VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: Looking outside the gut-brain axis: is liver a novel player in AD and PD?

Applicant: Colucci Rocchina

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered?*
- *This project has perspectives for **international collaborations, applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The project aims to evaluate the effect of a rotenone inhibitor on the development of AD and PD, in order to better assess the involvement of the liver-gut-brain network. The rationale of the project is clear, but the sequence of the experiments and methodologies can be better explained. The team aim at the characterization in 2 different animal models, which might be ambitious. The risks have been evaluated and the backup plans described. The project does not involve other components of the DSF. The idea of this project can serve as starting point to submit competitive funds but needs rewriting.

Reviewer n. 2

The project has significant scientific relevance, as there is growing evidence of the liver's role in brain function, though a definitive connection has not yet been established. Therefore, in my view, the project is both original and innovative. The department's know-how is not provided, neither for the Padua group nor for the Pisa group, other than the indication that they can conduct these experiments. Only two operational units are mentioned, without any description of their merits or prior experience.

The project could have a significant impact on the study of PD and AD, as it would suggest a key role for the liver in systemic inflammation and, consequently, in CNS health. In this regard, it would provide baseline data on liver condition in mouse models of AD and PD. Moreover, it could offer functional insights into the role of the NLRP3 inhibitor in systemic inflammation.

In my view, the plan could be realistically feasible, but the various steps of the study are not clearly outlined and remain very vague.

The methods and materials could have been further detailed and clarified. For example, the number of mice intended for use in the experiment is not reported, making it difficult to assess the statistical power the study might achieve. It is also not specified how the data will be analyzed, as the authors merely state that analyses and correlations will be performed.

All analyses are conducted on liver and blood samples, but there is no parallel assessment of what may occur at the CNS level, as no experiments are included to document its involvement. The PD mouse model is chosen to simulate late-stage disease, while the AD model is used to mimic early stages of neurodegeneration. In my view, it would have been more appropriate to use a single disease model in both early and late stages and investigate that in greater depth. Furthermore, the authors plan to use 'a gut-directed specific NLRP3 inhibitor,' but its mechanism of action and the rationale for its targeted activity are not explained. The contingency plan does not appear to have been thoroughly considered, as no information is provided regarding the mice or their housing conditions. The treatment planned for the PD mice could be harmful and potentially lead to their death, preventing the collection of biological material needed for the experiment. The authors merely state that 'The arrangement of WPs allows us to monitor the progress of

the project in real time', without clearly explaining the procedure. This statement is vague and provides no meaningful clarification.
The project is not well structured and does not clearly outline the research steps, although it does have considerable potential given the underlying hypothesis.

Reviewer n. 3

While the proposed project appears scientifically valid, it is complicated (from my standpoint at least) to fully understand the level of innovation and originality brought from the proposed activities. Material and methods are available, but it is hard to fully evaluate the plan and its feasibility, also due to the way it is written and elaborated (in some part it appears as a cut/paste exercise). The risk and mitigation strategy are not clear as well and, for example, a review of the initial hypothesis cannot be considered a mitigation action.

Reviewer n. 4

The project addresses a highly relevant and emerging topic: the potential involvement of the liver within the gut–brain axis and its contribution to the pathogenesis of Alzheimer's and Parkinson's disease. The conceptual framework is well grounded in recent literature, and the proposed integration of peripheral and central mechanisms is scientifically interesting and innovative.
However, several limitations significantly affect the overall robustness and feasibility of the scientific plan:
- Overambitious scope: The project proposes an extremely large number of endpoints, methodologies, and analyses in two different animal models within a relatively short timeframe and limited budget. This raises substantial concerns regarding feasibility and prioritization of objectives.
- Insufficient methodological detail: Although many techniques are listed, the methodological description remains mostly narrative. Essential experimental parameters (sample size per group, randomization procedures, blinding, inclusion/exclusion criteria, replicates, expected variability) are missing. This prevents a real assessment of the reproducibility and reliability of the proposed work.
- Lack of statistical analysis plan: No statistical strategy is provided. There is no indication of the tests to be used, how multiple comparisons will be handled, or how the data structure will be managed. This represents a major weakness for a project heavily reliant on quantitative biological endpoints.
- No power analysis: The absence of any sample-size justification based on statistical power prevents evaluation of whether the proposed experiments will be adequately powered to produce scientifically meaningful outcomes.
Collaboration with the partner unit (UNIPI) insufficiently described: While the presence of two research units is mentioned, the operational structure of the collaboration is vague. The division of responsibilities, harmonization of protocols, quality control, and coordination are not adequately explained.
- Risk assessment is present but superficial: The risks listed are plausible, but mitigation strategies remain general and lack quantitative or procedural detail.
- Potential for future development: The topic is promising and could indeed lead to competitive funding, but this potential is weakened by the methodological gaps.
Overall, the project is conceptually strong and innovative but requires substantial refinement in experimental design and strategic planning to ensure feasibility and scientific rigor.

Competence and expertise of the applicant.

- *What are the **merits and scientific expertise of the applicant**?*
- *Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1

Prof. Colucci is associate professor in pharmacology, with a solid publication record, including several papers on gut microbiota and its correlation with Alzheimer' and Parkinson' diseases. There is no doubt that she has the expertise to lead this project.

Reviewer n. 2

The applicant has solid expertise in gastrointestinal pharmacology, inflammation, and neurodegenerative diseases, with research focused on the mechanisms linking intestinal and neurodegenerative conditions.

She also has experience studying the NLRP3 inflammasome showing appropriate merits and scientific expertise.

Reviewer n. 3

The applicant has a long experience and scientific competence in the pharmacology of the area of interest, which are considered appropriate and sufficient for the proposed project. However, the proposal is not properly presented, and this is under applicant's responsibility. It appears indeed difficult to understand the level of innovation and originality, together with the effective feasibility of the project.

Reviewer n. 4

The applicant demonstrates solid expertise in pharmacology, neurogastroenterology, neuroinflammation, and experimental models of neurodegenerative diseases. The publication record is strong and highly relevant to the project's aims, and the PI has clear experience with the molecular and histological techniques required. However, the methodological weaknesses in the proposal—particularly the absence of a statistical and power-analysis framework—raise some concerns about the PI's approach to quantitative experimental planning. Despite this, the applicant's background and scientific track record are highly suitable for leading a project of this thematic nature.

Competence and expertise of the research team.

- *Does the research **team bring complementary expertise** to the project?*
- *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*

Reviewer n. 1

the team comprises the PI and a research unit from the university of Pisa (associate professor and resident) who will perform the in vivo experiments.

Reviewer n. 2

The research team possesses the appropriate and complementary expertise needed to successfully carry out the proposed research programme. The project does not involve any international research collaborations.

Reviewer n. 3

While team members have good expertise there is not a clear complementarity with the applicant's and this is again reflected in the proposal structure and quality.

Reviewer n. 4

The research team includes complementary expertise across two institutions, with UNIPD contributing molecular and ex-vivo analyses and UNIPI providing competence in in-vivo experimentation and histology. The scientific profiles of both units are coherent with the proposed work.

However, several issues reduce the perceived strength of the collaborative structure:

- The collaboration plan is insufficiently detailed: workflow, data sharing, standardization of protocols, animal-handling coordination, and joint quality-assurance processes are not clearly specified.
- The large volume of techniques and analyses proposed raises concerns regarding the actual personnel availability and operational capacity required to complete all work packages.
- While international collaborations are mentioned as a possibility, no concrete partnerships or networks are described.

Overall, the team possesses the necessary scientific expertise, but the operational planning of the collaboration needs clearer articulation to ensure effective execution of the proposed research.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE INTERNA

Project: Looking outside the gut-brain axis: is liver a novel player in AD and PD?
Applicant: Colucci Rocchina

Punti di forza:
Il progetto affronta una tematica di interesse nel campo delle patologie neurodegenerative, inserendosi nel filone di ricerca emergente sull'interazione tra organi periferici e sistema nervoso centrale. L'approccio multidisciplinare proposto e l'utilizzo di modelli murini già consolidati indicano una buona padronanza tecnica delle metodologie sperimentali previste

Criticità
Il piano di lavoro risulta molto ambizioso e articolato, ma al tempo stesso ampio e non sempre sufficientemente focalizzato. Il numero elevato di parametri molecolari, funzionali e istologici, pone interrogativi sulla fattibilità di completare tutte le attività nei 24 mesi previsti. Nonostante il PI si proponga di supervisionare l'intero progetto, buona parte di esso è condotto in collaborazione con l'Università di Pisa, dove verrà svolta la maggior parte delle attività sperimentali.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: LSD for Nicotine Dependence and Comorbidities: Uncovering Neurobiological Mechanisms and Therapeutic Feasibility in a Preclinical Model

Applicant: Comai Stefano

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered?*
- *This project has perspectives for **international collaborations, applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The project aims at elucidating the tole of psychedelic drugs on the nicotine dependence, in order to find possible therapeutic strategies. The plan is ambitious, as it involves 2 long term in vivo studies, but the know-how of the research team is consolidated. The team is international, and the data obtained can be applied to other funding applications and to expand the research network. The risks and possible backup plan have been evaluated.

Reviewer n. 2

The proposed project concerns an area of strong medical need, which in spite of strong efforts at both an academic and industrial level, has not yet generated the desired results; the proposed approach may generate innovation, but at the same time has un underestimated high risk of failure, particularly in the definition of effective required microdose and the related therapeutic window (in my opinion this aspect is not properly approached in the proposal). Also, one question remains open from my point of view: is there an implicit risk of overdosing in the use of a psychedelic substance as drugs? On the other hand, the plan appears scientifically valid and feasible, with all the material and methods available, and based on a solid know-how. Also, there is a proper network in place, with an international collaboration already established. Finally, the plan is overdetailed for what concerns the experimental methods, and in my opinion the time dedicated to the project by the individual scientists is low in view of the described activities and the desired results.

Reviewer n. 3

The project is scientifically innovative and explores an aspect that is both highly relevant and significant at a global level. The approach is novel, and preliminary data suggest that developing experiments in this direction could yield results capable of addressing a currently much-debated therapeutic gap.

The project is built on departmental know-how, as well as on additional national and international collaborations. It can be realistically carried out and has the potential for significant future impact. If the results are adequate, it could open new strategies for addressing nicotine withdrawal, a problem that persists worldwide. The study has already been approved by the Ethics Committee of the University of Padua and by the Ministry of Health.

The work packages (WPs) are clearly presented, as are the materials and methods of the experiments, making them understandable even to non-specialists. A strong point is the consideration of sex differences, as different psychological effects are acknowledged in order to evaluate distinct mechanisms across sexes. The study is well described. However, among the elements and methods for evaluating the results, nothing is reported that differentiates the effects of a single dose of LSD from those of microdosing.

The risk assessment and contingency plan are well-structured, and address all identified concerns.
The project could yield innovative findings with translational impact. For this reason, it holds promising application prospects, as it addresses a global issue: nicotine withdrawal symptoms. The project also benefits from well-established international collaborations, which further strengthen the application.
The project has a strong start-up research character, making it attractive for competitive funding due to its enhanced translational relevance.

Reviewer n. 4

The project addresses an important topic — the potential use of LSD to reduce nicotine dependence and its psychiatric comorbidities. The mechanistic questions are scientifically relevant, and the integration of behavioral, electrophysiological, and neurochemical approaches is methodologically strong. The project is also innovative in comparing microdosing versus a full psychedelic dose and in including sex-dependent analyses.
However, several major limitations affect the **translational impact and feasibility**:

- **Low translational plausibility of the central hypothesis**: LSD is unlikely to become a clinically acceptable treatment for nicotine dependence due to substantial safety, ethical, and regulatory barriers. This significantly limits the clinical relevance of the proposed work, even if the preclinical effects are positive.
- **Use of oral nicotine self-administration (OSA) as the addiction model raises concerns**. Although OSA can model voluntary consumption, it is not a gold-standard paradigm for nicotine addiction. Intravenous self-administration (IVSA) is the most validated method to assess reinforcing properties and drug-seeking behavior in rodents.
- **Pharmacokinetic limitations of oral nicotine**: Nicotine is subject to extensive first-pass metabolism**,** making brain exposure highly variable and uncertain. Without direct quantification of nicotine levels, the robustness of the addiction phenotype is questionable.
- **Uncertain mechanistic interpretation**: If nicotine intake is inconsistent or sub-threshold due to metabolic variability, it becomes difficult to attribute behavioral or neurophysiological changes to addiction- or withdrawal-related states.
- **High conceptual interest but limited therapeutic relevance**: The main strength of the project lies in its potential to elucidate **serotonergic and dopaminergic mechanisms**, rather than in its ability to propose a realistic therapeutic strategy for smoking cessation.

Overall, the project is **mechanistically valuable but translationally weak**, and the choice of model significantly limits the validity of the conclusions.

Competence and expertise of the applicant.

- *What are the **merits and scientific expertise of the applicant**?*
- *Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1

Stefano Comai is associate professor in Pharmacology, with a strong know-how in exploiting behavioural models to elucidate mechanisms of neurodisorders. The publication record demonstrate that he has the capability to successfully complete this project.

Reviewer n. 2

The applicant's expertise and competence are well documented and supportive for the proposed project; however, the presented plan raises a few questions (see above) which should have been anticipated by the applicant.

Reviewer n. 3

The applicant has excellent expertise and merits. His research focuses on the neurobiological mechanisms of psychiatric disorders and the development of novel therapeutic strategies. The characteristics of the applicant are absolutely in line and appropriate for the proposal project.

Reviewer n. 4

The PI has long experience and sufficient expertise to carry out the proposed work.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

Competence and expertise of the research team. - *Does the research **team bring complementary expertise** to the project?* - *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*
Reviewer n. 1 The team comprises an international collaborator (McGill University, Canada), one Italian collaborator (San Raffaele) and 1 group of the DSF. The international consolidated network, with broad expertise in the topic, can successfully fulfil the proposed research. **Reviewer n. 2** The team is well structured and has also an international profile already in place. **Reviewer n. 3** The research team possesses complementary expertise that aligns well with the project objectives. Furthermore, the group's international collaborations could contribute to the project's success and facilitate the dissemination of its results. **Reviewer n. 4** The project is based on a solid collaborative effort with personnel expert in this research domain.

COMMISSIONE INTERNA

Project: LSD for Nicotine Dependence and Comorbidities: Uncovering Neurobiological Mechanisms and Therapeutic Feasibility in a Preclinical Model
Applicant: Comai Stefano

Punti di forza:
Il progetto risulta interessante poiché affronta la problematica della dipendenza da nicotina e le sue comorbidità psichiatriche, proponendo un approccio innovativo basato sull'impiego di LSD, in particolare attraverso il paradigma del microdosing. Positiva la collaborazione internazionale.
Criticità:
Il piano di lavoro è molto articolato, con un numero elevato di test comportamentali e analisi neurobiologiche che richiederanno una gestione estremamente efficiente dei tempi e delle risorse. L'impegno dichiarato del PI e del team non appare pienamente congruo con la fattibilità complessiva del progetto. Inoltre, non sono riportati le specifiche né il codice del progetto per la sperimentazione animale.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: Monoamine oxidase inhibitors to hit a key player in neuroinflammation associated with neurodegenerative diseases

Applicant: Dalla Via Lisa

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered?*
- *This project has perspectives for **international collaborations, applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The aim of the project is to evaluate the inhibitory effect of synthetic and natural derivates as MAO-B inhibitors. There are 5 WP, which can be a lot in 2 years, but the research team components and expertise are commensurate to the workload. There is a clear risk assessment for each WP of the project (form very low to medium), and possible alternative plans are proposed. The international collaboration can surely promote further application to international funds, and in general improve the outreach of the projects.

Reviewer n. 2

The proposal fits some of the required features, but miss some others, like originality and potential for innovation (quite low). Materials are available and methods in place, together with a solid background and know-how, particularly from the pharmacological point of view. However, it remains quite unclear whether the objective is indeed to run a mechanistic study or to identify novel and selective MAO-B inhibitors. With reference to the second point, I doubt that this may be achieved by studying a small set of compounds, even though preliminary data indicate their activity as inhibitors, and considering the impressive mass of data already generated in the years and reported in literature by a large number of groups. As well, the natural product approach will hardly generate novel hits, and this part of the work seems not well integrated in the proposed projects. More specifically, little attention is given to possible issues deriving from low and non-selective MAO-B activity, and this minimally considered also in the risk analysis.

Reviewer n. 3

The project is not scientifically significant due to its focus on developing affordable treatments for neurological diseases already present. While it may not be entirely original or innovative, the research could have the potential to identify new compounds to counteract neurodegenerative disorders.

The project leverages the departmental know-how, involving three research groups from the University of Padua, particularly two groups from the Department of Pharmaceutical and Pharmacological Sciences. It could have a significant impact on future developments, as the identification of new compounds to counteract neurodegenerative diseases is highly sought after and has the potential to address the clinical need for anti-inflammatory neuroprotective drugs.

The study plan is well detailed but also highly complex, encompassing numerous experiments and work packages over the two-year project period. Nevertheless, the proposal brings together a team of experts with complementary expertise and there is an international collaboration with the University of Colima (Mexico). The work packages (WPs) are well presented, and each experiment is clearly explained, allowing a full understanding of the intended objectives. The principal investigator has already obtained the necessary authorizations for animal cell culture work.

The contingency plan is well developed, with numerous risks identified and specific measures outlined to address potential issues.
The project is based on collaboration between three units of the University of Padua (two from the Department of Pharmaceutical and Pharmacological Sciences and one from the Department of Molecular Medicine) and the University of Colima (Mexico), thereby establishing international collaborations.
Furthermore, the project is strongly oriented towards new applications and networking.
The project clearly has a start-up research character and the potential to attract funding, given the urgent clinical need addressed and the necessity to discover new anti-inflammatory neuroprotective drugs for the treatment of neurodegenerative diseases.

Reviewer n. 4

The project focuses on the development and characterization of novel MAO-B inhibitors for neuroinflammation associated with neurodegenerative diseases. The biochemical rationale of targeting MAO-B in reactive glia is well described, and the PI provides a strong medicinal chemistry and enzymology background. The integration of synthetic compounds and natural products is also methodologically comprehensive.
However, the proposal is significantly limited by issues concerning **novelty, translational value, and clarity of objectives**:
Limited novelty of the scientific concept: MAO-B inhibitors have been extensively explored for decades, and **multiple MAO-B inhibitors (rasagiline, selegiline, safinamide)** are already in clinical use. Their biological profile, safety issues, and mechanistic impact on neuroinflammation have been well documented. The project does not sufficiently articulate what makes these newly synthesized compounds fundamentally different or capable of overcoming the known limitations of current MAO-B inhibitors.
Weak translational rationale for Alzheimer's disease:
Although MAO-B levels may be elevated in AD, **clinical MAO-B inhibitors have not demonstrated efficacy in slowing cognitive decline in AD patients**, and no convincing evidence supports MAO-B inhibition as a disease-modifying strategy in Alzheimer's disease. The proposal nonetheless positions these inhibitors as a potential treatment for AD without addressing this significant discrepancy with clinical data.
Lack of focus on clear, biologically meaningful endpoints:
The proposal describes a wide range of in vitro assays (MAO kinetics, cytotoxicity, inflammatory markers, oxidative stress, NF-κB signaling), but the **overall endpoint strategy remains diffuse**. It is unclear how hits will be prioritized beyond MAO-B inhibition, and no clear criteria are provided for defining a "lead compound" beyond enzymatic potency and reduction of inflammatory markers.
Scientific impact limited to incremental medicinal chemistry optimization:
The work may yield new scaffolds or structure–activity relationships, but its potential to change therapeutic strategies for neurodegeneration is modest.
Overall, the project is methodologically well structured but conceptually and translationally limited, largely due to the mature nature of the MAO-B target and the absence of a compelling rationale for its relevance in AD.

Competence and expertise of the applicant.

- *What are the **merits and scientific expertise of the applicant**?*
- *Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1

Prof. Lisa Dalla Via is associate professor in Medicinal Chemistry, with a consolidate track record of publication regarding the mechanism of action of active molecules, including MAO inhibitors. There is no doubt that she has the capability to lead the project and coordinate the research group.

Reviewer n. 2

Applicant's expertise and competence is valid in supporting most of the proposed activities. However, I found some unclear planning and description of both activities and objectives, and these raise some questions in terms of feasibility, particularly from a drug discovery point of view.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

Reviewer n. 3
The applicant demonstrates the expertise and competence required to carry out the project. Moreover, she collaborates with three different research groups—two from the University of Padua and one from the University of Colima in Mexico—demonstrating her ability to engage in international collaborations.
Reviewer n. 4
The PI has the background to carry out the proposed work.

Competence and expertise of the research team.
- *Does the research **team bring complementary expertise** to the project?*
- *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*

Reviewer n. 1
The team includes 2 research groups of UNIPD (other than the proponent), and an international collaboration, with the University of Colima. The teams have a broad and complementary expertise. The research group includes 2 early-stage researchers from Padova and 1 form the partner university.
Reviewer n. 2
There is appropriate complementarity amongst team members; however, the two side (small molecules and natural products) appears not completely integrated within the project plan.
Reviewer n. 3
The research group is well-structured and demonstrates complementary expertise necessary to carry out the research program described in the project. Moreover, the involvement of the University of Colima, Mexico, gives the project an international dimension and could contribute to its overall success.
Reviewer n. 4
The Team is well integrated and the knowhow to carry out the project are present.

COMMISSIONE INTERNA

Project: Monoamine oxidase inhibitors to hit a key player in neuroinflammation associated with neurodegenerative diseases

Applicant: Dalla Via Lisa

Punti di forza:
Il progetto è scritto in modo sufficientemente chiaro e lineare. Il team è internazionale ed è sufficientemente specificato il ruolo di ciascun componente.

Criticità:
L'approccio si basa su saggi standard, non su tecniche avanzate. La descrizione delle implicazioni del progetto risulta piuttosto generica. Il progetto presenta alcuni spunti propositivi che non sempre risultano sufficientemente integrati in un quadro progettuale coerente.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: Ultrasmall Copper Clusters as Tumor-selective Catalyst for Enhanced Chemodynamic Therapy and Radiotherapy

Applicant: Gandin Valentina

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered**?***
- *This project has perspectives for **international collaborations**, **applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The project is focused on enhancing the synthetic methodologies for phosphine–Cu derivatives with an emphasis on sustainability. The resulting Cu(I) complexes will undergo comprehensive characterization to assess their physico-chemical and pharmacological properties. Both the project plan and timeline are well-defined, and the chosen methodologies are suitable for evaluating the synthesized compounds. Since the proposed materials closely resemble previously studied systems, the overall risk is low; nonetheless, potential minor challenges have been considered. Overall, the work appears to represent a consolidation of existing data rather than an initial exploratory investigation.

Reviewer n. 2

The research proposal is very clear and well written; all the required features are essentially met, and the proposed work is based on a solid group's know-how, with a potential for a further expansion and start-up research in case of positive outcome. There are though some minor question marks concerning the project organisation and comments I think useful submitting to the team:

Three university are mentioned, but only two are explicited

To make the project a bit more ambitious and to decrease the overall risk, could it be useful to consider some more phosphine scaffolds?

The risk of radiosensitising efficiency is perhaps underestimated due to the lack of a specific protocol

The presence of a specialist in in-vitro biology could help in designing/discussing the relative tests and results

Reviewer n. 3

The project demonstrates high value in terms of scientific relevance and innovation, as it aims to optimize copper clusters for chemodynamic therapy (CDT) and radiotherapy (RT), developing new compounds to combat various types of cancer and overcome issues related to therapy resistance.

The project effectively leverages departmental know-how, with experiments organized to utilize the complementary expertise of the research groups involved. It has the potential for significant future impact and considers the sustainability of the experiments. While the plan appears realistically feasible, the description of the research program lacks some information needed to define the project timeline and to assess whether the experiments can be completed as scheduled.

I do not have the expertise to evaluate WP1 and WP2. However, the various reported work packages (WPs) are generally well described, guiding the reader through the experimental workflow. However, in some cases, the methodology is not detailed enough. For instance, in WP3, the specific cell types used are not reported; the text only refers to cancer-free or tumoral cells or generic organoids, without specifying the

analyses planned to derive mechanistic insights. This lack of detail prevents assessment of the number of cell types and organoids required to evaluate the project's feasibility.
WP0 appears to rely solely on the principal investigator, without considering the support of the overall team for Task 0.3: Risk Management.
Milestones and the timeline are appropriate and consistent with the deliverables.
The risk assessment is well structured; however, some aspects are presented in a generic manner, and the contingency plan is not fully detailed.
The project's aims could foster collaborative networks, including at an international level, attracting interest in advancing metal-based clusters toward late-preclinical and clinical development as innovative anticancer strategies.
The project's aims have the potential to generate opportunities for commercial exploitation, giving it a start-up character and the ability to attract funding. The results could lead to patents of potential interest from multiple perspectives.

Reviewer n. 4

The project presents a scientifically solid chemical framework aimed at developing ultrasmall copper clusters for chemodynamic therapy (CDT) and radiotherapy enhancement. The chemical synthesis is very well articulated, and the preliminary results demonstrate strong rationale for further exploration. The proposal is conceptually appealing and positioned at the intersection of medicinal inorganic chemistry and nanotechnology, where the PI and team have strong expertise.
Limitations and weakes

Biological assays are poorly outlined.

In WP3 ("Efficacy in cell models"), the proposal states that efficacy and toxicity will be tested *"on a wide panel of human cancer cell lines"*, but no specific cell lines are identified. Likewise, non-tumor controls and resistant models are mentioned only in general terms. Without specifying cancer types, phenotypes, or justification for model selection, it is difficult to assess whether the biological evaluation is coherent and adequate.

Lack of detail on biological methodologies.

Although the proposal mentions that "standard colorimetric/fluorimetric assays (MTT, SRB, NR, ADH)" will be used, no explicit testing pipeline, viability criteria, exposure times, or quality control measures are provided. Moreover, the mechanistic studies (Task 3.3) list an extensive array of endpoints (ROS probes, damage markers, organelle stress, immunogenic cell death markers), but again without methodological structure or prioritization.
Overall, the biological plan lacks operational clarity.

Feasibility of biological WPs is uncertain

Given the lack of methodological detail, undefined cell lines, and limited biological expertise, it is difficult to assess whether the project can complete the full mechanistic and radiobiology characterization described in WP3.
Despite these limitations, the chemical component of the project is strong and innovative, and the preliminary synthetic work is convincing. The project is likely to generate valuable chemical insights, but the biological evaluation may not reach the depth needed to support robust structure–activity conclusions or translational claims

Competence and expertise of the applicant.

- *What are the **merits and scientific expertise of the applicant**?*
- *Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1

Valentina Gandin is associate professor of medicinal chemistry at the DSF, with a consolidate record of publications. Her research focuses on the development of anticancer agents, in particular metal-based agents. There is no doubt that she can lead the project, coordinate the research group in order to achieve the completion of the project.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

Reviewer n. 2
The applicant has a long-lasting experience and competence to properly support the proposed activities from both the experimental and theoretical standpoints. Also, as evident from the project plan, she has a good managerial approach in coordinating the team activities.
Reviewer n. 3
The applicant has demonstrated merits and scientific experience, as evidenced by numerous publications and grants in which she serves as principal investigator.
The qualifications and experience reported in the applicant's CV are sufficient and appropriate to carry out the proposed project.
Reviewer n. 4
The PI shows sufficient expertise to carry out the proposed work.

Competence and expertise of the research team.
- *Does the research **team bring complementary expertise** to the project?*
- *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*

Reviewer n. 1
The research team does not include international collaborations, but it comprises research groups of the DSF and of the University of Roma Tre. The expertise is complementary. The team includes 2 Ph.D. students.
Reviewer n. 2
The team is well organised and members have complementary experience. As mentioned above, a more specialistic experience for what concern the biological experimental activities could be of help, above all in case of positive results.
Reviewer n. 3
The research team has complementary expertise sufficient to cover the entire project plan, but no international collaborations are included.
Reviewer n. 4
Team largely chemistry-oriented, with limited biological expertise.
The PI's expertise is primarily synthetic and structural chemistry, strongly supported by the preliminary data. However, the biological component—representing a substantial portion of the project—is not backed by personnel with extensive experience in cancer biology, mechanistic cellular assays, or radiobiology. Only one team member appears to have relevant biological background, and even then not specifically in advanced cancer cell models or radiotherapy-associated assays. This imbalance may affect feasibility.

COMMISSIONE INTERNA

Project: Ultrasmall Copper Clusters as Tumor-selective Catalyst for Enhanced Chemodynamic Therapy and Radiotherapy

Applicant: Gandin Valentina

Punti di forza:
Il progetto è descritto in modo lineare e scorrevole. Sono presenti dati preliminari. Il team presenta competenze complementari.

Criticità:
L'analisi dei rischi come anche la descrizione di metodi e modelli sperimentali non risultano sempre adeguate.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: From Binding to Function: Identification and Optimization of CXC-type chemokine receptor 4 (CXCR4) Agonists for Neuromuscular Junction Regeneration in Amyotrophic Lateral Sclerosis (ALS) Therapy

Applicant: Mattarei Andrea

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered?*
- *This project has perspectives for **international collaborations**, **applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The project concerns the identification of CXCR4 agonists (small molecules with clinical applicability) at therapeutic strategy of ALS. The proposal presents preliminary data, which strengthen the feasibility of the project, which is articulate in 3 aims, which a clear research plan. There is an extensive risk analysis for each of the steps of the project, with the first aim resulting the most problematic. There is a clear explanation of contingency plan. The possible results can surely serve as starting point for future applications.

Reviewer n. 2

The project is highly innovative and exceptionally challenging. It demonstrates strong elements of originality and innovation. The results of the project could have a significant impact on future developments, given the substantial unmet needs in ALS treatment. The plan is ambitious and challenging, yet realistically feasible.

This section of the project is clearly reported and well described, allowing the reader to gain deeper insight into the project workflow. The milestones and timeline appear to be appropriate.

Given the challenging nature of the project, a contingency plan is provided, and it is well structured.

Indeed, the main risk associated with the initial phase of the project lies in the possibility that none of the selected CXCR4 binders will exhibit the desired functional agonist activity.

The project offers strong prospects for international collaborations, practical applications, and networking opportunities.

The proposed project addresses a critical unmet need in amyotrophic lateral sclerosis (ALS) by targeting the earliest site of pathology—the neuromuscular junction (NMJ)—through a regenerative pharmacological strategy. As such, it has a strong start-up potential and is well positioned to attract funding.

Reviewer n. 3

The proposed project addresses a critical unmet need (ALS) by an innovative and quite original approach, involving a regenerative pharmacological strategy. The plan appears feasible and materials and methods are available. The project has an intrinsic high risk being based on a single source of test compounds, which however is quite robust and should provide at least some initial hit. In case of failure of the Aim 1, the recovery strategy (analogues of NUCC-390) in my opinion is quite weak; this compound has already been optimised, and I doubt on the possibility of identifying a novel IP space in a short period. On the other hand, should the proponent have already identified potential IP space, I suggest this be considered a priority activity for the optimisation work. Furthermore, it should be clarified whether the effective target of the project is one or more chemotype leads; or one lead and potential back-ups from the same chemotype. This

is indeed not completely clear going through the different sections. As well, the list of in-silico activities should have been written in a more concise and logic mode. Finally, the proposal is based on a solid know-how of the participating groups as well as on a solid collaboration between them.

Reviewer n. 4

This is an ambitious and scientifically compelling drug-discovery proposal addressing a clinically urgent and mechanistically well-justified target in ALS. The concept of leveraging the *regenerative window* at the neuromuscular junction (NMJ) to delay motor decline—supported by the group's strong preliminary data. It is highly innovative, and the use of DNA-encoded library (DEL) screening represents a powerful, state-of-the-art strategy to identify novel CXCR4 agonists.

The proposal also benefits from excellent methodological integration:

DEL high-throughput screening,**nanodisc-embedded CXCR4 SPR affinity profiling**, **functional signaling assays in primary motor neurons**, **axonal growth assays**, and a **computational + medicinal chemistry SAR campaign**.

However, despite its conceptual strength, the project appears **overambitious for the 24-month PRID duration**. Specifically:

Overly broad scope within limited time

The project attempts to progress through the full early drug-discovery pipeline—from identifying hits in a 4.2-billion compound DEL, to functional screening, to design–synthesis–testing cycles of SAR optimization. Even with the strong team and the cost-free DEL access, this workflow usually requires multiple iterative rounds and dedicated medicinal chemistry resources over several years.High density of experimental tasks

AIMs 1–3 include:

SPR kinetic profiling on two platforms,

primary motor neuron cultures and multiple signaling assays (Ca^{2+}, cAMP FRET, ERK1/2 WB),

microfluidic axonal elongation assays,

extensive computational modeling (docking + MD),

synthetic routes to produce analogue libraries (Task 3.1),

evaluation of ADMET and drug-likeness.

This number of parallel, highly specialized tasks risks exceeding the effective capacity of the two-year PRID framework.

Dependency on external deliverables

The DEL screening and resynthesis (via WuXi AppTec) involves external timelines that may not be fully predictable (Task 1.3). Any delays here would compress the already tight validation and optimization schedule. Despite these concerns, the project is **methodologically rigorous**, based on strong preliminary data, and positioned toward an impactful therapeutic innovation in ALS.

Competence and expertise of the applicant.

- *What are the **merits and scientific expertise of the applicant**?*
- *Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1

The PI is associate professor in medicinal chemistry with consolidate expertise in the field of medicinal chemistry, including the synthesis of agonist of the CXCR4 Receptor.

Reviewer n. 2

The applicant has an excellent CV, clearly demonstrating strong merits and scientific expertise.

The applicant's merits and scientific expertise are fully appropriate for coordinating this research group.

Reviewer n. 3

The proponent has a solid and well documented experience covering most of the proposed activities. The overall risk of the project is not completely estimated, and this is under the proponent's responsibility

Reviewer n. 4

The PI has the expertise to carry out the proposed work and to coordinate the team assembled.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

Competence and expertise of the research team. - *Does the research **team bring complementary expertise** to the project?* - *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*
Reviewer n. 1 The research team comprises 3 Ph.D. students and 5 PI from the DSF and another Department of UNIPD. The team has a broad and complementary expertise, that can manage the complexity of the proposed research. **Reviewer n. 2** The research team demonstrates strong expertise and complementarity, covering all aspects of the project. At present, the project does not involve any international collaborations. **Reviewer n. 3** Well integrated team with good network and potential for international collaborations. Each member ha the required competence to support all the proposed activities. **Reviewer n. 4** The team is very strong and well structured, with highly complementary expertise.

COMMISSIONE INTERNA

Project: From Binding to Function: Identification and Optimization of CXC-type chemokine receptor 4 (CXCR4) Agonists for Neuromuscular Junction Regeneration in Amyotrophic Lateral Sclerosis (ALS) Therapy

Applicant: Mattarei Andrea

Punti di forza:
Il progetto presenta una solida base scientifica, con approcci innovativi e ben mirati. Materiali e metodi sono ben descritti e appaiono fattibili. Il team presenta competenze definite e complementari.

Criticità:
Il progetto appare piuttosto ambizioso, considerato il finanziamento disponibile. Il numero di attività è elevato, con concreto rischio di non rispettare le tempistiche previste.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: Peridroplet Mitochondria: Key Players in Cisplatin Resistance of Gynecological Cancers

Applicant: Montopoli Monica

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered?*
- *This project has perspectives for **international collaborations**, **applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The project evaluates the role of mitochondria in the resistant mechanisms to cisplatin in gynaecological cancer. 7 WP are very ambitious, and impose a tight schedule. Several tasks are reported as “medium risk”, so feasibility can be a problem. The experiments are not well described; it is unclear if the proponent has the already the capability of performing them or if they need a complete new set up.

Reviewer n. 2

The proposed project is aimed at developing previous and interesting finding by the same research group; the proposed activities, while not greatly original, are scientifically significant and apparently feasible in the given timelines, possibly generating innovative results, which may in turn represent the basis for a clinical application and start-up research. Methods and materials are available, at least for starting the activities. The working plan is overall reasonable; however, it appears in most parts quite generic, and lack of specific details are evident (eg which small molecules/repositioning drugs are considered? Is any specific drug design plan defined? Who will run the bioinformatic activities? Etc). As well, the risk analysis is not extended to key milestones (while the possible failure in M1 is quoted, what about possible failures in M2, M3 and M4?). Finally, the team is expanded and involve collaborations at an international level. However, to attract further funds, I think a more ambitious target should be pursued (rational drug design) to possibly generate some IP

Reviewer n. 3

The project addresses an important topic in cancer therapy, the resistance to cisplatin. For this reason, understanding the underlying mechanisms is crucial, and the use of PDM provides an original and valuable approach.

The project does not describe the know-how of the applicant’s laboratory or of the collaborating teams, aside from the cell lines already available in the applicant’s lab. The project could have a significant impact in the future, and the proposed drug repositioning strategy may help accelerate potential clinical translation. However, the Materials and Methods section does not sufficiently detail all procedures, making it difficult to fully assess the feasibility of the project.

The Materials and Methods are presented superficially, without detailing the specific analyses to be performed. The described work packages outline the workflow but lack methodological detail. The methodologies to be used are not clearly reported. The milestones are generic and overly concise, and no deliverables are provided. The timeline appears appropriate.

The risk assessment and contingency plan are presented by considering the main problems that may be encountered during the project, including potential delays in experimental progress due to unforeseen technical issues or resource limitations.

The concept underlying the project has the potential to foster international collaborations and networking. If the results are promising, there will be opportunities to attract competitive funding.

Reviewer n. 4

The proposal addresses a highly relevant and clinically challenging problem: cisplatin resistance in gynecological cancers, focusing on the emerging role of peridroplet mitochondria (PDM) and lipid–mitochondria interactions in metabolic rewiring. This is a scientifically timely and original framework, well aligned with recent evidence indicating that metabolic plasticity drives chemoresistance. The PI presents a strong rationale supported by preliminary data showing differences in LD abundance, mitochondrial morphology, autophagy/mitophagy, and lipid uptake between sensitive and resistant lines.

While each WP is conceptually strong, the **scope is exceptionally broad for a two-year PRID**, covering an amount of work comparable to a multi-year, multi-grant program. Many work packages rely on extensive, labor-intensive methodologies (e.g., proteomics, lipidomics, metabolomics, mitophagy modulation, functional genomics via sh/siRNA), each of which could constitute a standalone project. The timeline (M1–M8, page 5) allocates only **3–6 month windows** for tasks that typically require much longer setup and iteration.

Main limitations:

1. Overambitious breadth of the experimental plan

The number of WPs (seven total) and the diversity of techniques—lipidomics, proteomics, EM, metabolic assays, RNA-seq, pathway analyses, 3D spheroids, screening for small molecule modulators—make it unlikely that all objectives can be completed within 24 months, even with skilled personnel.

2. High methodological load without clear prioritization

The project attempts to characterize:
PDM structure
PDM proteome
PDM lipidome
PDM involvement in mitophagy
Expression of multiple tethering proteins
Global metabolic rewiring (OCR, ATP, acetyl-CoA)
Transcriptomics of metabolic genes
3D functional validation
Bioinformatics discovery + model building

3. Heterogeneity of goals

Some WPs focus on mechanistic biology, others on therapeutic screening, others on computational integration. A more focused approach (e.g., concentrating on one mechanistic axis such as PDM–LD tethering and mitophagy) would strengthen coherence.

Despite these limitations, the scientific concept is compelling and the preliminary data solid, and the study could yield important insights if appropriately narrowed.

Competence and expertise of the applicant.
*What are the **merits and scientific expertise of the applicant**?*
*Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1

The PI is Associate professor, with good publication record, and clear expertise to lead the project

Reviewer n. 2

The Applicant's competence and expertise are valid concerning part of the activities. However, the overall proposal misses to provide important details concerning critical activities (eg bioinformatic and risk analysis) which are under the applicant's responsibility. Finally, the time dedicated to the project is not clearly defined,

Reviewer n. 3

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

The applicant does not describe her research activity in the CV, although it can be inferred from the attached publications. Furthermore, the applicant does not report having held any principal investigator positions in other projects. **Reviewer n. 4** The PI has the expertise to carry out the proposed work.
Competence and expertise of the research team. - *Does the research **team bring complementary expertise** to the project?* - *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*
Reviewer n. 1 The research team comprises two early-stage researchers (Ph.D. student and Postdoc) who will perform the activities. Furthermore, there are 2 components from other Italian Institutions with complementary expertise with the PI. **Reviewer n. 2** The team is well integrated concerning the pharmacological/medical aspects; however it apparently lacks drug discovery expertise as well as chemo- and bioinformatic competences **Reviewer n. 3** The team is composed of researchers with extensive experience and complementary expertise in cancer research and the study of pharmacological resistance. No information is provided to evaluate the complementarity of the team, and the project does not include any international collaborations. **Reviewer n. 4** The team is well assembled.

COMMISSIONE INTERNA

Project: Peridroplet Mitochondria: Key Players in Cisplatin Resistance of Gynecological Cancers

Applicant: Montopoli Monica

Punti di forza:
Il progetto affronta un problema clinico rilevante, quale la resistenza al cisplatino nei tumori ginecologici. L'attenzione alle interazioni tra lipid droplet e peridroplet mitochondria rappresenta un elemento di originalità, con il potenziale di identificare nuovi meccanismi molecolari e bersagli terapeutici per superare la resistenza ai trattamenti.

Criticità:
Il progetto prevede un piano di lavoro molto articolato, che richiederà una gestione attenta dei tempi e delle risorse. Il ruolo dei diversi partecipanti al progetto non è delineato in modo chiaro, così come non è dichiarato l'impegno temporale del PI. Il progetto non è di facile interpretazione e alcuni aspetti risultano confusionari.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: Development of a structural mass spectrometry-based assay to detect human transthyretin aggregates in the plasma of ATTR amyloidosis patients

Applicant: Spolaore Barbara

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered?*
- *This project has perspectives for **international collaborations**, **applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The project concerns the development of a methodology, it is very ambitious, with 4 tasks with a tight time-schedule, considering also the variability of the collection of samples from patients. It is based on preliminary data, and this mitigate the risk of failure. The team put together two groups of the DSF. If successful, this project has the potential to become the starting point for a start-up.

Reviewer n. 2

The proposed project is based on a good departmental know-how, is scientifically significant and with potential for innovation, while does not present any significant originality. It could also have an important impact on the development of diagnostic tools available at the clinical level in the field of interest. The project is feasible, with appropriate working plan and timelines, and materials and technologies are already available at the departmental level. The team is based on local competencies, but the members have an extended international and national network. I do not envisage great potential for supporting dedicated start-up research based in case of positive outcomes of the project (low potential for IP generation).

Reviewer n. 3

The project is original and innovative, as it aims to develop a methodology for diagnosing the disease through non-invasive blood analyses. It is significant given the possibility to diagnose the pathology severity by blood analyses.

The project leverages the departmental expertise, thanks also to up-to-date instrumentation and the availability of specialized facilities. The results of the project could have a significant impact on the future development and discovering of specific pharmacological treatments. Given the expertise of the involved research group, the proposed plan appears realistically feasible.

All these points are adequately addressed and clearly reported, following the experimental workflow and providing detailed insight into the experimental methodologies. Milestones are clearly outlined, and the proposed timeline appears appropriate.

The project is challenging and has highlighted some critical points that could be better addressed; however, a contingency plan has been established.

This project could offer opportunities for international collaboration, given the global interest in studying ATTR pathology.

Given the strong interest in ATTR research, the project holds potential for international collaboration, may attract funding, and could have the potential to evolve into a start-up initiative.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

Reviewer n. 4
The project addresses a highly relevant question in ATTR amyloidosis: the biochemical and structural characterization of circulating transthyretin (TTR) aggregates and the development of a proteolysis-based assay for their detection. The preliminary data are strong and support the rationale for extending the analysis to patient-derived samples.
However, several feasibility concerns and timeline uncertainties limit the robustness of the experimental plan:
1. Unclear timelines for patient recruitment and sample availability
The proposal requires plasma samples from multiple patient subgroups (ATTRwt-CM early onset, late onset, ATTRv-CM, healthy controls, and later therapy-treated patients).
Yet, no timeline is provided for when and how many patients will realistically be enrolled, nor is it clear whether the required sample numbers (at least 10 per group) can be collected early enough to feed into Tasks 1–4. Because many downstream analyses depend on timely availability of these samples—such as aggregate enrichment (Task 2) and limited proteolysis assays (Task 3)—any delay in recruitment could significantly undermine the feasibility of the entire project.
2. Task 4 relies on therapies that may not be available during the project window; it requires plasma samples from patients undergoing treatment with tafamidis and acoramidis..
This raises concerns about whether Task 4 can be completed as described, and whether meaningful comparisons between treated and untreated patients will be possible.
Given that ATTRwt-CM and ATTRv-CM are relatively uncommon conditions, predictable recruitment cannot be assumed.
Overall assessment
The proposal is scientifically important and methodologically sophisticated, with strong potential to advance understanding of ATTR amyloidosis. However, the feasibility is weakened by unclear timelines, reliance on patient samples with uncertain availability, and dependence on therapies not yet accessible

Competence and expertise of the applicant.
- *What are the **merits and scientific expertise of the applicant**?*
- *Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1
Associate professor in biochemistry, with good publication record and the expertise to lead the project
Reviewer n. 2
The applicant has a well-documented experience and competencies with respect to the proposed project and related experimental activities. She has directly contributed to the generation of preliminary results which represents the starting point for a further exploration towards the development of novel diagnostic detection methods.
Reviewer n. 3
The applicant has strong expertise in protein structure and dynamics, particularly using HDX-MS and limited proteolysis, with experience in studying transthyretin aggregation and protein–nucleic acid interactions. Combined with the team's expertise, the applicant is well-qualified to lead the proposed project.
Reviewer n. 4
The analytical expertise of the PI and the team is clearly well aligned with the proposed experimental approaches.

Competence and expertise of the research team.
- *Does the research **team bring complementary expertise** to the project?*
- *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*

Reviewer n. 1
The research team includes 2 groups of the DSF, with different expertise, and includes one doctoral student.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

Reviewer n. 2
The team brings the required complementarity to the applicant's expertise, while no international collaboration is declared
Reviewer n. 3
The team possesses complementary and excellent expertise. The project is not involved in any international collaborations.
Reviewer n. 4
The team shows the expertise to conduct the study.

COMMISSIONE INTERNA

Project: Development of a structural mass spectrometry-based assay to detect human transthyretin aggregates in the plasma of ATTR amyloidosis patients

Applicant: Spolaore Barbara

Punti di forza:
Il progetto è scritto in modo chiaro e comprensibile, con potenziali ricadute cliniche. Sono presenti dati preliminari. Buona composizione del team.

Criticità:
Il progetto è ambizioso e la fattibilità non emerge in modo chiaro. L'approccio non è innovativo. Non sempre risulta definito il ruolo dei singoli componenti.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE ESTERNA

Project: Advanced hybrid and targeted lipid-based systems for the delivery of plasmid DNA

Applicant: Grigoletto Antonella

General assessment of scientific quality and innovation - Assessment of scientific plan

- *Is the project **scientifically significant**, **original** and **innovative**?*
- *Is the project **built on a departmental know-how**? Has the project a significant **impact** for future development? Is the **plan realistically feasible**?*
- *Are the research **methods, materials, work packages, tasks, milestones and timeline appropriate** and in agreement with deliverables?*
- *Are the risk assessment and the contingency plan properly considered**?***
- *This project has perspectives for **international collaborations**, **applications, networking?***
- *Has the project the character of **start-up research** that can **attract in the future competitive and non-competitive funds**?*

Reviewer n. 1

The project concerns the development of lipid nanoparticles for the delivery of pDNA, with application as therapeutics for muscular diseases. Indeed, despite the wide use of LNP as mRNA delivery systems, the delivery to the nucleus of DNA-loaded LNP requires a different approach to the formulation. The research team has the adequate knowledge to develop and characterize the LNP, the proposed program is ambitious, but feasible in 24 months, considering the complementary know-how of the team. There are contingency plans for the crucial steps of the project, on the formulative side there are no doubt that Dr. Grigoletto has the expertise to tone the formulation to achieve adequate loading and to demonstrate preliminary *in vitro* efficacy. The results of the project can serve as preliminary data for further grant application, also in collaborative project with international groups.

Reviewer n. 2

The project will be carried out in accordance with the department's established expertise. The expected positive outcomes could have a substantial impact on future developments. The plan appears realistically feasible, although the scheduling of the in vitro and in vivo analyses seems constrained within an overly limited timeframe.

The research methods, materials, work packages, tasks, and milestones are clearly described; however, the timelines for WP4 and WP5 appear too limited to achieve the required results.

Risk assessment and the contingency plan appear to be appropriately addressed, also considering the nature of the applicant's contract.

It could contribute to expanding the existing know-how to further optimize LNPs. The project has a start-up character, and positive results could lay the foundation for a patent and for the development of new pharmacological approaches for the treatment of various diseases.

The project is both interesting and ambitious. The methodologies are clearly described and appropriately prioritized. However, the timeline could be better optimized, particularly for WPs 3 and 4, which are scheduled within an excessively constrained procedural period.

Reviewer n. 3

The proposed project is scientifically significant, sufficiently innovative and with some element of originality. It is based on the departmental know-how and feasible, with some potential and not completely estimated risk (the risk of failure in obtaining the desired targeted systems is not considered). Working plan and timelines are reasonable and material and methods are available, with external collaborations available in case. Finally, the positive outcomes of the project could generate an appropriate IP, which in turn could generate start-up research

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

Reviewer n. 4
This project aims to develop next-generation lipid nanoparticle (LNP) systems for safe and effective plasmid DNA delivery, overcoming current limitations such as poor endosomal escape and off-target liver accumulation. By integrating nature-inspired strategies the project seeks to enhance cellular uptake, intracellular trafficking, and site-specific release of genetic material. These advanced, biocompatible LNP formulations have the potential to enable long-term gene expression and lay the groundwork for novel gene therapies for diseases with unmet clinical needs, such as muscular dystrophy and cystic fibrosis. The project is well structured, the objectives are very well cleared outlined and may have an important impact in the field. As a weakness I do foresee possible over ambitiousness with the risk of not being able to completely achieve what has been proposed.

Competence and expertise of the applicant.
- *What are the **merits and scientific expertise of the applicant**?*
- *Are they **appropriate and sufficient for the proposed project**?*

Reviewer n. 1
Antonella is a young scientist with a proven experience in drug delivery, including the development of lipid-based nanoparticles for nucleic acid delivery and immunoliposomes. Antonella' publication list and participation to international conferences confirm her experience in the field. There si no doubt that she has the capability of conduct the research proposed.
Reviewer n. 2
The applicant reports adequate merits and scientific expertise to lead the project given extensive experience in polymer conjugation of therapeutic proteins, peptides and drugs using natural and synthetic polymers, (PEG -polyethylene glycol-, polyglutamic acid and hyaluronic acid) and in polymer modification and characterization.
The applicant's experience is appropriate for the proposed project and aligns with the collaborator's needs to complete all the work packages
Reviewer n. 3
The applicant has competence and long and documented experience concerning the project activities, with a good international experience and network
Reviewer n. 4
The applicant has an appropriate background to successfully lead the work.

Competence and expertise of the research team.
- *Does the research **team bring complementary expertise** to the project?*
- *Is the project involved in **international research collaborations** that can significantly contribute to the success of the project?*

Reviewer n. 1
The project includes only National collaborations. Nevertheless, the research group comprises different expertise from the DSF and from the University of Milano.
Reviewer n. 2
The research team possesses excellent and complementary expertise, enabling coverage of all aspects of the proposed project. At present, the project is not involved in any international collaboration.
Reviewer n. 3
The team is well integrated, and complementary expertise is in place, with potential international collaborations available in case.
Reviewer n. 4
The team is well structured and brings together the appropriate interdisciplinary expertise to ensure the successful execution of the proposed research.

VALUTAZIONI PROGETTI DI RICERCA DI DIPARTIMENTO PRID – ANNO 2025

COMMISSIONE INTERNA

Project: Advanced hybrid and targeted lipid-based systems for the delivery of plasmid DNA

Applicant: Grigoletto Antonella

Punti di forza:
Il progetto si fonda su una solida base scientifica e affronta una tematica di grande rilevanza nel campo della gene therapy, proponendo strategie innovative per superare limiti tuttora irrisolti dei sistemi lipidici per la formulazione di DNA plasmidico. L'approccio razionale, l'elevato livello di competenza del gruppo di ricerca e la disponibilità di risultati preliminari rafforzano la credibilità e il potenziale impatto della proposta.

Criticità:
L'articolazione del progetto in numerosi work package, che includono lo sviluppo formulativo, validazioni in vitro avanzate e studi in vivo, rende il piano di lavoro particolarmente ambizioso e il cronoprogramma di 24 mesi potenzialmente limitante per il completamento di tutte le attività previste.