Ministero della Salute – Direzione Generale della Ricerca e dell'Innovazione in Sanità

Rendiconto 5 per mille ANNO 2023
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Ente della Ricerca Sanitaria
Denominazione Ente: IRCCS Istituto Clinico Humanitas
Codice fiscale: 10125410158
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Dati del rappresentante legale: Riccardo Bui

RESPONSABILE SCIENTIFICO	TITOLO PROGETTO	FINANZIAMENTO ASSEGNATO
PELLEGATTA GAIA	Unveiling the role of sphingosine-1-phosphate receptor 1 as a potential target to treat fibrosis in patients affected by Eosinophilic Esophagitis	100.000,00
PUCCINI ALBERTO	Circulating tumor DNA (ctDNA) to detect minimal residual disease (MRD) and to guide decision-making process in locally advanced gastroesophageal cancer patients	300.000,00
SELMI CARLO	Assessment of the immune, microbiota and metabolomic status of fibromyalgia and psoriatic arthritis patients: correlation with pain, intestinal permeability, nociceptor status, glucose and insulin levels	295.090,00
STEFANINI GIULIO	Glucagon like peptide-1 receptor agonists to prevent coronary artery disease progression in diabetics with acute coronary syndrome: the PROGRESSION-GLP1 study	297.505,00
FERRANTE GIUSEPPE	Pathophysiological insights and tailored therapeutic approaches in patients with ST-segment elevation myocardial infarction and plaque erosion: an optical coherence tomography imaging study with single-cell analysis	300.000,00
CATTANEO DARIO	Personalizzazione delle terapie antibiotiche ed antifungine nel paziente complesso mediante approcci combinati di farmacologia clinica e microbiologia	128.702,78
MOROSI LAVINIA	Analisi della distribuzione spaziale di farmaci e mataboliti in campioni di tessuto tumorale raccolti alla chirurgia tramite mass spectrometry imaging	338.000,00

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Titolo del progetto: Unveiling the role of sphingosine-1-phosphate receptor 1 as a potential target to treat fibrosis in patients affected by Eosinophilic Esophagitis

Data di inizio progetto: 01/01/2025	**Data di fine progetto**: 31/12/2027
Fondi 5 per mille assegnati al progetto: **€ 100.000,00**	**Di cui:** **Quota da sostenere entro l'anno di rendicontazione: € 0,00** **Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni): € 100.000,00**

VOCI DI SPESA	Quota da sostenere entro l'anno di rendicontazione	Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni)
Personale di ricerca (borsista, a contratto e di ruolo in quota parte)	0,00	0,00
Apparecchiature (ammortamento, canone di locazione/leasing)	0,00	0,00
Materiale d'uso destinato alla ricerca (per laboratori di ricerca, acquisto farmaci ecc.)	0,00	75.000,00
Spese di organizzazione (manifestazioni e convegni, viaggi e missioni ecc.)	0,00	5.000,00
Elaborazione dati	0,00	5.000,00
Spese amministrative	0,00	15.000,00
Altro	0,00	0,00
TOTALE	**0,00**	**100.000,00**

Data, 10/07/2025

Il Legale Rappresentante

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Titolo del progetto: Unveiling the role of sphingosine-1-phosphate receptor 1 as a potential target to treat fibrosis in patients affected by Eosinophilic Esophagitis

Eosinophilic esophagitis (EoE) is a chronic immune-mediated inflammatory disease of the oesophagus. Initially considered a rare condition, it is emerging in developing countries and currently affects an estimated 34.4/100 000 people in Europe and North America (1). It is characterized by isolated eosinophilic infiltration of the oesophagus resulting in oesophageal dysfunction. In the absence of treatment, oesophageal inflammation and symptoms tend to persist and worsen over time (3). An exacerbated Th2 response with increased expression of interleukin (IL)-4, IL-5, IL-13, and eotaxins (4) promotes the proliferation, recruitment, and accumulation of eosinophils in the oesophageal wall. The persistence of inflammation induces oesophageal structural changes (5) leading to fibrotic remodelling and impaired oesophageal function. The molecular mechanisms involved in these processes are still unknown. Currently, the existing anti-inflammatory treatments are not effective in reversing fibrosis (6). As a result, oesophageal fibrosis has a significant impact on the quality of life of EoE patients, requiring invasive endoscopic procedures such as endoscopic dilatations.
The lack of a specific fibrosis target and the paucity of EoE preclinical and clinical studies (6) do not allow clinicians to predict whether patients will progress towards a fibrostenotic phenotype of the disease and, if so, how to treat fibrosis.
Sphingosine-1-phosphate (S1P) is a pleiotropic lipid mediator of several cellular processes such as cell migration, proliferation, differentiation, cytokine production.
Emerging evidence suggests that S1P is also an important regulator of the fibrotic process through the activation of its 5 receptors (S1PR1-5).
Importantly, S1P/S1PR1 regulates inflammation in terms of recruitment and activation of eosinophils, lymphocytes, natural killer cells and dendritic cells (7,8). A dysregulation of

S1PRs and a pathological increase of S1P was found in other type of immunological diseases, including asthma and IBD (9). Selective SP1R modulators have yielded promising results in immune-mediated diseases treatment (10, 11, 12, 13). Among the promising molecules, Ozanimod (CelgeneTM) and Etrasimod (ArenaTM), respectively S1PR1,5 and S1PR1,4,5 agonists, showed significant clinical, endoscopic, and histological remission in Ulcerative colitis patients in comparison to the placebo group. In June 2021 Etrasimod (APD334) has received Orphan Drug Designation status from the FDA in and is currently being evaluated in EoE in a phase 2b, randomized trial (NCT04682639) (14).
By using an Opal multiplex technique for the detection of multiple biomarkers, we observed a significant increase of S1PR1 expression in oesophageal tissue from 13 EoE patients at diagnosis compared to 5 healthy controls. Interestingly, the S1PR1 increase was particularly strong in patients with a EoE fibrostenotic phenotype compared to an inflammatory phenotype. Additionally, S1PR1 did not co-localised with immune cells per se, and its expression appeared to be distributed homogenously between epithelial cells and the lamina propria, where the deposition of fibrotic tissue occurs. It is plausible that S1P/S1PR1 axis plays a critical role in the recruitment not only of the predominant inflammatory cells (eosinophils, mast cells, basophils, and both B and T lymphocytes) but also promotes the activation of fibroblasts, which in turn sustain fibrotic remodelling of the tissue. Based on these promising data we aim to validate S1PR1 as a new potential therapeutic target to prevent and treat fibrosis in patients affected by EoE.

Objectives and outcomes
Primary outcome
To characterise the expression of S1PR1 in a large cohort of patients affected by EoE and to correlate S1PR1 expression with the fibrostenotic disease phenotype at diagnosis (T0).
Secondary outcome:
To define the effect of S1PR1 modulation in reversing fibrosis in EoE patients.

Study procedures: prospective and observational study
1. S1P and S1PR1 expression. Oesophageal biopsies from EoE patients will be prospectively enrolled by newly diagnosed EoE patients and matched healthy control over a period of 24 months.
Subject enrolled will be divided into 3 groups: 50 healthy subjects, 30 EoE with inflammatory phenotype (EoEI) and 20 EoE with fibrostenotic (EoEF) phenotype. For each patient, oesophageal biopsies will be collected at diagnosis during an upper GI endoscopy (T0). Patients' biopsies will be further embedded in paraffin block and cut in sections. At T0, S1P and S1PR1 expression will be evaluated on oesophageal sections by Multiplex immunohistochemistry and immunofluorescence staining. Moreover, tissue proteins and RNA will be extracted from the section to confirm the staining results with western blot and gene expression analysis (qPCR), respectively. Together with S1PR1, we will evaluate the expression of proteins and genes related to fibrosis as well as pro-inflammatory and pro-fibrotic (TGFβ) cytokines expression.
2.Organ culture. At T0, from each patient and healthy subject enrolled, at least 2 oesophageal biopsies will be collected and further processed to perform organ culture. We will divide the treatment condition in 3 groups: healthy subject, EoEI (inflammatory) and EoEF (fibrostenotic). Briefly, each biopsy will be cultured for 24 hours in either control medium (no treatment) or Ozanimod (S1PR1 agonist).
After 24 hours:
-Medium will be collected to characterize change in cytokine production and tissue will be processed to perform:

-RNA-sequencing for transcriptomic analysis: to define the different molecular pathways between EoE patient and healthy controls and between inflammatory and fibrostenotic EoE patients at baseline and upon S1PR1 agonist modulation (Ozanimod)
- Protein and gene expression analysis: to confirm on protein and gene level the changes detected by RNA-sequencing and to assess the changes upon S1PR1 agonist modulation in terms of fibrosis-related protein and gene expression.
3. Blood collection for organoids generation. Based on the observation of expression of S1PR1 on the epithelial cells, we will explore its involvement of the fibrotic processes in EoE patients. To this end, human induced pluripotent stem cell-derived intestinal organoids (HIOs) will be generated from EoE patients and subsequently subjected to Ozanimod. Peripheral blood mononuclear cells (PBMC) will be reprogrammed (15). Once generated, HIOs will be subjected to repeated inflammatory stimuli (such as IFNγ, TNFα, TGF-β and IL-1β) and then, treated with Ozanimod or vehicle for the evaluation of fibrotic pathways through quantitative RT-PCR, immunofluorescence and western blot. Moreover, proliferation and expression of genes and proteins related to epithelial to mesenchymal transition (EMT), associated with wound healing and tissue regeneration will be analysed.

Inclusion criteria:
EoE patients
-Patients≥ 18 years
-Patients with a newly confirmed histological diagnosis of EoE
-Patients performing esophagogastroduodenoscopy according to the clinical guidelines for EoE diagnosis
Healthy subjects
Subjects who perform esophagogastroduodenoscopy for other diagnostic reason and who are not affected by EoE
Exclusion criteria
-Patients not able to comply with the study procedures
-Patients with other gastrointestinal disorders such as active Helicobacter pylori infection, history of achalasia, oesophageal varices, Inflammatory Bowel Disease
-Patients affected by Eosinophilic granulomatosis with polyangiitis vasculitis.
-Patients affected by Hypereosinophilic syndrome, defined by multiple organ involvement and persistent blood eosinophil count > 1500 eosinophils/μL.
Impact of the study: Patients with EoE are at risk of potentially life-threatening complications due to esophageal fibrosis. Understanding and identifying a potential fibrotic target in EoE is a complex challenge with significant clinical implications. The main aim of our study is to gain a better understanding of the molecular pathways beyond fibrosis, which is crucial for the development of an anti-fibrotic agent that could dramatically change the natural history of the disease.

References:
1. Lucendo A.J., Molina-Infante J., Arias Á., et al. Guidelines on eosinophilic esophagitis: Evidence-based statements and recommendations for diagnosis and management in children and adults. United Eur. Gastroenterol. J. 2017, 5, 335-358.
2. Arias, Á., Lucendo A.J. Incidence and prevalence of eosinophilic oesophagitis increase continiously in adults and children in Central Spain: A 12-year population-based study. Dig. Liver Dis. 2019, 51, 55–62.
3. Straumann A., Spichtin HP, Grize L., et al. Natural history of primary eosinophilic esophagitis: A follow-up of 30 adult patients for up to 11.5 years. Gastroenterology. 2003, 125, 1660–1669.
4. Rothenberg, M.E. Biology and treatment of eosinophilic esophagitis. Gastroenterology. 2009, 137, 1238–1249.

5. Alexander J., Katzka D. Editorial: Expanding a narrow perspective on narrow calibre oesophagus in eosinophilic oesophagitis— Authors' reply. Aliment. Pharmacol. Ther. 2015, 41, 148–149.
6. Lupher M.L., Gallatin WM. Regulation of fibrosis by the immune system. Adv. Immunol. 2006, 89, 245–288.
7. Sugita K., Kabashima K., Sakabe J., et al. FTY720 regulates bone marrow egress of eosinophils and modulates late-phase skin reaction in mice. Am J Pathol. 2010; 177(4):1881-1887
8. Cohen J. A., Comi G., Selmaj et al. Safety and efficacy of ozanimod versus interferon beta-1a in relapsing multiple sclerosis (RADIANCE): a multicentre, randomised, 24-month, phase 3 trial. Lancet Neurol. 2019; 18(11):1021-1033
9. Sun X., Ma S. F., Wade et al. Functional variants of the sphingosine-1-phosphate receptor 1 gene associate with asthma susceptibility. J Allergy Clin Immunol. 2010; 126(2):241-9
10. Vaclavkova A., Chimenti S., Arenberger P., et al. Oral ponesimod in patients with chronic plaque psoriasis: a randomised, double-blind, placebo-controlled phase 2 trial. Lancet. 2014; 384(9959):2036-45
11. Sandborn, W. J., Feagan, B. G., Wolf, D. C., et al. Ozanimod Induction and Maintenance Treatment for Ulcerative Colitis. N Engl J Med. 2016; 374(18):1754-62
12. Sandborn, W. J., Peyrin-Biroulet, L., Zhang, J., et al. Efficacy and Safety of Etrasimod in a Phase 2 Randomized Trial of Patients with Ulcerative Colitis. Gastroenterology. 2020; 158(3):550-561
13. Pérez-Jeldres T., Tyler C.J., Boyer J.D., et al. Targeting Cytokine Signaling and Lymphocyte Traffic via Small Molecules in Inflammatory Bowel Disease: JAK Inhibitors and S1PR Agonists. Front Pharmacol. 2019; 13;10:212
14. Racca F., Pellegatta G., Cataldo G., et al. Type 2 Inflammation in Eosinophilic Esophagitis: From Pathophysiology to Therapeutic Target. Front Physiol. 2022;12; 12:815842. doi: 10.3389/fphys.2021.815842. eCollection 2021.
15. Rizzo G., Rubbino F, Elangovan S., et al. Dysfunctional Extracellular Matrix Remodeling Supports Perianal Fistulizing Crohn′s Disease by a Mechanoregulated Activation of the Epithelial-to-Mesenchymal Transition. Cell Mol Gastroenterol Hepatol. 2023; 15(3): 741–764.

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Dati del rappresentante legale: Riccardo Bui

Titolo del progetto: Circulating tumor DNA (ctDNA) to detect minimal residual disease (MRD) and to guide decision-making process in locally advanced gastroesophageal cancer patients

Data di inizio progetto: 01/01/2025	**Data di fine progetto**: 31/12/2027
Fondi 5 per mille assegnati al progetto: **€ 300.000,00**	**Di cui:** **Quota da sostenere entro l'anno di rendicontazione: € 0,00** **Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni): € 300.000,00**

VOCI DI SPESA	Quota da sostenere entro l'anno di rendicontazione	Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni)
Personale di ricerca (borsista, a contratto e di ruolo in quota parte)	0,00	60.000,00
Apparecchiature (ammortamento, canone di locazione/leasing)	0,00	0,00
Materiale d'uso destinato alla ricerca (per laboratori di ricerca, acquisto farmaci ecc.)	0,00	190.000,00
Spese di organizzazione (manifestazioni e convegni, viaggi e missioni ecc.)	0,00	0,00
Elaborazione dati	0,00	5.000,00
Spese amministrative	0,00	45.000,00
Altro	0,00	0,00
TOTALE	**0,00**	**300.000,00**

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Il Legale Rappresentante

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Titolo del progetto: Circulating tumor DNA (ctDNA) to detect minimal residual disease (MRD) and to guide decision-making process in locally advanced gastroesophageal cancer patients

1. Background

Gastric cancer is the 7th most common cancer worldwide and 5th in mortality, while esophageal cancer and gastroesophageal junction cancer are considered the 8th in incidence and 6th in mortality (1,2). Despite the improvements in terms of outcomes related to neoadjuvant approach in both gastric and esophageal cancers, the risk of recurrence after perioperative chemotherapy (CT) or chemoradiotherapy (CRT) followed by surgery remains high, especially among those patients who do not achieve a pathological complete response (3). In addition, no validated prognostic nor predictive biomarker is available to select patients and to guide clinicians in shared-decision making (4). Circulating tumor DNA (ctDNA) represents a promising non-invasive biomarker option, easily accessible through liquid biopsy, to predict patient outcomes in response to perioperative cancer therapy and surgical resection. ctDNA sequencing can serve as prognostic and predictive biomarker and for molecular residual disease (MRD), as it has already been demonstrated in different cancer types, and Phase II and III clinical trials are ongoing globally to validate this technique (5-8). However, data in gastroesophageal cancer are lacking.

Due to these reasons, we aim to investigate the clinical utility of a ctDNA assay to detect minimal residual disease (MRD) and guide clinician choices during standard perioperative treatment strategy and surveillance in stage II and III gastroesophageal cancers patients (e.g. intensification of adjuvant chemotherapy or more frequent radiological assessment and follow up).

Here we present our prospective, interventional, monocentric study (Figure 1) designed to establish the prognostic role of ctDNA and MRD detection in our patients with gastroesophageal cancers.
In order to set a highly sensitive and specific ctDNA test to be implemented in this study, a small cohort of patients (N=20) with gastroesophageal cancer will be tested.

2. Study design
Prospective, interventional, single center study.
3. Study objectives
3.1 Primary Study Objective
This project is conceived to explore the clinical utility of ctDNA to detect minimal residual disease (MRD) and to guide clinician choices during standard perioperative treatment strategy and surveillance in stage II and III gastroesophageal cancers patients.
Secondary objectives:
• To correlate the ctDNA status at different timepoints with outcomes.
• To correlate the change of serial ctDNA measurements during treatment with outcomes.
3.2 Study endpoints
1) To evaluate the sensitivity and specificity of liquid biopsy at different timepoints in gastroesophageal cancer patients' management.
2) Correlation between ctDNA status and Disease-free survival (DFS), Overall Survival (OS) and clinical and pathological response.
3) Correlation between the ctDNA changes and Disease-free survival (DFS), Overall Survival (OS) and clinical and pathological response.
4. Statistical analysis
Due to the descriptive intent of the study, a formal statistical hypothesis to be tested is not identified. Consequently, the calculation of the required sample size has not been performed. Data will be summarized as frequencies and proportions or as median and range. Chi-square will be used to evaluate differences between groups in case of categorical data, while the Wilcoxon test for continuous variables. Survival Curves will be estimated by Kaplan-Meier method and differences among subgroups will be evaluated using the log-rank test. The Cox Proportional hazard model will be used to estimate Hazard ratio and corresponding 95% confidence intervals. Statistical significance will be set at 0.05 (two sides).
5. Study population
Participants of any gender who are 18 years of age or older diagnosed with stage II or III gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, esophageal adenocarcinoma or esophageal squamous carcinoma.
5.1 Inclusion Criteria
1) Newly histologically documented stage II or III gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, esophageal adenocarcinoma or esophageal squamous carcinoma referred to our Institution.
2) Candidate to undergo standard neoadjuvant or perioperative treatment with induction chemoradiation, or perioperative chemotherapy followed by surgery.
3) Age ≥ 18 years of age.
5.2 Exclusion Criteria
1) Stage I, recurrent, or metastatic gastroesophageal cancer;
2) Received prior therapy for gastroesophageal cancer;
3) No baseline tumor biopsies or inadequate amount of tissue from biopsies available to send for assay development.
6. Study procedures
For every enrolled patient, the following samples will be retrieved:
- FFPE tumor biopsy (or surgery);
- At diagnosis: 2 blood sample in EDTA (5 ml) + 2 blood sample in Paxgene (10 ml);

- After neoadjuvant treatment (or before surgery): 2 blood sample in EDTA (5 ml) + 2 blood sample in Paxgene (10 ml);
- 4-8 weeks after surgery: 2 blood sample in EDTA (5 ml) + 2 blood sample in Paxgene (10 ml);
- At 6 months of follow up: 2 blood sample in EDTA (5 ml) + 2 blood sample in Paxgene (10 ml);
- At 12 months of follow up: 2 blood sample in EDTA (5 ml) + 2 blood sample in Paxgene (10 ml);

In one year, we plan to collect around 200 patients. Patients' medical records are also reviewed.

First year: enrollment

Second and third year: follow up.

7. Expected results

This prospective analysis is thought to explore the potential prognostic value of ctDNA in patients affected by locally advanced gastroesophageal cancer undergoing neoadjuvant or perioperative treatments. This subgroup of patients represents a challenge for clinicians not only for the right therapeutic strategy to choose but also
for the follow-up management. While the role of liquid biopsy has being largely studied in CRC and other type of cancers, data are lacking for upper GI cancers. Therefore, information obtained by this analysis will increase our knowledge on the possible applications of this new technology. This study entails the use of liquid biopsy, providing precise information for each patient with high sensitivity and specificity. This method will enable a more precise analysis of the mutational landscape and MRD in our population.

References:

1. Grad, C., Grad, S., Fărcaş, R. A., Popa, S. & Dumitraşcu, D. L. Changing trends in the epidemiology of gastric cancer. Med Pharm Rep 96, 229–234 (2023).
2. Uhlenhopp, D. J., Then, E. O., Sunkara, T. & Gaduputi, V. Epidemiology of esophageal cancer: update in global trends, etiology and risk factors. Clin J Gastroenterol 13, 1010–1021 (2020).
3. Asplund J., Kauppila J.H., Mattsson F., Lagergren J. Survival Trends in Gastric Adenocarcinoma: A Population-Based Study in Sweden. Ann. Surg. Oncol. 2018; 25:2693–2702. doi: 10.1245/s10434-018-6627-y.
4. Lordick F, Carneiro F, Cascinu S, Fleitas T, Haustermans K, Piessen G, Vogel A, Smyth EC; ESMO Guidelines Committee. Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2022 Oct;33(10):1005-1020. doi: 10.1016/j.annonc.2022.07.004. Epub 2022 Jul 29. PMID: 35914639.
5. Kotani D, Oki E, Nakamura Y, et al. Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. Nat Med. 2023 Jan;29(1):127-134. doi: 10.1038/s41591-022-02115-4. Epub 2023 Jan 16. PMID: 36646802; PMCID: PMC9873552.
6. Tie J, Cohen JD, Lahouel K, et al. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. N Engl J Med. 2022 Jun 16;386(24):2261-2272. doi: 10.1056/NEJMoa2200075. Epub 2022 Jun 4. PMID: 35657320; PMCID: PMC9701133.
7. Tie J, Cohen JD, Wang Y, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. Gut. 2019 Apr;68(4):663-671. doi: 10.1136/gutjnl-2017-315852. Epub 2018 Feb 2. PMID: 29420226; PMCID: PMC6265124.
8. Zhou J, Wang C, Lin G, et al. Serial Circulating Tumor DNA in Predicting and Monitoring the Effect of Neoadjuvant Chemoradiotherapy in Patients with Rectal Cancer: A Prospective Multicenter Study. Clin Cancer Res. 2021 Jan 1;27(1):301-310. doi: 10.1158/1078-0432.CCR-20-2299. Epub 2020 Oct 12. PMID: 33046514.

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Titolo del progetto: Assessment of the immune, microbiota and metabolomic status of fibromyalgia and psoriatic arthritis patients: correlation with pain, intestinal permeability, nociceptor status, glucose and insulin levels.

Data di inizio progetto: 01/01/2025	**Data di fine progetto**: 31/12/2027
Fondi 5 per mille assegnati al progetto: **€ 295.090,00**	**Di cui:** **Quota da sostenere entro l'anno di rendicontazione: € 34.500,00** **Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni): € 260.590,00**

VOCI DI SPESA	Quota da sostenere entro l'anno di rendicontazione	Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni)
Personale di ricerca (borsista, a contratto e di ruolo in quota parte)	30.000,00	41.500,00
Apparecchiature (ammortamento, canone di locazione/leasing)	0,00	0,00
Materiale d'uso destinato alla ricerca (per laboratori di ricerca, acquisto farmaci ecc.)	0,00	139.226,50
Spese di organizzazione (manifestazioni e convegni, viaggi e missioni ecc.)	0,00	5.100,00
Elaborazione dati	0,00	5.000,00
Spese amministrative	4.500,00	39.763,50
Altro (Shotgun metagenomic sequencing)	0,00	30.000,00
TOTALE	**34.500,00**	**260.590,00**

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Titolo del progetto: Assessment of the immune, microbiota and metabolomic status of fibromyalgia and psoriatic arthritis patients: correlation with pain, intestinal permeability, nociceptor status, glucose and insulin levels.

BACKGROUND AND RATIONALE

Chronic pain is defined as pain that persists or recurs for more than 3 months, and one paradigmatic example is fibromyalgia (FM) in the absence of musculoskeletal impairment1. The pathogenesis of pain has been studied and **evidence suggests that its processing is altered in the brain of patients with FM, causing 'nociplastic pain'**, where reversible changes to the nervous system increase the sensitivity of the control system that usually decides how to interpret a stimulus, whether as painful or not. **An imbalance has been proved between nociceptive and anti-nociceptive neurotransmitters and receptors2.** The stimuli might derive from the periphery, although a clear source has not been identified. A peripheral joint source of pain has been proposed, considering the high prevalence of FM among patients with rheumatic diseases3, 4.
Several hypotheses have been proposed for the activation of nociceptive pathways. A stimulation from an activated immune system has been suggested, as some studies have shown alterations suggesting a possible role of cytokines5, 6, innate immune cells7, and adaptive immunity8-10, although evidence show conflicting results. **Patients with FM have an increased risk for metabolic syndrome (MetS) and the coexisting metabolic syndrome may increase the severity of fibromyalgia** 11. At the core of MetS lies a disruption in glucose, lipid, and protein metabolism, leading to the accumulation and dysfunction of adipose tissue, insulin resistance, and secretion of proinflammatory cytokines. **This cascade can induce a chronic, low-grade**

inflammatory state, exacerbated by the leakage of proinflammatory molecules from the gut into the
bloodstream due to a compromised gut barrier integrity, the so called "leaky gut" 12. Consistently, insulin resistance seems to characterize all patients with FM regardless of age, gender, and ethnicity13, but how this links to inflammatory alterations or neuropathic impairment remains to be elucidated.
Preclinical studies have shown **a positive correlation between neuropathic pain and insulin resistance in obese rats**, and decreased expression of insulin receptors in skeletal muscle has been postulated as a possible underlying mechanism14. In line with these findings, García et al. proposed that the nociceptive hypersensitivity induced by insulin resistance in the mouse model is due to the modulation of several ion channels on primary afferent neurons15. Dysfunction of the cerebral microcirculation has also been proposed to explain central pain in patients with FM, and impaired cerebrovascular reactivity has been demonstrated with advanced imaging methods in patients with insulin resistance16, 17.18 Recently, a link between MetS, depression and FM has similarly been found in patients with irritable bowel syndrome19. An impaired gut-brain axis, which connects the gut microbiota with the brain through the enteric nervous system, have been proposed as a possible pathogenetic mechanism20. **Alterations in the composition of the gut microbiota have also been recently reported in FM, with impaired proportions of bacteria participating in the metabolism of neurotransmitters in the host, associated with changes in neurotransmitter metabolism21, 22**. Nonetheless, the exact role of gut microbiota in impairing immune cell function and phenotype, or nociceptor activity in FM has yet to be elucidated. Moreover, alterations in gut microbiota composition are known to play a pivotal role also in the development of metabolic diseases such as obesity23, MetS24, and NAFLD25, although the precise mechanisms underlying these metabolic changes remain unclear. Recent research has shed light on the significance of gut barrier function, revealing that its maintenance relies on finely tuned mechanisms influenced by the microbial composition. The **gut barrier acts as a protective shield, preventing the entry of enteric microbiota and potent immunostimulatory molecules into the bloodstream,** while allowing the absorption of essential nutrients and fluids. Moreover, we have described that beneath the epithelium lies another cellular barrier known as the **gut vascular barrier (GVB), which regulates entry into the portal circulation and the liver's access.** Therefore, if a molecule or microorganism breaches the epithelial barrier, it will be confined to the lamina propria unless the GVB is compromised as well. Indeed, **bacterial entry into the systemic circulation requires disruption of the GVB**26. The GVB is strictly connected with another vascular barrier that we have identified in the brain choroid plexus (the plexus vascular barrier, PVB) which becomes visible only when the GVB is disrupted as a mechanism of defense of the brain from intestinal inflammation27. **This suggests a direct crosstalk between the gut and the brain via vascular networks**. **Increased intestinal permeability has also been associated with post-prandial glucose peaks in type 1 diabetes18** . Also, patients with FM are characterized by an increase in intestinal mucosal permeability, suggesting that **a leaky-gut may be responsible for controlling blood glycemia and consequently insulin levels in MetS or FM patients**.
In addition, the gastrointestinal (GI) tract is richly innervated by intrinsic enteric neurons as well as extrinsic neurons. This enteric nervous system (ENS) collectively governs various aspects of tissue function, including intestinal movement, nutrient absorption, immune response, and pain perception28. Enteric neurons can be categorized into two main groups based on their neurotransmitters: nitric oxide synthase 1 (Nos1)+ neurons, which utilize nitric oxide (NO), and choline acetyltransferase (Chat)+ neurons, which employ acetylcholine (Ach). **Nos1+ neurons function as inhibitory motor neurons** or secretomotor neurons and they co-express vasoactive intestinal peptide (VIP), a 28-

amino acid peptide that interacts with two high-affinity large G protein-coupled receptors, VIPR1
and VIPR2. **Chat+ neurons are diverse and include excitatory motor neurons, interneurons, and sensory neurons29**.
Nociceptors are specialized sensory neurons expressing ion channels like Nav1.8 and TRPV1 and they play a pivotal role in pain transmission, eliciting withdrawal responses and avoidance behaviors30. A recent study has demonstrated that **gut permeability is influenced by nociceptors present in the gut**, which stimulate the secretion of mucus from neighboring intestinal goblet cells via the calcitonin gene-related peptide (CGRP) - receptor activity modifying protein 1 (Ramp1) pathway. **Mice lacking nociceptors exhibit reduced mucus thickness under normal conditions, whereas nociceptor activation promotes mucus production**31. Neurotransmitters involved in nociceptor activation or inhibition are released by the gut microbiota as final mediators or their precursors, hence, changes in microbiota composition can also lead to different production of neurotransmitters and neuronal signals32. Similarly, several metabolites released by bacteria can have an analgesic effect; butyrate, for instance, may regulate CGRP release to decrease visceral hyperalgesia. **There is therefore a bidirectional network between the microbiota, nociceptors, mucosal and immune barriers which is mediated by several microbial mediators**, including neurotransmitters thus controlling neural activity30.
Besides FM, in a subgroup of patients affected by inflammatory arthritis, pain may persist despite good control of the gross inflammatory state33, a condition named 'residual pain'. As a paradigm, **patients affected by psoriatic arthritis (PsA), an inflammatory arthritis involving peripheral joint, spine, as well as skin, nails, and enthesis34, may suffer from considerable residual pain burden**, which affects functional status and quality of life35. Residual pain occurs more frequently in women, elderly, and obese patients, independent of treatment36.
The involvement of the interleukin 23–T-helper-17 cell pathway driving inflammation37 and a role of gut microbiota38 and increased gut permeability39 have been described in PsA. Further, insulin resistance has a high prevalence in patients with PsA and is associated with disease activity, as in FM. However, the features of residual pain have not been clearly characterized. Pain does not appear greatly reduced after treatment with biologics, suggesting a minor role of inflammation, at least that inducing the rheumatic disease40. The pathogenic mechanism underlying residual pain have not been studied and the burden of this is reflected in the difficulties of managing these patients, in whom it is challenging to understand whether it is necessary to introduce a new therapy or treat pain as a symptom.
Therefore, PsA patients appear to be an appropriate population for comparison with patients with primary chronic fibromyalgia pain.

HYPOTHESIS AND AIMS
We herein hypothesize, for the first time, that the modification in microbiota composition which is characteristic of FM and PsA patients, leads to a change in intestinal permeability (leaky-gut), increased glucose uptake, low, but persistent systemic inflammation, modification of neurotransmitters and hormones involved in nociceptor responses, and establishment of insulin resistance leading to disease symptoms and pain. Identification of new biomarkers and targetable signaling pathways may lead to new therapeutic approaches. The ultimate goal is to identifying novel therapeutic strategies targeting both pain perception and the disease mechanisms.

Based on our research hypothesis, we structured the design of the proposed project considering the following working packages (WPs):
WP 1 Pain profiling (Selmi).

Pain assessment will allow stratification of patients based on pain outcomes and provide insights into the mechanisms of pain in FM and PsA; furthermore, by comparing deeply profiled pain perception in FM and PsA with residual pain, we expect to improve shared decision making for treatment regimens (including pharmacological and nonpharmacological therapies), as well as increase patients' perceptions of disease activity and the quality of their pain sensation.
WP 2 Leaky gut analysis (Selmi - Rescigno)
In WP2 we will assess the leaky gut status in patients affected by FM or PsA through biological and functional assays.
WP 3 Insulin resistance (Lania)
Systematic assessment of both insulin resistance and pain in patients with FM or PsA will confirm the relationship between these two entities and further clarify whether neuropathic changes induced by insulin resistance occur early in the course of the disease. The relationship between chronic pain, the degree of insulin resistance and other determinants of the metabolic syndrome will also be assessed.
WP 4 Adaptive immune response (Lugli)
The leaky gut is associated with low grade inflammation. Hence the status of the adaptive immune response will be evaluated in this WP.
WP 5 Innate immune response and cytokines (Di Mitri, Jaillon, Garlanda)
Here we will evaluate the associations between leaky gut, insulin resistance, and the innate immune response. We will profile the composition and activation of immune cell subsets and we will measure the abundance of soluble factors, to finally identify targets for therapeutic intervention.
WP 6 Metagenomics and metabolomics (Rescigno)
Microbiota composition and its produced metabolites are associated with insulin resistance, leaky-gut, MetS, FM and AsP and pain development. In this WP we will analyze microbiota and metabolomics composition.
WP 7 Data integration (Shulzhenko and Morgun)
The integration of biological parameters (immunity, metabolomics, microbiota and clinical data) will allow us identifying molecular and microbial pathways involved in IR induction, pain development and disease symptoms.

STUDY DESIGN AND METHODS
WP1: Clinical study
Patients
Patients affected by fibromyalgia (n=30) according to the 2010/2011 ACR criteria and 2016 revised version41, not taking non-steroidal anti-inflammatory drugs (NSAIDs) for at least one month prior to enrolment, will be enrolled.
In parallel, 30 patients with PsA, classified according to CASPAR criteria42, who reached remission or low disease activity according to DAPSA (Disease Activity Index for Psoriatic Arthritis) score43 or who achieved minimal disease activity (MDA)44 after any treatment with disease-modifying anti-rheumatic drugs (DMARDs) for at least three months, but who still complain of residual pain with a visual analog scale >20 mm on a 0-100 scale45, 46, with 100 indicating the worst assessment.
As a control, 30 age-sex matched healthy subjects will be recruited.
Specifically, patients aged ≥18 years, referred to the Rheumatologic Outpatient Clinic of the Humanitas Research Hospital and capable of expressing written informed consent will be enrolled if they fulfill the following criteria.
Inclusion criteria
Subjects fulfilling all of the following inclusion criteria are eligible for the study:

patients aged ≥18 years;

- diagnosis of fibromyalgia according to the 2010/2011 ACR criteria and 2016 revised version41, not taking NSAIDs for at least one month prior to enrolment;
- diagnosis of PsA, classified according to CASPAR criteria42, who reached remission or low disease activity according to DAPSA (Disease Activity Index for Psoriatic Arthritis) score43 or who achieved minimal disease activity (MDA)44 after any DMARD for at least three months, but who still complain of residual pain with a visual analog scale >20 mm on a 0-100 scale45, 46, with 100 indicating the worst assessment;
- able to provide written informed consent.

Exclusion criteria
The presence of any one of the following exclusion criteria will lead to the exclusion of the subject.
- current or previous treatment with steroids;
- pregnancy and/or lactation;
- current or anamnestic malignancy.

Recruitment
Patients attending the Rheumatologic Outpatient Clinic of the Humanitas Research Hospital and eligible according to inclusion/exclusion criteria will be enrolled and will undergo a rheumatologic evaluation with clinimetric assessment. For patients with FM, Widespread Pain Index (WPI) and Symptom Severity Scale (SSS) score41 will be evaluated. For patients with PsA, DAPSA43 and MDA44 will be assessed. A complete pain profiling will be performed as shown below.
As a control 30 age-sex matched healthy subjects will be recruited from Humanitas employees.
Blood, saliva and stools will be collected.
Anthropometry and body composition
Bioelectrical impedance analysis will be performed to measure weight (kg), body fat (kg and %) and skeletal muscle mass (kg). Height (cm) will be measured using a stadiometer. BMI will be calculated as weight (kg) divided by height (m) squared and categorised using the international criteria: underweight (<18.5 kg/m2), normal-weight (18.5–24.9 kg/m2), overweight (25.0–29.9 kg/m2) and obese (≥30.0 kg/m2). Waist circumference (cm) will be measured with the participant standing at the middle point between the ribs and ileac crest.
In all cases, the measurements will be made at least two hours after the last meal, released from clothing and metal objects and having remained standing at least 5 minutes before the assessment.
Pain profiling
Quantitative Sensory Testing (QST)
The QST tests chosen are listed below. These tests can be fully applied at the bedside.
1. Mechanical detection threshold (MDT) assessed using modified von Frey filaments
2. Mechanical pain threshold (MPT) assessed using blunt mechanical pinprick stimuli
3. Stimulus-response-functions: mechanical pain sensitivity (MPS) for blunt mechanical pinprick stimuli and dynamic mechanical allodynia for stroking light touch (PinPrick and OptiHair2 set stimulators)
4. Wind-up ratio — the perceptual correlate of temporal pain summation for repetitive pinprick stimuli
5. Vibration detection threshold with Rydel-seiffer Tuning forks 64Hz
6. Pressure pain threshold with Algometer FPX50
7. Conditioned pain modulation using ischemic pain via blood pressure cuff

These will all be undertaken by a trained member of staff. Quantitative Sensory Testing will require the patient to be exposed to stimuli that elicit sensations of light and heavy touch and
pressure. This is a patient assessment methodology grounded in scientific rigour, is used globally in clinical research and is well established at the institution. The methodology and inciting stimulus will be communicated in written and verbal consenting, allowing patient withdrawal at any point.

Numerical Rating Scale (NPRS-11)

The Numerical Rating Scale (NPRS-11) is an 11-point scale for self-report of pain. The participant selects a whole number (integers 0–10) that best reflects the intensity of their pain. The anchors are 0 = no pain and 10 = extreme pain/worst possible pain. The NPRS can be administered verbally (therefore also by telephone) or graphically for self-completion.

DN4 (Douleur Neuropathique en 4 Questions)

DN4 (Douleur Neuropathique en 4 Questions) is a screening tool for neuropathic pain consisting of interview questions (DN4-interview) and an examination of the participant.

Pain Catastrophising Scale (PCS)

The PCS is a questionnaire developed to help quantify an individual's pain experience, asking about how they feel and what they think about when they are in pain.

Pain Anxiety Symptoms Scale (PASS-20)

The Pain Anxiety Symptoms Scale-20 (PASS-20) is a questionnaire which assesses 4 factorially distinct components of pain-related anxiety (ie, cognitive, fear, escape/avoidance, physiological).

PROMIS (Patient-Reported Outcomes Measurement Information System®)

PROMIS is a set of person-centred measures that evaluates and monitors physical, mental, and social health in adults and children.

Gastrointestinal symptoms evaluation

Rome IV Diagnostic Questionnaire for Functional Gastrointestinal Disorders

The Rome IV Diagnostic Questionnaire was developed to screen for functional gastrointestinal disorders, serve as inclusion criteria in clinical trials, and support epidemiologic surveys

Food Dietary Assessment

Food Registry

For the assessment of food consumption, a Food Registry will be used. Each patient and healthy control individual will be instructed to register three non-consecutive days of dieting (two weekdays and one weekend day) and should include detailed foods or preparations. Later the data will be tabulated in the DietoSystem software and the mean 3-day intake will be adjusted to reduce intrapersonal and interpersonal variation. The items examined will be total calories, carbohydrates, proteins, lipids, vitamins (A, C, B12, D and E) and minerals (folate, selenium, zinc, iron, calcium and magnesium). Energy and nutrient intakes of the study population will be compared to the Italian LARN references.

Self-assessment evaluation for anxiety and depression

Beck's Depression Inventory (BDI)

The Beck Depression Inventory (BDI) is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression.

Beck Anxiety Inventory (BAI)

The Beck Anxiety Inventory (BAI) is a widely used 21-item self-report inventory used to assess anxiety levels in adults.

WP2: Leaky gut assessment

Leaky-gut assessment in plasma:

We will analyze potential biomarkers related to leaky gut in the plasma of FM and PsA patients. Blood plasma and serum will be collected at a single time point: LPS,

lipopolysaccharide binding protein (LBP), soluble CD14 (sCD14), zonulin, hCRP, PV-1 and calprotectin. Moreover, we will measure the neuropeptide CGRP, the VIP and the neurotransmitter acetylcholine (ACh) .

Functional assay:

FM and PsA patients with increased plasma biomarkers of leaky-gut will undergo a functional test to confirm altered permeability. This assessment involves employing a multi-saccharide test to detect saccharides in urine samples following oral administration. The Mass-QGASTROPACK® I test (AB-Analitica, Padova, Italy) is utilized for this purpose, following the manufacturer's instructions. This test relies on quantifying four sugars excreted in the urine after ingestion. The presence of these sugars in abnormal quantities indicates potential abnormalities in the structure or function of specific gastrointestinal regions, including the stomach, small intestine, and large intestine. Specifically, mannitol serves as an internal control, assessing small bowel epithelial surface and integrity. Sucrose, lactulose, the lactulose-to-mannitol ratio (L/M), and sucralose function as markers for gastroduodenal, small intestinal, small intestinal normalized for total small intestinal absorption surface, and colonic permeability, respectively.

Leaky-gut, immune and nociceptor assessment in colon biopsies:

Patients with FM and PsA who have been found to have increased intestinal permeability (via plasma biomarkers and functional testing) will undergo a colonoscopy to obtain a colon biopsy.

Mucosal barrier status will be evaluated by assessment of PV-1 (marker of GVB permeability in CD31/ CD34+ endothelial cells), ZO-1 (marker of intestinal epithelial barrier) staining by immunofluorescence. MUC2, Ramp1, nociceptor distribution will also be evaluated (TRPV1+ and CGRPα +) 47. On a limited number of samples (n=15), immune cells, nociceptor and ENS spatial interaction will be evaluated by spatial transcriptomics on FFPE samples (GeoMx DSP).

WP3: Metabolic profile (Lania)

Metabolic profile assessment (insulin sensitivity):

Metabolic profile will be assessed by evaluating fasting glucose, fasting insulin, hemoglobin A1c (HbA1c), lipid profile, body mass index (BMI). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Triglyceride-Glucose (TyG) Index and Quantitative Insulin Sensitivity Check Index (QUICK1) will be used to assess insulin sensitivity.

WP 4: Adaptive immune response (Lugli)

Leaky gut and microbial translocation have been linked to the chronic activation of the adaptive immune system 48. Our lab has pioneered the development of high-dimensional flow cytometry analysis of the immune system 49, 50and related data analysis51. Frequency of immune cell populations in circulation related to immune activation, proliferation, differentiation, effector functions and cytotoxic/T helper cell activity will be evaluated by high-dimensional flow cytometry capable to measure 40+ markers. We have developed automated bioinformatics approaches for the unbiased and rapid analysis of large and complex datasets, to identify the dynamics of immune cell populations in disease and in response to treatment52, 53. By using these approaches, we will make it possible to correlate

adaptive immune signatures with clinical and inflammatory characteristics of the different patient cohorts (see WP5). Sex and age-matched healthy controls will be included as a comparison. We expect to identify novel cell populations in patients that are absent, or underrepresented in healthy controls, and thus possibly involved in the pathogenesis/perpetuation of the disease. The combination of markers expressed on the surface will make it possible to further isolate these cells for downstream omics analysis, such as at the transcriptomic (RNA-seq) or epigenomic (ATAC-seq) level.

These investigations will inform us on the possible functions of these cells, that will be further evaluated with dedicated functional assays *in vitro*.

WP 5: Innate immune response and cytokines (Di Mitri, Jaillon, Garlanda)

The first objective of WP5 will be to assess the local and systemic inflammatory status observed in plasma of patients, and to compare them with healthy controls. Specifically, we will assess the presence of a wide panel of inflammatory and anti-inflammatory biomarkers via a multiplex assay (e.g. CRP, PTX3, IL-1β, IL-6, IL-10, TNF-α, TGF-β, CXCL1, CXCL2, G-CSF, sIL-1R2). We will then assess whether the expression level of these molecules is associated with pathology activity or severity.

The second objective will be to study the presence and activation status of innate immune cells (e.g. monocytes and natural killer cells) in blood using flow cytometry analysis. This analysis will be performed in parallel with WP4. We will improve the characterization of innate immune cells through different approaches, including multiparametric flow cytometry analysis (up to 50 colors). In particular, we will assess the presence of cells expressing T cell immune checkpoint ligands (e.g. PD-L1, PD-L2, VISTA, OX40L, 4-1BBL, CD86), myeloid checkpoints (e.g., SIRP□, CD200R), and other molecules indicating differences in maturation or activation states (such as CD101, CD36, CD24, CXCR2, CXCR4, CD177, MHCII, CD80, CD86 for neutrophiles and monocytes, CD16, CD57, KIR, NKG2A, CD27, CD62L, NKG2D for NK cells). We will include original markers of the IL-1 system, including IL-1R2 and IL-1R8, which we are developing as markers of leukocyte dysfunction/activation in inflammatory conditions. As for WP4 activities, we will take advantage of CRUSTY, a versatile platform for rapid analysis and visualization of high-dimensional flow cytometry data developed in our institute51. These results will be complemented by transcriptomic analysis performed on isolated peripheral blood mononuclear cells (PBMC) from blood. We will apply deconvolution analysis to gene expression data to visualize the heterogeneity of different cell subsets present in patient samples.

WP6: Metabolomic and microbiota profiling (Rescigno)

Microbiota composition of patients affected by FM or PsA will be analyzed in oral and fecal samples. Metabolomics will be measured in plasma and fecal samples. All samples will be collected at a single time point (baseline).

Fecal and buccal swab samples will be stored at -80°C until DNA extraction. Bacterial DNA will be extracted from all the samples using DNeasy PowerSoil Pro Kits (Qiagen) according to the manufacturer's procedures. DNA concentration will be measured using the NanoDrop spectrophotometer (Thermo Fisher scientific) and stored at −80 °C. Shotgun metagenomic sequencing will be performed fecal (~ 7.5 Gb/sample) and buccal swab (~ 15 Gb/sample) samples: libraries will be prepared using Illumina® DNA prep according to the manufacturer's procedures.

The second aim of WP6 will be to identify metabolites in the plasma and feces of patients affected by FM or PsA. An untargeted metabolomics analysis will be performed. Samples will be collected and flash frozen, stored and maintained at -80 °C until processed. Samples will be spiked with internal standard, lyophilized overnight and then extracted with 400 µL of cold MeOH, 100 µL of H2O and 1 mL of cold tert-methylbutylether (MTBE). After centrifugation two phases can be observed: the upper organic phase and the bottom aqueous phase were collected and dried with a vacuum concentrator. Aqueous phases are analyzed using HILIC chromatographic column in negative mode with mobile phase (MP) at pH 10.5 and positive mode with MP at pH 3. Organic phases are analyzed with a C18 chromatographic column in positive and negative mode with MP pH respectively at 9.0 and 3.0. For quality control (QC) in each condition, a pooled sample is generated by taking a small volute of each sample extract. QC are analyzed along with technical blanks to assure method reproducibility during the entire analysis time. Analysis is performed using UHPLC (Vanquish Flex, Thermofisher Scientific) online with a high-resolution mass spectrometer (HRMS) (Orbitrap IQ-X

Tribrid Mass Spectrometer, Thermofisher scientific). Compound identification is achieved using the AcquireX method on pooled samples, and compound quantitation on each sample is based on peak integration of extracted ion chromatograms. Data mining and analysis is performed with Compound Discoverer Ver 3.3 (Thermofisher Scientific).

WP7: Data integration and analysis (Shulzhenko and Morgun**, visiting professors)**

Omics data from the different results obtained in the previous WPs will be integrated with clinical data through Transkingdom Network Analysis (TkNA) which is a unique analytical framework for inferring causal factors underlying host–microbiota and other multi-omic interactions. This analysis will allow us identifying causality and pathways involved in disease development. The analysis will be performed in collaboration with Natalia Shulzhenko and Andrey Morgun that are visiting professors of Humanitas University54.

EXPECTED RESULTS AND IMPACT

The present project takes advantage of unique lines of expertise strictly collaborating to develop precision and personalized medicine which requires the identification of biomarkers useful for early assessment of individual risk, diagnosis and identification of risk factors related to chronic pain. In this regard, monitoring the innate and adaptive immune responses, encompassing both cellular and humoral components, microbiota, metabolomics form the same patients and correlating them with the leaky-gut, glucose and insulin levels, as well as nociceptor activity, could contribute to the identification of new biomarkers, as previously demonstrated for other conditions55-57. We may further envision that alterations observed may lead to new therapeutic approaches to limit the quality-of-life impairment secondary to chronic pain.

FEASIBILITY, PITFALLS AND CAVEATS

Humanitas research center is characterized by the close proximity of the hospital and research laboratories thus allowing close interactions between clinicians and researchers. We expect to enroll all of the patients in the first two years thus allowing to finish sample analysis within the proposed time frame (36 months). We do not envisage technical issues as all of the techniques are in place. Whatever the results will be informative.

MILESTONES

M1: determine the expression level of soluble molecules and their association with the activity or severity of the pathology.
M2: determine the presence, maturation, and activation status of innate immune cells in patient samples.
M3: determine the microbiota composition of FM and PsA patients.
M4: determine metabolomics of FM and PsA patients.
M5: correlate above parameters with glucose, insulin, glycate hemoglobin, insulin sensitivity indexes and lipid profile and increased intestinal permeability
M6: evaluate the status of nociceptors and neurotransmitters in FM and PsA patients with increased intestinal permeability.

BIBLIOGRAPHY(in bold references from the group)

1.Treede RD, Rief W, BarkeA, . . . Wang SJ. 2019. Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). *Pain*160: 19-27
2.Sarzi-Puttini P, Giorgi V, Marotto D, . . . Atzeni F. 2020. Fibromyalgia: an update on clinical characteristics, aetiopathogenesis and treatment. *Nat Rev Rheumatol*16: 645-60

3.Haliloglu S, Carlioglu A, Akdeniz D, . . . Kosar A. 2014. Fibromyalgia in patients with other rheumatic diseases: prevalence and relationship with disease activity. *Rheumatol Int*34: 1275-80
4.Fan A, Pereira B, Tournadre A, . . . Dubost JJ. 2017. Frequency of concomitant fibromyalgia in rheumatic diseases: Monocentric study of 691 patients. *Semin Arthritis Rheum*47: 129-32
5.Uçeyler N, Valenza R, Stock M, . . . Sommer C. 2006. Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. *Arthritis Rheum*54: 2656-64
6.Bazzichi L, Rossi A, Massimetti G, . . . Bombardieri S. 2007. Cytokine patterns in fibromyalgia and their correlation with clinical manifestations. *Clin Exp Rheumatol*25: 225-30
7.Caxaria S, Bharde S, Fuller AM, . . . Sikandar S. 2023. Neutrophils infiltrate sensory ganglia and mediate chronic widespread pain in fibromyalgia. *Proc Natl Acad Sci U S A*120: e2211631120
8.Banfi G, Diani M, Pigatto PD, . . . Reali E. 2020. T Cell Subpopulationsin the Physiopathology of Fibromyalgia: Evidence and Perspectives. *Int J Mol Sci*21
9.Fineschi S, Klar J, Gustafsson KA, . . . Dahl N. 2022. Inflammation and Interferon Signatures in Peripheral B-Lymphocytes and Sera of Individuals With Fibromyalgia. *Front Immunol*13: 874490
10.Verma V, Drury GL, Parisien M, . . . Diatchenko L. 2022. Unbiased immune profiling reveals a natural killer cell-peripheral nerve axis in fibromyalgia. *Pain*163: e821-e36
11.Çakit O, Gümüştepe A, Duyur Çakit B, . . . Genç H. 2021. Coexistence of fibromyalgia and metabolic syndrome in females: The effects on fatigue, clinical features, pain sensitivity, urinary cortisol and norepinephrine levels: A cross-sectional study. *Arch Rheumatol*36: 26-37
12.Camilleri M, Vella A. 2022. Whatto do about the leaky gut. *Gut*71: 424-35
13.Pappolla MA, Manchikanti L, Candido KD, . . . TrescotAM. 2021. Insulin Resistance is Associated with Central Pain in Patients with Fibromyalgia. *Pain Physician*24: 175-84
14.Zhai X, Sun C, Rong P, . . . WangS. 2016. A Correlative Relationship Between Chronic Pain and Insulin Resistance in Zucker Fatty Rats: Role of Downregulation of Insulin Receptors. *J Pain*17: 404-13
15.Garcia G, Gutierrez-Lara EJ, Centurion D, . . . Murbartian J. 2019. Fructose-Induced Insulin Resistance as a Model of Neuropathic Pain in Rats. *Neuroscience*404: 233-45
16.Frosch OH, Yau PL, Osorio RS, . . . Convit A. 2017. Insulin resistance among obese middle-aged is associated with decreased cerebrovascular reactivity. *Neurology*89: 249-55
17.Mountz JM, Bradley LA, Modell JG, . . . Mountz JD. 1995. Fibromyalgia in women. Abnormalities of regional cerebral blood flow in the thalamus and the caudate nucleus are associated with low pain threshold levels. *Arthritis Rheum*38: 926-38
18.Damci T, Nuhoglu I, Devranoglu G, . . . Ilkova H. 2003. Increased intestinal permeability as a cause of fluctuating postprandial blood glucose levels in Type 1 diabetic patients. *Eur J Clin Invest*33: 397-401
19.Bayrak M. 2020. Metabolic syndrome, depression,and fibromyalgia syndrome prevalence in patients with irritable bowel syndrome: A case-control study. *Medicine (Baltimore)*99: e20577
20.Minerbi A, Fitzcharles MA. 2020. Gut microbiome: pertinence in fibromyalgia. *Clin Exp Rheumatol*38 Suppl 123: 99-104
21.Clos-Garcia M, Andrés-Marin N, Fernández-Eulate G, . . . Falcón-Pérez JM. 2019. Gut microbiome and serum metabolome analyses identify molecular biomarkers and altered glutamate metabolism in fibromyalgia. *EBioMedicine*46: 499-511
Minerbi A, Gonzalez E, Brereton NJB, . . . Shir Y. 2019. Altered microbiome composition in individuals with fibromyalgia. *Pain*160: 2589-602

23.Ley RE, Turnbaugh PJ, Klein S, . . . Gordon JI. 2006. Microbial ecology: human gut microbes associated with obesity. *Nature*444: 1022-3
24.Fan Y, Pedersen O. 2021. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol*19: 55-71
25.Aron-Wisnewsky J, Vigliotti C, WitjesJ, . . . Clément K. 2020. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol*17: 279-97
26.Spadoni I, Zagato E, Bertocchi A, . . . Rescigno M. 2015. A gut-vascular barrier controls the systemic dissemination of bacteria. *Science*350: 830-4
27.Carloni S, Bertocchi A, Mancinelli S, . . . Rescigno M. 2021. Identification of a choroid plexus vascular barrier closing during intestinal inflammation. *Science*374: 439-48
28.Veiga-Fernandes H, Mucida D. 2016. Neuro-Immune Interactions at Barrier Surfaces. *Cell*165: 801-11
29.Klose CSN,Veiga-Fernandes H. 2021. Neuroimmune interactions in peripheral tissues. *Eur J Immunol*51: 1602-14
30.Lagomarsino VN, Kostic AD, Chiu IM. 2021. Mechanisms of microbial-neuronal interactions in pain and nociception. *Neurobiol Pain*9: 100056
31.Yang D, Jacobson A, Meerschaert KA, . . . Chiu IM. 2022. Nociceptor neurons direct goblet cells via a CGRP-RAMP1 axis to drive mucus production and gut barrier protection. *Cell*185: 4190-205.e25
32.Chen Y, Xu J, Chen Y. 2021. Regulation of Neurotransmitters by the Gut Microbiota and Effects on Cognition in
Neurological Disorders. *Nutrients*13
33.Trouvin AP, Attal N, Perrot S. 2022. Assessing central sensitization with quantitative sensory testing in inflammatory rheumatic diseases: A systematic review. *Joint Bone Spine*89: 105399
34.Ritchlin CT, Colbert RA, Gladman DD. 2017. Psoriatic Arthritis. *N Engl J Med*376: 957-70
35.Liu V, Fong W, Kwan YH, . . . Leung YY. 2021. Residual Disease Burden in Patients With Axial Spondyloarthritis and Psoriatic Arthritis Despite Low Disease Activity States in a Multiethnic Asian Population. *J Rheumatol*48: 677-84
36.van Mens LJJ, Turina MC, van de Sande MGH, . . . Baeten DLP. 2018. Residual disease activity in psoriatic arthritis: discordance between the rheumatologist's opinion and minimal disease activity measurement. *Rheumatology (Oxford)*57: 283-90
37.Veale DJ, Fearon U. 2018. The pathogenesis of psoriatic arthritis. *Lancet*391: 2273-84
38.Wang L, Wang Y, Zhang P, . . . Huang F. 2022. Gut microbiota changes in patients with spondyloarthritis: A systematic review. *Semin Arthritis Rheum*52: 151925
39.Hecquet S, Totoson P, Martin H, . . . Verhoeven F. 2021. Intestinal permeability in spondyloarthritis and rheumatoid arthritis: A systematic review of the literature. *Semin Arthritis Rheum*51: 712-8
40.Dougados M, Taylor PC, Bingham CO, 3rd, . . . Kessouri M. 2022. The effect of tofacitinib on residual pain in patients with rheumatoid arthritis and psoriatic arthritis. *RMD Open*8
41.Wolfe F, Clauw DJ, Fitzcharles MA, . . . WalittB. 2016. 2016 Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin Arthritis Rheum*46: 319-29
42.Taylor W, Gladman D, Helliwell P, . . . Mielants H. 2006. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum*54: 2665-73
43.Schoels MM, Aletaha D, Alasti F, . . . Smolen JS. 2016. Disease activity in psoriatic arthritis (PsA): defining remission and treatment success using the DAPSA score. *Ann Rheum Dis*75: 811-8

44.Coates LC, Fransen J, Helliwell PS. 2010. Defining minimal disease activity in psoriatic arthritis: a proposed objective target for treatment. *Ann Rheum Dis*69: 48-53
45.Wolfe F, Michaud K. 2007. Assessment of pain in rheumatoid arthritis: minimal clinicallysignificant difference, predictors, and the effect of anti-tumor necrosis factor therapy. *J Rheumatol*34: 1674-83
46.Berghea F, Berghea CE, Zaharia D, . . . Vlad VM. 2021. Residual Pain in the Context of Selecting and Switching Biologic Therapy in InflammatoryRheumatic Diseases. *Front Med (Lausanne)*8: 712645
47.Spencer NJ, Hibberd T, Xie Z, . . . Hu H. 2022. How should we define a nociceptor in the gut-brain axis? *Front Neurosci*16: 1096405
48.Brenchley JM, Price DA, Schacker TW, . . . Douek DC. 2006.Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med*12: 1365-71
49.Brummelman J, Haftmann C, Núñez NG, . . . Lugli E. 2019. Development, application and computational analysis of high-dimensional fluorescentantibody panels for single-cell flow cytometry. *Nat Protoc*14: 1946-69
50.Galletti G, De Simone G, Mazza EMC, . . . Lugli E. 2020. Two subsets of stem-like CD8(+) memory T cell progenitors with distinct fate commitments in humans. *Nat Immunol*21: 1552-62
51.Puccio S, Grillo G, Alvisi G, . . . Lugli E. 2023. CRUSTY: a versatile web platform for the rapid analysis and visualization of high-dimensional flow cytometry data. *Nat Commun*14: 5102
52.De Biasi S, Gibellini L, Lo Tartaro D, . . . Cossarizza A. 2021. Circulating mucosal-associated invariant T cells identify patients responding to anti-PD-1 therapy. *Nat Commun*12: 1669
53.WischnewskiV, Maas RR, Aruffo PG, . . . Joyce JA. 2023. Phenotypic diversity of T cells in human primary and metastatic brain tumors revealed by multiomic interrogation. *Nat Cancer*4: 908-24
54.Newman NK, Macovsky MS, Rodrigues RR, . . . Morgun A. 2024. Transkingdom Network Analysis (TkNA): a systems framework for inferring causal factors underlying host-microbiota and other multi-omicinteractions. *Nat Protoc*
55.Jaillon S, Ponzetta A, Di Mitri D, . . . Mantovani A. 2020. Neutrophil diversity and plasticity in tumour progression and therapy. *Nat Rev Cancer*20: 485-503
56.Mantovani A, Garlanda C. 2023. Humoral Innate Immunity and Acute-Phase Proteins. *N Engl J Med*388: 439-52
57.Masetti M, Carriero R, Portale F, . . . Di Mitri D. 2022. Lipid-loaded tumor-associated macrophages sustain tumor growth and invasiveness in prostate cancer. *J Exp Med*219

Ministero della Salute – Direzione Generale della Ricerca e dell'Innovazione in Sanità

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Dati del rappresentante legale: Riccardo Bui

Titolo del progetto: Glucagon like peptide-1 receptor agonists to prevent coronary artery disease progression in diabetics with acute coronary syndrome: the PROGRESSION-GLP1 study

Data di inizio progetto: 01/12/2024	**Data di fine progetto**: 30/11/2027
Fondi 5 per mille assegnati al progetto: **€ 297.505,00**	**Di cui:** **Quota da sostenere entro l'anno di rendicontazione: € 70.772,92** **Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni): € 226.732,08**

VOCI DI SPESA	Quota da sostenere entro l'anno di rendicontazione	Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni)
Personale di ricerca (borsista, a contratto e di ruolo in quota parte)	61.541,67	95.158,33
Apparecchiature (ammortamento, canone di locazione/leasing)	0,00	0,00
Materiale d'uso destinato alla ricerca (per laboratori di ricerca, acquisto farmaci ecc.)	0,00	86.179,25
Spese di organizzazione (manifestazioni e convegni, viaggi e missioni ecc.)	0,00	5.000,00
Elaborazione dati	0,00	5.000,00
Spese amministrative	9.231,25	35.394,50
Altro	0,00	0,00
TOTALE	**70.772,92**	**226.732,08**

Data, 10/07/2025

Il Legale Rappresentante

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Titolo del progetto: Glucagon like peptide-1 receptor agonists to prevent coronary artery disease progression in diabetics with acute coronary syndrome: the PROGRESSION-GLP1 study

Background:
Myocardial revascularization represents the most commonly performed therapeutic intervention worldwide and the gold standard of treatment for patients with acute coronary syndromes (ACS). Owing to clinical and technological advancements in stent design and revascularization strategies, percutaneous coronary intervention (PCI) provides excellent outcomes during short and long-term follow-up. However, a relevant portion of ACS patients undergoing PCI require a repeat revascularization procedure in the following years, largely due to CAD progression on non-culprit segments considered as the main underlying mechanisms. In the landmark Prospective Natural History Study of Coronary Atherosclerosis (PROSPECT) trial, including 697 patients with ACS undergoing intravascular imaging guided PCI, one out of five patients had a repeat revascularization at three years, with a similar proportion of events attributable to culprit lesions (10.9%) and CAD progression in non-culprit sites (10.5%).
Both clinical and angiographic factors might contribute to an increased risk of myocardial revascularization failure over time. Specific coronary atherosclerotic plaque features which might predispose to recurrent ACS events have been mainly investigated through invasive intravascular imaging studies. However, intracoronary imaging is notably underused in clinical practice and is not recommended to systematically search for non-culprit plaques, especially in the absence of angiographically obstructive lesions. Coronary computed tomography angiography (CCTA) is a noninvasive test that enables evaluation of all coronary arteries and their branches in patients with known or suspected CAD. The high accuracy of CCTA for detection and exclusion of CAD is well reported, and

recent advances in computed tomographic technologies now allow for coronary atherosclerotic quantification and characterization, with high diagnostic performance when compared with invasive reference standards. If routinely performed in patients with manifest CAD, CCTA could offer the unique opportunity to describe the natural history of atherosclerosis before ACS occurrence, while accounting for the totality of atherosclerotic features in all coronary arteries and their branches at the patient level. At the same time, novel tools are being developed to implement the diagnostic accuracy of CCTA in the presence of coronary stents thereby allowing to recognize subclinical in-stent restenosis. Across clinical factors, diabetes mellitus is one of the most common determinants of CAD progression after PCI with newest generation drug eluting stents, and diabetic patients present an increased risk of both target lesion failure and non-target lesion revascularization compared to non-diabetic individuals. While several pathogenic mechanisms might justify the accelerated atherosclerosis associated with diabetes, including lipid metabolism dysregulation, endothelial dysfunction and chronic inflammation, very little is known concerning the relative effectiveness of the different classes of antidiabetic medications to promote or retard the progression of atherosclerosis. Injectable glucagon like peptide-1 receptor (GLP-1R) agonists mimic endogenous GLP-1 by stimulating pancreatic insulin secretion with a low risk of hypoglycemia. Seven randomized clinical trials and a subsequent meta-analysis have shown a consistent reduction in major adverse cardiovascular events associated with GLP1-R agonists (GLP1-RA) when administered in diabetic patients at high cardiovascular risk. As a consequence, GLP1-RA have been recommended as first-line antidiabetic medications in diabetic patients with atherosclerotic cardiovascular disease by the 2021 guidelines of the European Society of Cardiology on cardiovascular disease prevention. Several potential indirect cardiovascular effects of GLP1-RA have been proposed to explain the strong prognostic benefit associated with these compounds. Among these, preclinical evidence suggests GLP1-RA could prevent the development and progression of atherosclerotic lesions by suppressing macrophage foam cell formation and potentially driven by anti-inflammatory mechanisms.

Aim:
To evaluate the effects of GLP1-RA on CAD progression and correlate the findings with immune and inflammatory modulation.

Preliminary findings:
The PROGRESSION trial (ClinicalTrials.gov Identifier: NCT03890822) is a single-center, prospective observational study conducted at Humanitas Research Hospital IRCCS (Milan, Italy), which actively enrolled 100 patients with non-ST-segment elevation acute coronary syndromes from 2019 to 2022. In order to be included, patients were required to undergo PCI and to present at least one non-culprit lesion with indication to medical therapy alone. Enrolled subjects underwent repeated CCTA at discharge and at 1 year, with the aim to evaluate the incidence and predictors of CAD progression after ACS. Progression of CAD is assessed based on percent atheroma volume and total atheroma volume, precisely and automatically quantified with the QAngio CT software (Medis Medical Imaging Systems, Leiden, NL).
A preliminary analysis of 16 patients was performed, 7 (44%) of these were treated with GLP1-ra. Baseline characteristics, including age, sex, cardiovascular risk factors, body mass index, and medical therapy at discharge did not differ between groups. Median baseline PAV was 25.7 (5.6-34.0)% in the GLP1-ra group and 25.3 (14.4-28.0)% in the no GLP1-ra group (p=0.68). At one year, a signal towards a lower PAV increase in the GLP1ra group was observed (-1.9 [-3.5, -0.2]% vs. 0.4 [-1.8, 2.5]%, p=0.10). Whereas, absolute change in TAV was not significantly different between the GLP1ra (-2.5 [-29.0-1.2] mm3) and no GLP1ra (14.4 [-4.9-51.2], p=0.37) groups.

Methods:
The PROGRESSION GLP1-RA study will prospectively enroll 25 diabetic patients with ACS, following the same angiographic inclusion criteria specified for the PROGRESSION trial. After PCI and guideline-directed medical therapy optimization, therapy with GLP1-RA will be initiated as suggested by current international guidelines, unless contraindicated. CCTA will be performed at baseline (within 2 weeks from discharge) and at 1 and 3 years of follow-up. These patients will be compared with diabetic patients already enrolled in the PROGRESSION trial and not on GLP1-RA treatment (n=25), who will be scheduled an additional CCTA at 3 years.
We will compare 1) atherosclerotic progression in non-culprit sites on native vessels and 2) the degree of subclinical in-stent restenosis with and without GLP1-ra. The primary endpoint will be the absolute change in percent atheroma volume (PAV) at 1 and 3 years, , as detected by CCTA. A key secondary endpoint was absolute change in total atheroma volume (TAV) at 1 and 3 years, as detected by CCTA.
In addition, all patients will undergo collection of peripheral blood mononucleated cells (PBMC) and plasma, at baseline and at follow-up.
We will perform single cell RNA sequencing and single proteomics sequencing (CITE-SEQ) on PBMC. We will obtain an average of 2500 cells per sample, in order to perform an accurate genes analysis. Moreover, the proteomics analysis will confirm and strengthen the RNA cluster analysis that will be performed for every samples. This analysis will clusterize and characterize the immune cells. The cell-to-cell interaction and cell abundance will provide information about the cellular response and function. The genes and pathways differentially activated in aggravate condition, but down modulated during GLP1-ra treatment, are the new, specific, and potentially highly efficient therapeutic targets. Plasma will be also examined for the analysis of the level of key pro-inflammatory cytokines, including IL-6, TNFa and CCL2. The plasma collected will be also used to perform a metabolomic assay, in order to find markers associated to the pathologic state and to the cell-related activation state. Lastly, we will determine the mutational state of genes involved in clonal hematopoiesis of indetermined potential (CHIP), the mutation of which has been shown to be associated with increased risk of CAD by mean of enhanced inflammation.

Relevance:
Since CAD remains the first cause of mortality worldwide, risk stratification aiming at identifying patients at higher risk of CAD progression over time is, therefore, pivotal to impact on patients prognosis. Moreover, new treatments are required to target and minimize CAD progression among patients at higher risk. This study will allow the implementation of 1) a more granular bioimaging-based risk stratification to identify those subjects at increased risk of CAD progression, and 2) targeted therapies to limit disease progression and prevent potentially fatal events.

References
Head SJ, Milojevic M, Taggart DP, Puskas JD. Current Practice of State-of-the-Art Surgical Coronary Revascularization. Circulation. 2017 Oct 3;136(14):1331-1345.
2020 ESC guidelines NSTE-ACS
Stefanini GG, Alfonso F, Barbato E, Byrne RA, Capodanno D, Colleran R, Escaned J, Giacoppo D, Kunadian V, Lansky A, Mehilli J, Neumann FJ, Regazzoli D, Sanz-Sanchez J, Wijns W, Baumbach A. Management of myocardial revascularisation failure: an expert consensus document of the EAPCI. EuroIntervention. 2020 Dec 4;16(11):e875-e890.
Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, Parise H, Templin B, White R, Zhang Z, Serruys PW; PROSPECT Investigators. A prospective natural-history study of coronary atherosclerosis. N Engl J Med. 2011 Jan 20;364(3):226-35.

Chang HJ, Lin FY, Lee SE, Andreini D, Bax J, Cademartiri F, Chinnaiyan K, Chow BJW, Conte E, Cury RC, Feuchtner G, Hadamitzky M, Kim YJ, Leipsic J, Maffei E, Marques H, Plank F, Pontone G, Raff GL, van Rosendael AR, Villines TC, Weirich HG, Al'Aref SJ, Baskaran L, Cho I, Danad I, Han D, Heo R, Lee JH, Rivzi A, Stuijfzand WJ, Gransar H, Lu Y, Sung JM, Park HB, Berman DS, Budoff MJ, Samady H, Shaw LJ, Stone PH, Virmani R, Narula J, Min JK. Coronary Atherosclerotic Precursors of Acute Coronary Syndromes. J Am Coll Cardiol. 2018 Jun 5;71(22):2511-2522.

Williams MC, Moss AJ, Dweck M, Adamson PD, Alam S, Hunter A, Shah ASV, Pawade T, Weir-McCall JR, Roditi G, van Beek EJR, Newby DE, Nicol ED. Coronary Artery Plaque Characteristics Associated With Adverse Outcomes in the SCOT-HEART Study. J Am Coll Cardiol. 2019 Jan 29;73(3):291-301.

Smilowitz NR, Mohananey D, Razzouk L, Weisz G, Slater JN. Impact and trends of intravascular imaging in diagnostic coronary angiography and percutaneous coronary intervention in inpatients in the United States. Catheter Cardiovasc Interv. 2018 Nov 15;92(6):E410-E415.

Truesdell AG, Alasnag MA, Kaul P, Rab ST, Riley RF, Young MN, Batchelor WB, Maehara A, Welt FG, Kirtane AJ; ACC Interventional Council. Intravascular Imaging During Percutaneous Coronary Intervention: JACC State-of-the-Art Review. J Am Coll Cardiol. 2023 Feb 14;81(6):590-605.

Budoff MJ, Dowe D, Jollis JG, et al. Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evalua- tion of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial. J Am Coll Cardiol 2008;52:1724–32.

DISCHARGE Trial Group. CT or Invasive Coronary Angiography in Stable Chest Pain. N Engl J Med. 2022 Apr 28;386(17):1591-1602.

Motoyama S, Ito H, Sarai M, Kondo T, Kawai H, Nagahara Y, Harigaya H, Kan S, Anno H, Takahashi H, Naruse H, Ishii J, Hecht H, Shaw LJ, Ozaki Y, Narula J. Plaque Characterization by Coronary Computed Tomography Angiography and the Likelihood of Acute Coronary Events in Mid-Term Follow-Up. J Am Coll Cardiol. 2015 Jul 28;66(4):337-46.

Van Royen N. TARGET AC sub-group analysis on diabetics and ACS patients. Barcelona (Spain) - European Society of Cardiology 2022

Wu PW, Tsay PK, Sun Z, Peng SJ, Lee CY, Hsu MY, Ko YS, Hsieh IC, Wen MS, Wan YL. Added Value of Computed Tomography Virtual Intravascular Endoscopy in the Evaluation of Coronary Arteries with Stents or Plaques. Diagnostics (Basel). 2022 Feb 3;12(2):390.

Rajagopal JR, Farhadi F, Richards T, Nikpanah M, Sahbaee P, Shanbhag SM, Bandettini WP, Saboury B, Malayeri AA, Pritchard WF, Jones EC, Samei E, Chen MY. Evaluation of Coronary Plaques and Stents with Conventional and Photon-counting CT: Benefits of High-Resolution Photon-counting CT. Radiol Cardiothorac Imaging. 2021 Oct 28;3(5):e210102

Sun Z, Almutairi AM. Diagnostic accuracy of 64 multislice CT angiography in the assessment of coronary in-stent restenosis: a meta-analysis. Eur J Radiol. 2010 Feb;73(2):266-73.

Gitto M, Vrachatis DA, Condorelli G, Papathanasiou K, Reimers B, Deftereos S, Stefanini GG. Potential Therapeutic Benefits of Sodium-Glucose Cotransporter 2 Inhibitors in the Context of Ischemic Heart Failure: A State-of-the-Art Review. Cardiovasc Hematol Agents Med Chem. 2022;20(2):90-102.

Kalra S, Baruah MP, Sahay RK, Unnikrishnan AG, Uppal S, Adetunji O. Glucagon-like peptide-1 receptor agonists in the treatment of type 2 diabetes: past, present, and future. Indian J Endocrinol Metab. 2016; 20:254–267

Kristensen SL, Rørth R, Jhund PS, Docherty KF, Sattar N, Preiss D, Køber L, Petrie MC, McMurray JJV. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet Diabetes Endocrinol. 2019 Oct;7(10):776-785.
Rakipovski G, Rolin B, Nøhr J, Klewe I, Frederiksen KS, Augustin R, Hecksher-Sørensen J, Ingvorsen C, Polex-Wolf J, Knudsen LB. The GLP-1 Analogs Liraglutide and Semaglutide Reduce Atherosclerosis in ApoE-/- and LDLr-/- Mice by a Mechanism That Includes Inflammatory Pathways. JACC Basic Transl Sci. 2018 Nov 21;3(6):844-857.
Joseph JJ, Deedwania P, Acharya T, Aguilar D, Bhatt DL, Chyun DA, Di Palo KE, Golden SH, Sperling LS;. Comprehensive Management of Cardiovascular Risk Factors for Adults With Type 2 Diabetes:

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Dati del rappresentante legale: Riccardo Bui

Titolo del progetto: Pathophysiological insights and tailored therapeutic approaches in patients with ST-segment elevation myocardial infarction and plaque erosion: an optical coherence tomography imaging study with single-cell analysis

Data di inizio progetto: 01/01/2025	**Data di fine progetto**: 31/12/2027
Fondi 5 per mille assegnati al progetto: **€ 300.000,00**	**Di cui:** **Quota da sostenere entro l'anno di rendicontazione: € 0,00** **Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni): € 300.000,00**

VOCI DI SPESA	Quota da sostenere entro l'anno di rendicontazione	Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni)
Personale di ricerca (borsista, a contratto e di ruolo in quota parte)	0,00	80.000,00
Apparecchiature (ammortamento, canone di locazione/leasing)	0,00	0,00
Materiale d'uso destinato alla ricerca (per laboratori di ricerca, acquisto farmaci ecc.)	0,00	150.000,00
Spese di organizzazione (manifestazioni e convegni, viaggi e missioni ecc.)	0,00	2.000,00
Elaborazione dati	0,00	2.000,00
Spese amministrative	0,00	45.000,00
Altro (patient insurance, support for core lab)	0,00	21.000,00
TOTALE	**0,00**	**300.000,00**

Data, 10/07/2025

Il Legale Rappresentante

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Il Legale Rappresentante

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Dati del rappresentante legale: Riccardo Bui

Titolo del progetto: Pathophysiological insights and tailored therapeutic approaches in patients with ST-segment elevation myocardial infarction and plaque erosion: an optical coherence tomography imaging study with single-cell analysis

BACKGROUND

Primary percutaneous coronary intervention (PCI) with drug-eluting stents (DES) implantation is the cornerstone therapy in patients with ST-segment elevation myocardial infarction (STEMI) (1). Nevertheless, risks of target lesion revascularization following PCI with DES, owing to stent thrombosis and restenosis due to neointimal hyperplasia or late neoatherosclerosis are not trivial (2). Furthermore, such one-size-fits-all therapeutic approach does not take into account the heterogeneity of the coronary pathologies and mechanisms underlying coronary thrombosis in STEMI. Indeed, in both autopsy data and in vivo clinical studies using high resolution intravascular optical coherence tomography (OCT) imaging, plaque rupture was the most frequent cause of coronary thrombosis (about 60%), plaque erosion without cap disruption, where flowing blood comes into direct contact with intimal surface lacking endothelial cells triggering coronary thrombosis, was found in about 30% of cases, and calcified nodule with disruption of fibrous cap was reported in about 5% of patients (3).

We reported the association of elevated systemic myeloperoxidase levels with plaque erosion and a differential expression of myeloperoxidase in thrombi overlying eroded plaques, compared with plaque rupture (4). Also increasing evidence lends support the idea that plaque erosion as a disease entity is wholly different from plaque rupture, with fundamental differences in the inflammatory mechanisms and pathogenesis of thrombosis (5). Nevertheless, a detailed single-cell level analysis of how immune cells are related to different coronary pathologies, i.e. plaque erosion vs plaque rupture, in acute STEMI is lacking.

Furthermore, previous observational studies, with the use of OCT, have reported an association between plaque erosion with lower residual percentage stenosis, compared with plaque rupture, after successful removal of thrombus burden (6,7). This finding has prompted several investigators to attempt a revascularization strategy consisting in thrombus removal and antithrombotic therapy only, without DES implantation, in patients with acute coronary syndromes and plaque erosion (6,7). Although such treatment approach was associated with good immediate clinical and angiographic results without vessel re-occlusion, nevertheless a high rate of target lesion revascularization at longer term follow-up was reported (6,7). The residual post-procedural percentage of area stenosis, that progressed over time, was found to be an independent predictor of target lesion revascularization at follow-up, thus underscoring the need for optimization of the acute angiographic result following thrombus removal.
Drug-coated balloons (DCB) are an emerging technology that allows to treat coronary artery disease without the need for permanent stent scaffold. DCB allow to achieve substantial acute gain (i.e. immediate increase in luminal diameter) compared with medical therapy and provide significant advantages, compared with plain old balloon angioplasty, in reducing the incidence of restenosis at follow-up, via the release of antiproliferative drugs to the vessel wall that offset vascular smooth cell proliferation, known to be a key pathogenetic mechanism of restenosis (8). A recent OCT study has also shown the potential of DCB to promote positive vessel remodeling (i.e. increased vessel dimension) at follow-up in about 50% of patients, with negligible late loss (i.e. reduction of luminal diameter at follow-up compared with the postprocedural result) (9). Recent meta-analyses of randomised clinical trials comparing DCB with DES have also shown the clinical safety and efficacy of DCB for the treatment of de-novo coronary artery disease in the setting of chronic coronary syndromes, reporting no thrombotic events in the early and late follow-up following DCB use (10). Evidence about the safety and effectiveness of DCB in STEMI patients is increasing (11).

AIMS

1) This is a proof-of concept study for OCT-guided primary angioplasty in patients with STEMI tailoring the use of DES or DCB according to different coronary pathologies, i.e. plaque rupture or plaque erosion, respectively. Specifically, the study aims at assessing the effects of angioplasty with DCB on acute and mid-term angiographic and clinical outcomes in patients with plaque erosion.
2) A key aim of the study is to investigate the differential immunological and inflammatory pathophysiological mechanisms of plaque erosion compared with plaque rupture by analysing the characteristics of specific cell types and individual cells simultaneously on a transcriptome-wide level and gene expression level.
3) The study will also investigate the relationship of different plaque phenotypes and specific immunological traits with microvascular obstruction, a key component of ischemia-reperfusion injury, as well as infarct size.

STUDY ENDPOINTS (Cohort of patients with plaque erosion)

-The primary endpoint of the study will be angiographic net gain in minimal lumen diameter (MLD) (mm) inside the DCB treated area in the per-protocol (PP) population, which will comprise patients who will receive the assigned treatment in the absence of bail-out stenting. Net gain is defined as follow-up MLD minus baseline MLD.
-Key secondary endpoints will be: device (DCB) success, procedure success, angiographic acute gain, acute/subacute/early/late vessel closure/thrombosis, angiographic late lumen loss (LLL) (i.e. post PCI MLD minus MLD at 6-month follow-up), MLD at 6 months, angiographic percent diameter stenosis at 6 months, binary restenosis rate (percent diameter stenosis [%DS] ≥50% at 6-month follow-up.
-Additional secondary endpoints:

-Vessel healing as assessed by OCT at 6 months following DCB, comprising evaluation of plaque morphology, vessel and lumen size, residual thrombus burden.

STUDY ENDPOINTS (entire STEMI cohort)

-Cell type composition, i.e.major cell types in the peripheral blood mononuclear cell (PBMCs), in patients with plaque erosion compared with plaque rupture, both at the site of the culprit lesion and the systemic circulation,

-Gene expression levels per cell type in patients with plaque erosion compared with patients with plaque rupture,

-Protein expression profiles in patients with plaque erosion compared with patients with plaque rupture,

-Clinical Device oriented Composite Endpoint (DoCE): the composite of cardiac death, target vessel myocardial infarction, clinically and/or physiologically indicated target lesion revascularization at 12 months in patients with plaque erosion compared with plaque rupture,

-Microvascular obstruction as assessed by angiographic indices of reperfusion such as myocardial blush grade, and postprocedural ECG ST resolution > 70%,

-Infarct size, as assessed by CK-MB and high sensitivity troponin release curve.

STUDY DESIGN

Prospective, multicentre, observational interventional trial enrolling about 150 patients with acute STEMI presenting within 24 hrs from symptom onset. All patients will undergo coronary angiography and primary PCI. Patients with OCT-documented plaque rupture will undergo DES implantation following thrombus aspiration. Patients with OCT-documented plaque erosion will undergo angioplasty with DCB following thrombus aspiration. Patients with plaque erosion will undergo coronary angiography and OCT evaluation of the treated target lesion at 6-month follow-up. All patients will be followed for up to 1 year for clinical endpoints. It is expected that the number of patients with plaque erosion will be about 50.

STUDY POPULATION

Inclusion criteria

Consecutive patients with a diagnosis of STEMI undergoing primary PCI within 24 hours from symptom onset, de novo coronary occlusion, written informed consent, age ≥18 yrs.

Exclusion criteria

Administration of thrombolytic, stent thrombosis, culprit lesion in bypass graft, impossibility to identify culprit lesion, known mechanical complication of acute myocardial infarction, unconscious patients, intubated patients, shock, women of child-bearing potential (e.g. below 55 years of age, who have not undergone tubal ligation, ovariectomy or hysterectomy and last menstruation within the last 12 months), participation in another interventional clinical trial, non-cardiac comorbid conditions with life expectancy of less than 1 year, patients unable or unwilling to give their informed consent.

Study duration

The estimated duration of the enrolment phase will be approximately 15 months. The duration of the follow-up phase will be 12 months. Total study duration is expected to be approximately 27 months. The First Patient-In is expected to be enrolled in November 1st 2024. The Last-1-year follow-up is expected on February 1st 2027. Our centre will enrol approximately 60 to 70 patients with STEMI, given the collaboration with an academic high-volume centre of primary PCI in Milan, eg Centro Cardiologico Monzino. This rate of enrolment corresponds to 1-1.2 patients per week which is a realistic target of enrolment.

References

1. Correction to: 2023 ESC Guidelines for the management of acute coronary syndromes: Developed by the task force on the management of acute coronary syndromes of the European Society of Cardiology (ESC). Eur Heart J. 2024;45(13):1145. doi:10.1093/eurheartj/ehad870
2. Shlofmitz E, Iantorno M, Waksman R. Restenosis of Drug-Eluting Stents: A New Classification System Based on Disease Mechanism to Guide Treatment and State-of-the-Art Review [published correction appears in Circ Cardiovasc Interv. 2019 Oct;12(10):e000044]. Circ Cardiovasc Interv. 2019;12(8):e007023. doi:10.1161/CIRCINTERVENTIONS.118.007023
3. Tomaniak M, Katagiri Y, Modolo R, et al. Vulnerable plaques and patients: state-of-the-art. Eur Heart J. 2020;41(31):2997-3004. doi:10.1093/eurheartj/ehaa227
4. Ferrante G, Nakano M, Prati F, et al. High levels of systemic myeloperoxidase are associated with coronary plaque erosion in patients with acute coronary syndromes: a clinicopathological study. Circulation. 2010;122(24):2505-2513. doi:10.1161/CIRCULATIONAHA.110.955302
5. Baaten CCFMJ, Nagy M, Bergmeier W, et al. Platelet biology and function: plaque erosion vs. rupture. Eur Heart J. 2024;45(1):18-31. doi:10.1093/eurheartj/ehad720
6. Jia H, Dai J, Hou J, et al. Effective anti-thrombotic therapy without stenting: intravascular optical coherence tomography-based management in plaque erosion (the EROSION study). Eur Heart J. 2017; 38:792–800. doi: 10.1093/eurheartj/ehw381
7. Yin Y, Lei F, Fang C, et al. Predictors of adverse prognosis in patients with acute coronary syndrome caused by plaque erosion with a nonstent strategy. J Am Heart Assoc. 2022; 11:e026414. doi: 10.1161/JAHA.122.026414
8. Jeger RV, Eccleshall S, Wan Ahmad WA, et al. Drug-Coated Balloons for Coronary Artery Disease: Third Report of the International DCB Consensus Group. JACC Cardiovasc Interv. 2020;13(12):1391-1402. doi:10.1016/j.jcin.2020.02.043
9. Ninomiya K, Serruys PW, Colombo A, et al. A Prospective Randomized Trial Comparing Sirolimus-Coated Balloon With Paclitaxel-Coated Balloon in De Novo Small Vessels. JACC Cardiovasc Interv. 2023;16(23):2884-2896. doi:10.1016/j.jcin.2023.09.026
10. Sanz Sánchez J, Chiarito M, Cortese B, et al. Drug-Coated balloons vs drug-eluting stents for the treatment of small coronary artery disease: A meta-analysis of randomized trials. Catheter Cardiovasc Interv. 2021;98(1):66-75. doi:10.1002/ccd.29111
11. Zhang Y, Chen D, Dong Q, et al. Drug-Coated Balloons for Acute Myocardial Infarction: A Metaanalysis of Randomized Clinical Trials. J Interv Cardiol. 2022;2022:4018771. Published 2022 Dec 27. doi:10.1155/2022/4018771
12. Johnson TW, Räber L, di Mario C, et al. Clinical use of intracoronary imaging. Part 2: acute coronary syndromes, ambiguous coronary angiography findings, and guiding interventional decision-making: an expert consensus document of the European Association of Percutaneous Cardiovascular Interventions. Eur Heart J. 2019;40(31):2566-2584. doi:10.1093/eurheartj/ehz332
13. Ali ZA, Landmesser U, Maehara A, et al. Optical Coherence Tomography-Guided versus Angiography-Guided PCI. N Engl J Med. 2023;389(16):1466-1476. doi:10.1056/NEJMoa2305861
14. Unverdorben M, Kleber FX, Heuer H, et al. Treatment of small coronary arteries with a paclitaxel-coated balloon catheter. Clin Res Cardiol. 2010;99:165–174.

Ministero della Salute – Direzione Generale della Ricerca e dell'Innovazione in Sanità

Rendiconto 5 per mille ANNO 2023
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Ente della Ricerca Sanitaria
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Dati del rappresentante legale: Riccardo Bui

Titolo del progetto: Personalizzazione delle terapie antibiotiche ed antifungine nel paziente complesso mediante approcci combinati di farmacologia clinica e microbiologia

Data di inizio progetto: 01/01/2025	**Data di fine progetto**: 31/12/2026
Fondi 5 per mille assegnati al progetto: **€ 128.702,78**	**Di cui:** **Quota da sostenere entro l'anno di rendicontazione: € 0,00** **Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni): € 128.702,78**

VOCI DI SPESA	Quota da sostenere entro l'anno di rendicontazione	Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni)
Personale di ricerca (borsista, a contratto e di ruolo in quota parte)	0,00	30.000,00
Apparecchiature (ammortamento, canone di locazione/leasing)	0,00	65.000,00
Materiale d'uso destinato alla ricerca (per laboratori di ricerca, acquisto farmaci ecc.)	0,00	14.397,36
Spese di organizzazione (manifestazioni e convegni, viaggi e missioni ecc.)	0,00	0,00
Elaborazione dati	0,00	0,00
Spese amministrative	0,00	19.305,42
Altro	0,00	0,00
TOTALE	**0,00**	**128.702,78**

Data, 10/07/2025

Il Legale Rappresentante

Si autorizza al trattamento dei dati ai sensi del d.lgs. 196/2003

Il Legale Rappresentante

Ministero della Salute – Direzione Generale della Ricerca e dell'Innovazione in Sanità

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Titolo del progetto: Personalizzazione delle terapie antibiotiche ed antifungine nel paziente complesso mediante approcci combinati di farmacologia clinica e microbiologia

Razionale del progetto

Nella pratica clinica quotidiana i farmaci vengono solitamente prescritti utilizzando schemi impostati sulla scorta di informazioni derivate dagli studi registrativi, e gli eventuali aggiustamenti posologici sono spesso attuati in modo empirico. Tuttavia, è esperienza comune che lo stesso farmaco, somministrato alla stessa dose, possa essere efficace nella maggioranza dei soggetti trattati risultando però scarsamente efficace e/o con effetti collaterali, a volte anche gravi, in alcuni pazienti. Di fatto, il notevole aumento di risposte imprevedibili ai farmaci sta diventando un problema sempre più rilevante nella società attuale. Alla luce di quanto sopra detto, trova utile ed efficace un approccio più razionale alla terapia farmacologica, in cui la posologia sia definita tenendo conto di tutte le variabili sopra elencate, delle evidenze scientifiche in continua evoluzione e di test diagnostici specifici in grado di valutare l'esposizione individuale del paziente al singolo farmaco.
Tali concetti hanno validità generale ma possono trovare particolare applicazione in setting clinici specifici, come nel campo delle malattie infettive. Sempre più frequentemente si osserva infatti che le posologie convenzionali di antibiotici e antifungini non sono sufficienti per ottenere valori target di farmacocinetica/farmacodinamica (PK/PD) nei pazienti complessi (critici, obesi, iperfiltranti, ipoalbuminemici, con insufficienza degli organi emuntori, sottoposti a procedure dialitiche ecc), potenzialmente aumentando il rischio di fallimento terapeutico. Per tale motivo, il ruolo del monitoraggio terapeutico (TDM, therapeutic drug monitoring) associato al dato microbiologico puntuale, sono cruciali per l'ottimizzazione della posologia dei diversi antibiotici e antifungini (si veda elenco sotto).

Tabella 1: Farmaci sottoposti a monitoraggio terapeutico

antibiotici	anifungini
Ampicillina	Itraconazolo
Cefepime	Isavuconazolo
Ceftazidime	Posaconazolo
Linezolid	Voriconazolo
Meropenem	
Piperacillina/tazobactam	
Sulbactam	
Vancomicina	
Gentamicina	

Scopo dello studio

Lo scopo di questo studio è di verificare se l'identificazione di approcci terapeutici personalizzati per le terapie antibiotiche ed antifungine basati su dati di TDM e di microbiologia puntuale possano migliorare l'outcome clinico di pazienti complessi rispetto all'utilizzo di posologie convenzionali.

La nostra ipotesi è che in questi pazienti ci possa essere una potenziale esposizione subottimale alle terapie infettive utilizzate alle posologie convenzionali.

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Titolo del progetto: Analisi della distribuzione spaziale di farmaci e mataboliti in campioni di tessuto tumorale raccolti alla chirurgia tramite mass spectrometry imaging

Data di inizio progetto: 01/04/2024	**Data di fine progetto**: 31/12/2026
Fondi 5 per mille assegnati al progetto: **€ 338.000,00**	**Di cui:** **Quota da sostenere entro l'anno di rendicontazione: € 169.000,00** **Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni): € 169.000,00**

VOCI DI SPESA	Quota da sostenere entro l'anno di rendicontazione	Quota accantonata, da sostenere, per progetti pluriennali (durata massima tre anni)
Personale di ricerca (borsista, a contratto e di ruolo in quota parte)	0,00	0,00
Apparecchiature (ammortamento, canone di locazione/leasing)	143.650,00	143.650,00
Materiale d'uso destinato alla ricerca (per laboratori di ricerca, acquisto farmaci ecc.)	0,00	0,00
Spese di organizzazione (manifestazioni e convegni, viaggi e missioni ecc.)	0,00	0,00
Elaborazione dati	0,00	0,00
Spese amministrative	25.350,00	25.350,00
Altro	0,00	0,00
TOTALE	**169.000,00**	**169.000,00**

Data, 10/07/2025

Il Legale Rappresentante

Si autorizza al trattamento dei dati ai sensi del d.lgs. 196/2003

Il Legale Rappresentante

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Titolo del progetto: Analisi della distribuzione spaziale di farmaci e mataboliti in campioni di tessuto tumorale raccolti alla chirurgia tramite mass spectrometry imaging

L'efficacia antitumorale è strettamente collegata alla distribuzione dei farmaci nel tessuto bersaglio in concentrazione sufficiente per essere attiva. Il tessuto tumorale presenta anomalie biomeccaniche associate alla resistenza, come l'aumento della rigidità della matrice e lo stress solido causato dalla crescita tumorale (Kalli et al., 2018). Queste anomalie ostacolano la penetrazione e la diffusione del farmaco, limitando la distribuzione e l'efficacia del trattamento. Vasi sanguigni e linfatici anomali, stroma reattivo e infiammazione nei tumori aumentano la pressione interstiziale, lo stress solido e l'ipossia, peggiorando ulteriormente la distribuzione del farmaco (Saggar et al., 2013; Fuso Nerini et al., 2014). I tumori solidi presentano anche uno stroma desmoplastico costituito da tessuto connettivo denso che aumenta la rigidità tissutale (Provenzano et al., 2012; Gkretsi et al., 2048; Morosi et al., 2021). Inoltre il reprogramming metabolico è uno degli hallmark del cancro la cui importanza è sempre più evidente. L'imaging con spettrometria di massa (MSI) offre una soluzione potente a questo problema, mappando e quantificando con precisione la distribuzione del farmaco nel tessuto con alta risoluzione spaziale e specificità. La pipeline analitica da noi sviluppata inoltre permette la mappatura simultanea dei metaboliti e dei lipidi nei tessuti tumorali fornendo informazioni su eventuali correlazioni tra la distribuzione spaziale dei farmaci e l'accumulo di specifici lipidi o alterazioni metaboliche, che potrebbero essere responsabili della scarsa efficacia terapeutica. Inoltre, MSI può integrarsi facilmente con dati istopatologici e altre tecniche di imaging, consentendo un approccio multimodale per migliorare la comprensione della distribuzione del farmaco nei tumori (Davoli et al., 2021).
Abbiamo sviluppato metodi per visualizzare diversi farmaci nel tessuto tumorale (ad esempio amoxicillina, cefazolina) in correlazione con analisi istologica e di

immunoistochmica sui sezioni adiacenti oltre che una innovativa pipeline analitica per la metabolomica spaziale untarget. I nostri dati dimostrano che la distribuzione intra-tumorale di piccole molecole in sezioni provenienti da vari modelli tumorali è estremamente eterogenea. In particolare, la distribuzione del farmaco appare molto scarsa e irregolare in tumori solidi con ampie aree necrotiche o fibrotiche e vascolarizzazione irregolare.
Il progetto quindi mira all' utilizzo dell'imaging con spettrometria di massa (MSI) per visualizzare la distribuzione dei farmaci nel tessuto tumorale in correlazione con un'analisi untageted dei metaboliti e lipidi, abbinato ad analisi istopatologica, al fine di approfondire la comprensione delle caratteristiche tumorali del tessuto che influenzano la penetrazione del farmaco stesso. Studiando la distribuzione degli antibiotici somministrati routinariamente prima dell'intervento chirurgico o di farmaci antitumorali con tossicità moderata (es. PARPi), miriamo a scoprire l'interconnessione tra distribuzione del farmaco, la morfologia tumorale locale e il suo stato metabolico.
L'obiettivo è quello di identificare caratteristiche morfologiche e/o metaboliche del tessuto tumorale nei campioni chirurgici che influenzano la distribuzione del farmaco. Questa analisi aiuterà a comprendere come le caratteristiche strutturali e metaboliche dei tumori, come la rigidità della matrice, lo stress solido, i vasi sanguigni e linfatici anomali, l'accumulo di determinati lipidi impattino sulla distribuzione di antibiotici e altre piccole molecole nel tessuto tumorale. La combinazione di dati MSI e analisi istopatologica fornirà approfondimenti completi sulla relazione complessa tra morfologia tumorale e distribuzione del farmaco, potenzialmente portando allo sviluppo di strategie terapeutiche più efficaci per i pazienti.