changes occurred mainly in

beginning of the process, from day 0 to day 3. cDC1 reprogramming was shown to be a stepwise process, imposing rapid epigenetic remodeling followed by gradual transcriptional changes.

Subsequently, Zimmermannova et al. addressed whether reprogrammed tumor-APCs processed and presented antigens and showed increased immunogenicity. Briefly, overexpressing PIB induced not only the expression of MHC class I and II but also the expression of costimulatory molecules, providing the critical signals for T-cell activation. This resulted in increased antigen presentation of endogenous antigens by tumor-APCs, as shown by immunopeptidomics and co-cultures with T cells recognizing model antigens endogenously expressed by cancer cells. In addition, cDC1 reprogramming promoted enhanced immune recognition and CD8+ T-cell killing of tumor-APCs.

To further analyze the functional properties of reprogrammed tumor-APCs, the study examined their ability to secrete proinflammatory cytokines, uptake antigens, and present them to CD8+ T cells. Reprogrammed cells acquired a cDC1 functional signature: they secreted cDC1-related cytokines and chemokines required for T-cell recruitment and activation, engulfed and processed proteins and dead cells, and cross-presented antigens to naïve CD8+ T cells. Notably, this system was not limited to cell lines; the demonstrated that patient-derived samples were permissive to cDC1 reprogramming at the single-cell level.

Since reprogramming cancer cells could potentially reduce tumorigenicity, transcriptional signatures were assessed during the reprogramming time course. Genes related to cell proliferation were downregulated, and tumor suppression genes were activated. Specifically, the downregulation of proliferation-related genes was common across most of the tested cell lines, highlighting an induction of cell cycle arrest with reprogramming. Additionally, both reprogrammed and partially reprogrammed cells showed slower cell division and a reduction in tumorigenic potential assessed both in vitro but also in vivo upon transplantation into immunodeficient mice.

Lastly, Zimmermannova et al. assessed the ability of in vitro-reprogrammed cells to promote antitumor immune responses in vivo. Injecting tumor-APCs intratumorally delayed tumor growth and extended survival in syngeneic mouse melanoma models. Moreover, combining the injection of tumor-APCs with ICB treatment fur-

ther reduced tumor growth and extended survival, emphasizing the synergy of cDC1 reprogramming and ICB in driving antitumor immunity in vivo.

The main findings of the current project include:

1. Demonstrated that the minimal cDC1 TF network elicits efficient cDC1 reprogramming in a broad panel of mouse and human cancer cell lines, as well as primary tumor samples.
2. Found that converting tumor cells into tumor-APCs, which exhibit characteristics similar to natural cDC1, promotes CD8+ T-cell activation, recognition, and the elimination of cancer cells. Reprogrammed tumor-APCs enabled presentation of endogenous tumor antigens on MHC-I, facilitating targeted CD8+ T-cell killing.
3. Showed that intratumoral injection of tumor-APCs elicited antitumor immunity, resulting in delayed tumor growth, extended survival, and improved response to ICB.

Implications for Future Research/Clinical Practice

Previously developed immune cell therapies and dendritic cell (DC)-based vaccines have a significant limitation, particularly the lack of methods to efficiently generate rare and specific immune subsets such as cDC1. Monocyte-derived DCs (moDCs) have been more frequently used in DC vaccines, since it is easier to isolate them from peripheral blood. However, moDC-based vaccines show limited clinical efficacy. Reprogramming cancer cells into cDC1, an immune subset crucial for antitumor immunity, shows great promise as a strategy to replenish this rare but potent immune compartment within the tumor microenvironment.

This study demonstrates that cDC1 reprogramming enforces antigen presentation, overcomes tumor evasion mechanisms, and enhances antitumor immunity. Importantly, cDC1 reprogramming was not restricted to specific cell types of origin; it progressed successfully in a wide range of cancer cell lines and primary tumor cells. The current project accelerated the preclinical development of an immunotherapy alternative that is feasibly applicable to all tumor types. Additionally, it could support the development of therapies based on in vitro tumor-infiltrating lymphocyte expansion and neoantigen discovery.

This study lays a strong foundation for future research into cancer-to-immune cell reprogramming and specification, particularly through in-depth molecular analyses. Specifically, the data generated—mRNA sequencing, single-cell mRNA sequencing, and ATAC-sequencing data at multiple time points and from various somatic cells—represents a valuable resource for understanding the gene dynamics underlying cDC1 reprogramming and specification. Zimmermannova et al. have advanced cDC1 reprogramming toward clinical application. Moreover, these extensive datasets will help answer critical questions, such as why some cancer cells reprogram more efficiently than others and how the cDC1 antigen processing and presentation machinery influences the quality of peptides displayed on the surface of reprogrammed cells.

In summary, converting tumor cells into immune cells that excel in antigen presentation represents an innovative therapeutic alternative, paving the way for the development of a novel cancer immunotherapy modality. This approach reverses the tumorigenic potential of cancer while promoting antigen presentation to enhance the antitumor activity of $CD8^+$ T cells. Building on this study, efforts to reprogram cancer cells in situ using adenoviral vectors are already spearheading the development of the next generation of cancer immunotherapies (Ascic et al., 2024, Science).

PAPER DISCUSSED

Zimmermannova O, Ferreira AG, Ascic E, et al. Restoring tumor immunogenicity with dendritic cell reprogramming. *Sci Immunol.* 2023;8(85):eadd4817. doi:10.1126/sciimmunol.add4817

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