LevoNano-IPF

Reactivating Mitochondrial Function: A Novel Therapeutic Paradigm for Idiopathic Pulmonary Fibrosis Using Levothyroxine Nanoparticles in Respirable Powder





Ruggero Bettini
University of Parma

NANOTECMEC Mid-Term Symposium

November 23rd & 24th, 2026

ERA4Health Partnership

Co-funded by the European Union

Consortium composition

Partners

- **University of Parma**, Department of Food and Drug (project coordinator)-P.I. Prof. Ruggero Bettini

- **University Paris-Saclay**, Institut Galien Paris-Saclay **UMR CNRS 8612-** Local Unit Lead Prof. Juliette Vergnaud

- **I3S**, Institute of Research and Innovation in Health, **University of Porto**- Local Unit Lead Prof. Raquel Soares

Collaborator

- **University-Hospital of Parma**, Department of nuclear medicine - Local Unit Lead Dr. Livia Ruffini

Project aim

Shaping a new therapeutic paradigm for the treatment of idiopathic pulmonary fibrosis through an innovative therapy aimed at **reactivating the mitochondrial function of lung epithelial cells** using levothyroxine nanoparticles in the form of dry powder for inhalation.

i) **proof of concept** of effectiveness of the new nanoformulation of levothyroxine in reversing lung fibrosis **in an animal** model including the assessment via PET imaging;

ii) in vitro **evaluation of the mechanism** of action of levothyroxine nanoformulation on fibrotic lung cell models;

iii) **pharmaceutical development** of a highly respirable powder containing levothyroxine nanocrystals;

iv) optimization of the production process of levothyroxine nanoformulation to drive the particle capture toward lung epithelial cell avoiding macrophagic clearance.

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Work plan and timeline
Starting date: February 3, 2025

WP	Task	Responsible	Trimester											
			1	2	3	4	5	6	7	8	9	10	11	12
1. Project management and coordination		UniPR	M1.1		D1.1	D4.1								
2. Production and optimization of LT4 nanoformulation	2.1 Nanosuspension production	UniPR				M2.2.1		M2.1.2						
2. Production and optimization of LT4 nanoformulation	2.2 Respirable powder production	UniPR							M2.2.2					
3. Cellular Uptake	3.1 Macrophages	UniPR								D3.1				
3. Cellular Uptake	3.2 Epithelial lung cells	UniPR								D3.2				
4. *In vitro* cellular response	4.1 Cell death and proliferation	UMR-CNRS 8612												
4. *In vitro* cellular response	4.2 Cell co-culture	UMR-CNRS 8612												
5. *In vivo* studies	5.1 Outcome of animal treatment	I3S, UniPorto												
5. *In vivo* studies	5.2 Biodistribution	I3S, UniPorto												
6. *In vivo* imaging with PET radiotracers	6.1 Radiotracer production and quality control process	UH PR										M6.1		
6. *In vivo* imaging with PET radiotracers	6.2 µPET/CT imaging	UH PR												

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Scientific progress

WP2 Optimization and production of the LT4 nanoformulation

Task 2.1 Nanosuspension production



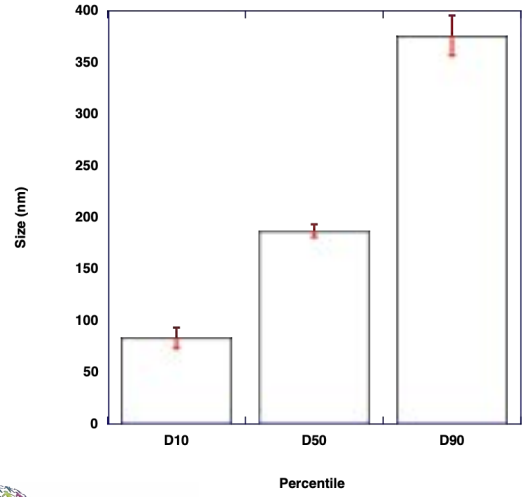
24h 25°C

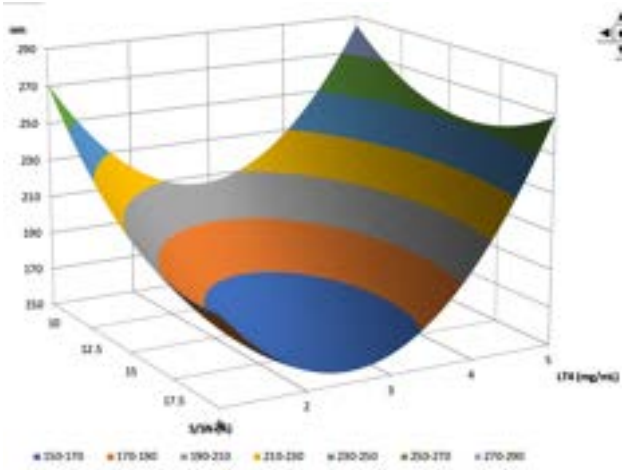


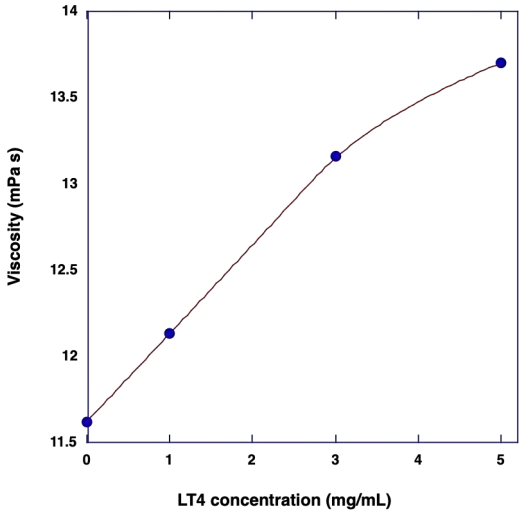
400 Bars,
15 cycles











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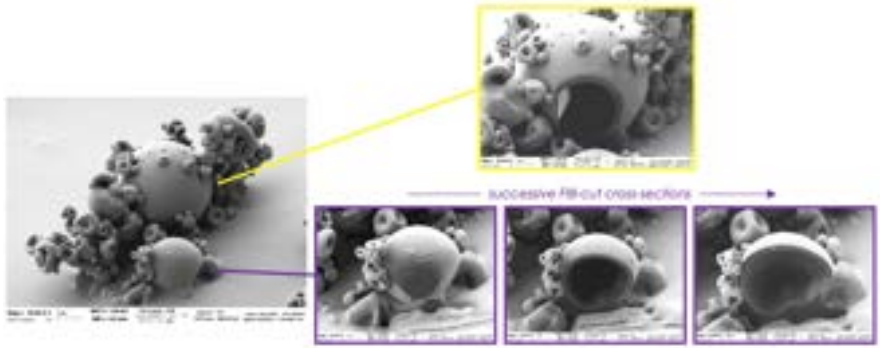
Scientific progress

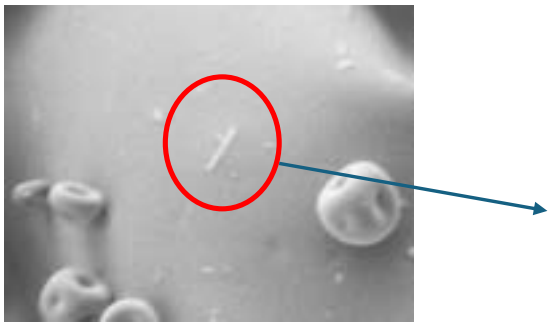


WP2 Optimization and production of the LT4 nanoformulation

Task 2.2 Respirable particle production

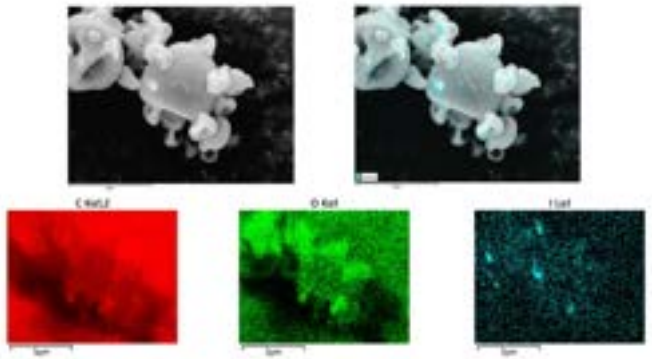
Focused Ion Beam-SEM





180 nm

Energy Dispersive X-Ray Analysis





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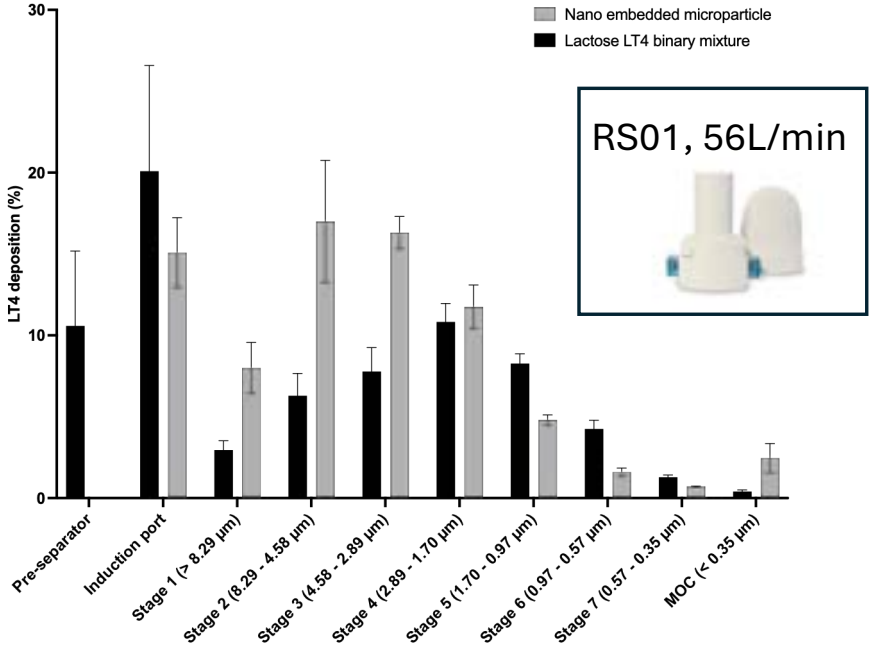
Scientific progress

WP2 Optimization and production of the LT4 nanoformulation

Task 2.2 Respirable powder characterization

	D_{v10} (µm)	D_{v50} (µm)	D_{v90} (µm)
Nano-embedded SD powder	1.55 (0.1)	4.28 (0.1)	15.0 (1.27)
Micronized LT4	0.73 (0.1)	1.62 (0.1)	4.19 (0.7)

MMAD (µm)	3.5 (0.15)
FPF % (< 5 µm)	75.04 (0.98)
eFPF % (< 2µm)	59.40 (0.23)



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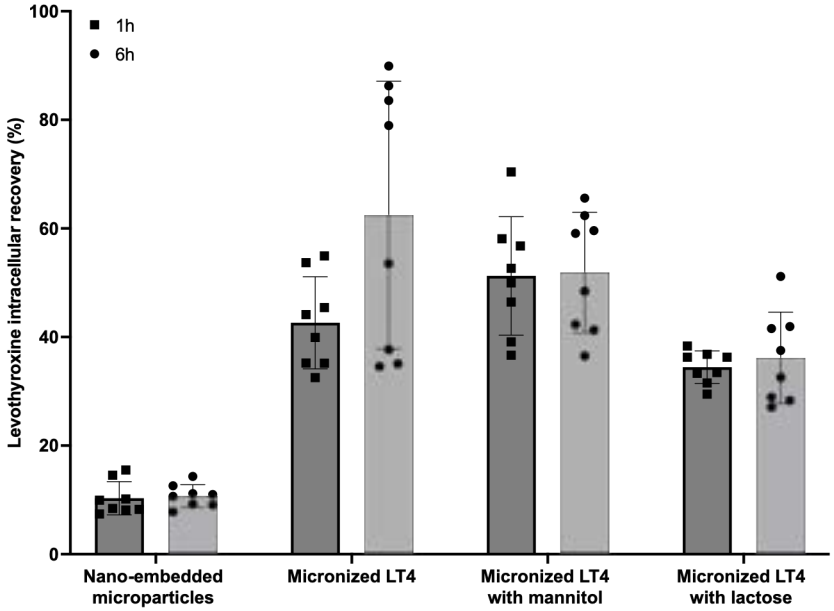
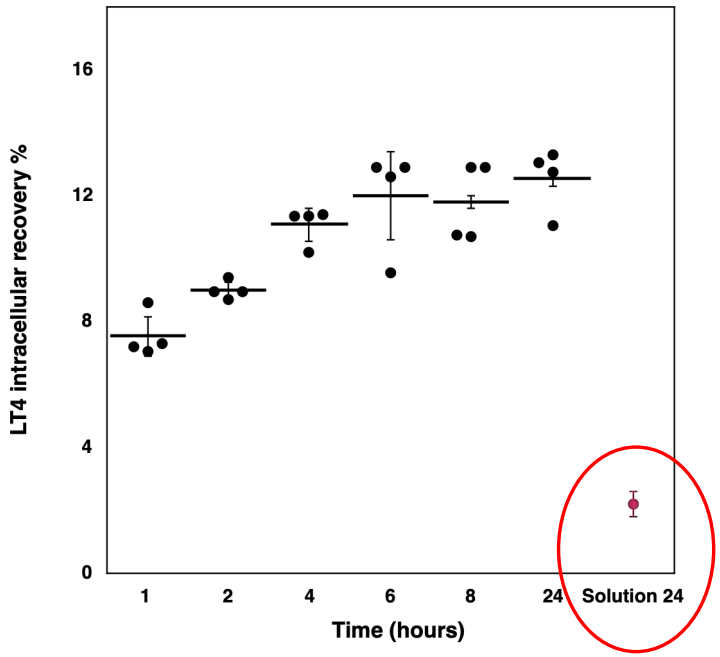
Scientific progress

WP3 Cellular Uptake

Task 3.1 LT4 macrophagic uptake via THP-1



Task 3.2 intracellular LT4 delivery in A549



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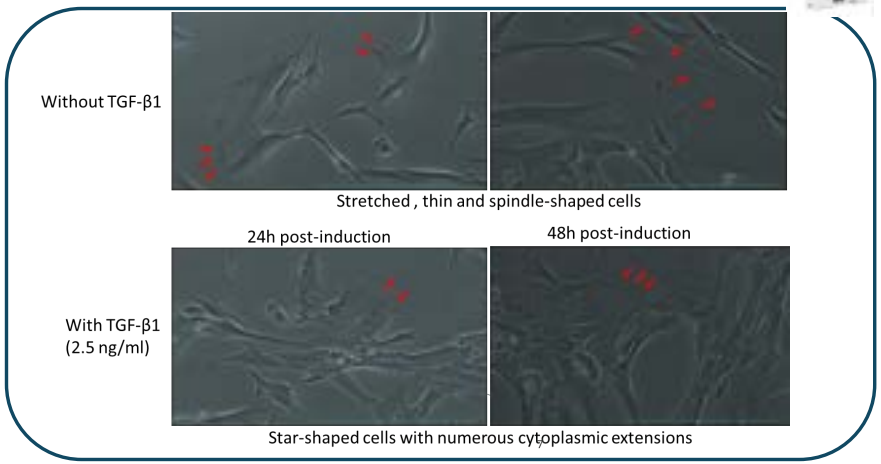
WP4 *In vitro* cellular response

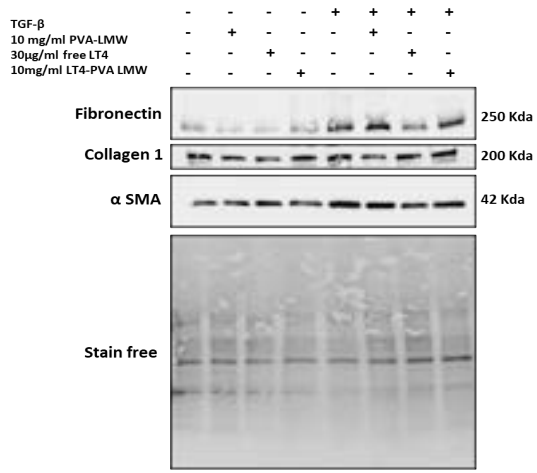
WP 4.1 Preliminary results with Fibroblasts and Myofibroblasts (IMR90 cells)

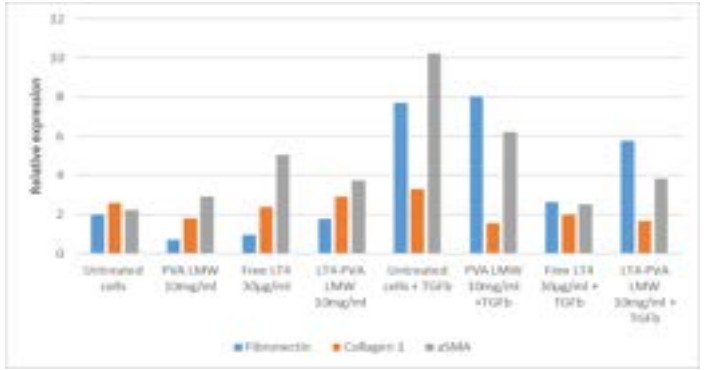
Morphological characterization





Effect on fibrosis markers expression





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Scientific progress

WP5 *In vivo* studies

- Preparation of the experimental protocol
- Submission to local Ethical Committee
- Granting of approval by the Ethical Committee
- Transfer from Unipr to I3S of the binary mixture of Lactose LT4 and spray-dried powders for familiarization with intratracheal powder administration using the PennWu device.



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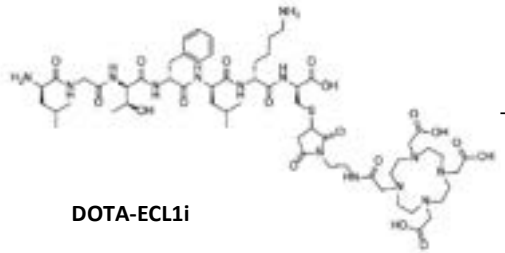
Scientific progress

WP6 *In vivo* imaging with PET radiotracers

Task 6.1 - Set-up of radiosynthesis and quality controls methods for ^{68}Ga-DOTA-ECL1i

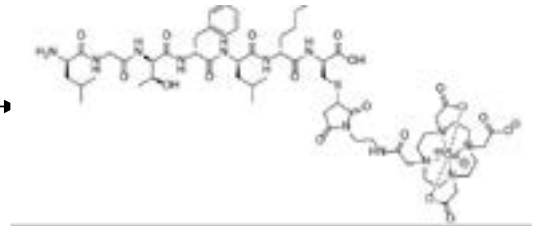
^{68}Ga-DOTA-ECL1i - *Imaging CCR2^{+} cells within the fibrogenic niche*



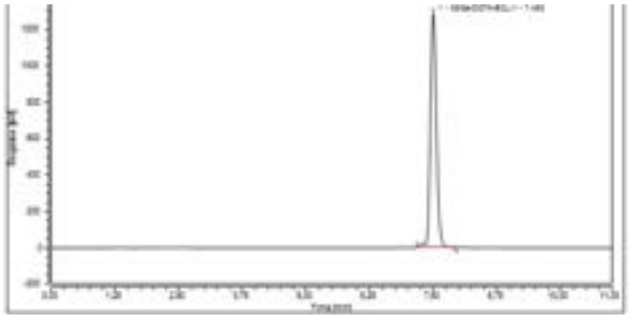
DOTA-ECL1i

95°C for 10 minutes

$^{68}GaCl_3$







	Radiochemical Purity (RCP%)	Radiochemical Yield (RCY%)	Molar Activity (Am, GBq/µmol)
Validation (20 µg precursor)	100.00	64	43.57 (range 1-60)
Performed preparations (n=5)	100.00	64	30.17 (range 1-60)

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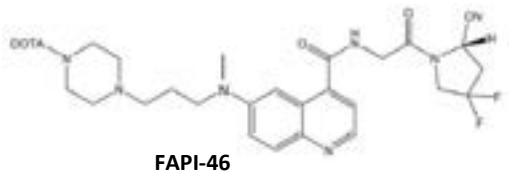
Scientific progress

WP6 *In vivo* imaging with PET radiotracers

Task 6.1. Set-up of radiosynthesis and quality controls methods for ^{68}Ga-FAPI-46

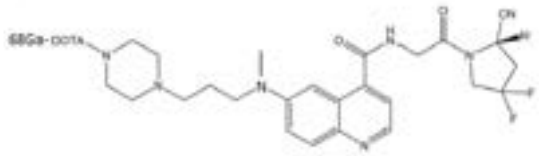
^{68}Ga-FAPI-46 - *Presence and activity of fibrogenesis*



FAPI-46

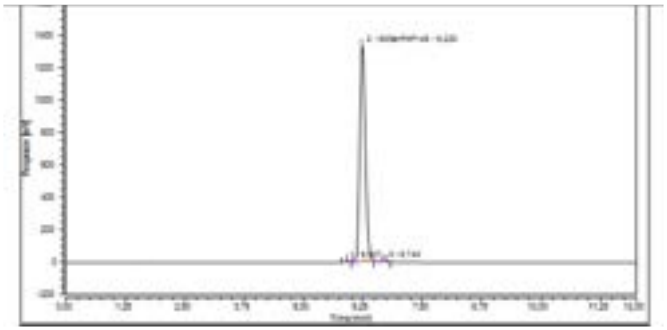
95°C for 10 minutes

$^{68}GaCl_3$



^{68}Ga-FAPI-46





	Radiochemical Purity (RCP%)	Radiochemical Yield (RCY%)	Molar Activity (Am, GBq/µmol)
Validation (20 µg precursor)	99.76	67.75	26.23 (range 1-60)
Performed preparations (n=2)	99.44	62.00	23.68 (range 1-60)

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Scientific progress

WP6 *In vivo* imaging with PET radiotracers

Task 6.2. µPET/CT imaging protocol in the bleomycin animal model



① **Preparation of the experimental protocol**

Completed ✓



② **Protocol transfer to animal facilities**

Completed ✓



③ **Submission to the Local Ethical Committee**

Completed ✓



④ **Submission to the Italian Ministry of Health**

Waiting for the final authorization

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Communication and dissemination

- Web Page:
https://www.levonanoipf.unipr.it/project/

- Linkedin page:
https://www.linkedin.com/company/levonano-ipf/posts/

***- Papers in peer reviewed journals**:*

Bazzoli G., Bianchera A., Trevisi G., Fattal E., Vergnaud J., Bettini R. "Engineered levothyroxine dry powder for inhalation to treat idiopathic pulmonary fibrosis"
Drug Delivery and Translational Research (2026) 16:3728-3745
https://doi.org/10.1007/s13346-026-02064-2

Bazzoli G., Bianchera A., Bettini R. "Levothyroxine dry powder for inhalation: spray drying or micronization
Journal of Drug Delivery Science and Technology (2026) 126:108772
https://doi.org/10.1016/j.jddst.2026.108772

Communication and dissemination

- Posters presentation in international conferences:

Bazzoli G., Bianchera A., Vergnaud J. Fattal E., Bettini R. “Respirable powders embedding levothyroxine nanoparticles for the treatment of pulmonary fibrosis”. 23rd International Symposium on Microencapsulation, Ferrara 10-12 September 2025

Bazzoli G., Bianchera A., Vergnaud J. Fattal E., Bettini R. “Levothyroxine nanoparticles embedded in respirable powders for idiopathic pulmonary fibrosis treatment”. 5th conference on Innovation in Drug Delivery, Turin, 1-3 October 2025

Bazzoli G., Bianchera A., Bettini R. “Levothyroxine respirable powders for the treatment of pulmonary fibrosis”. Avogadro Colloquia 2025, Rome 27-28 November 2025.

Bazzoli G., Bianchera A., Bettini R. “Levothyroxine dry powder for inhalation to treat idiopathic pulmonary fibrosis: micronized adhesive mixtures vs nano-embedded microparticle”. 15th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Prague, 23-26 March 2026

Bazzoli G., Bianchera A., Bettini R. *“Levothyroxine dry powder for inhalation: Spray drying or micronization?”*. Exploring the future of inhalation drug delivery 2nd edition, Parma, 8-10 September 2026

Implementation of RRI approach

Involving or not involving operational stakeholders is rooted in a balanced and ethical approach. Given the serious and life-threatening nature of the disease (prognosis often poor, with a median survival ranging from 2 to 5 years after diagnosis), the project recognizes the importance of not creating undue expectations in patients and their families, especially in the early stages. The decision-making process is guided by a commitment to transparency, fairness, and ethical considerations.

Patient advocacy groups and citizens may contribute to a subsequent, clinical-stage project; the consortium carefully manages expectations and communicates openly about the uncertainties inherent in this early-stage research, ensuring a responsible engagement with all stakeholders. However, in the event of positive results, the consortium is committed to transparently disseminating the findings through the project website

Implementation of RRI approach

Key element: drug repurposing

- The approach based on the use of a molecule (levothyroxine) of well-known toxicology through its repositioning for a different pathology through pulmonary administration, goes in the direction of developing a medicine that is not only effective but also safe for patients and affordable.

Emphasis on developing a therapy that is not only effective but also accessible to a broader population.

Innovation's affordability and accessibility are central in addressing societal needs.

Committment to ensure that the developed therapy is within reach for a diverse range of individuals facing IPF. This affordability aspect is crucial to preventing potential disparities in access to the treatment

Sustainability not only from the economical standpoint but also from the environmental point of view.

Environmental Risk Analysis could become, in the near future a decisive factor for bringing new medicines to the market

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Unforeseen Issues and Corrective Measures and Next Steps

- The project is progressing as planned at the submission stage. Some activities have even been brought forward compared with what was initially planned.

- The only major issue is the seven-month delay in the start of WP4, caused by the unavailability of the CNRS Paris-Saclay laboratories due to safety-related issues. To address this, it was decided to bring WP3 forward, so that in vitro data would be available to guide the in vivo studies.
- The consortium nevertheless expects to make up some of the lost time by accelerating WP4 activities.
- In any case, WP4 is expected to be completed well before the end of the project, which is scheduled for early February 2028.

- The two *in vivo* work packages (WP5 and WP6) are getting underway and are expected to proceed as planned.

Thank you

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