1 - General information

Project code: PNRR-MAD-2022-12375994

Project topic: C1) Malattie croniche non trasmissibili, ad alto impatto sui sistemi sanitari e socio-assistenziali: fattori di rischio e prevenzione

PI / Coordinator: Salonia Andrea

Applicant Institution: Ospedale San Raffaele - Milano

Call section: Malattie Croniche non Trasmissibili (MCnT) ad alto impatto sui sistemi sanitari e socio-assistenziali

Proposal title: Definition of a personalized signature of chronic inflammation and early aging predictive of the development of comorbidities in infertile men

Duration in months: 24

MDC primary: Endocrinologia

MDC secondary: Ematologia e Immunologia

Project Classification IRG: Immunology

Project Classification SS: Hypersensitivity, Autoimmune, and Immune

Project Keyword 1: Prevention of immune-mediated diseases: identification of at-risk populations, immuno-epidemiology of genetic and environmental factors, and interventions aimed at altering the immune response so as to modify or prevent disease expression

Project Request: **Animals:** ☐ **Humans:** ☒ **Clinical trial:** ☐

Project total financing request to the MOH: € 1.000.000

Free keywords: Male infertility

Declarations

In case of a Synergy grant application 'Principal Investigator'(PI) means 'corresponding Principal Investigator on behalf of all Principal Investigators', and 'Host Institution' means 'corresponding Host Institution'.

1) The Principal Investigator declares to have the written consent of all participants on their participation and on the content of this proposal, as well as of any researcher mentioned in the proposal as participating in the project (either as other PI, team member or collaborator).	☒
2) The Principal Investigator declares that the information contained in this proposal is correct and complete.	☒
3) The Principal Investigator declares that all parts of this proposal comply with ethical principles (including the highest standards of research integrity — as set out, for instance, in the European Code of Conduct for Research Integrity — and including, in particular, avoiding fabrication, falsification, plagiarism or other research misconduct).	☒
4) The Principal Investigator is only responsible for the correctness of the information relating to his/her own organisation. Each applicant remains responsible for the correctness of the information related to him and declared above.	☒

Personal data protection

The assessment of your grant application will involve the collection and processing of personal data (such as your name, address and CV), which will be performed pursuant to Regulation (EC) No 45/2001 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data. Unless indicated otherwise, your replies to the questions in this form and any personal data requested are required to assess your grant application in accordance with the specifications of the call for proposals and will be processed solely for that purpose. Details concerning the purposes and means of the processing of your personal data as well as information on how to exercise your rights are available in the privacy statement. Applicants may lodge a complaint about the processing of their personal data with the European Data Protection Supervisor at any time.

Abstract

Infertility is a disease of nearly endemic proportions, affecting up to 15% of couples of reproductive ages. Overall, a pure male factor infertility underlies the problem in at least 30% of cases. General health status in infertile men is gaining increasing clinical attention throughout the last decades. Indeed, infertile men were shown to be at increased risk of non-oncologic and oncologic morbidity and mortality compared to age-comparable fertile ones. Despite emerging and solid epidemiological studies, the common ground fostering overall health status and male infertility is still far from being understood. Notwithstanding the numerous hypotheses that have been considered to explain the potential link overall men's health status and male infertility - e.g., including genetic, endocrinological, and metabolic abnormalities - the exact nature of these associations remains unclear. Recent clinical findings showed evidence of onset of age-related comorbidities 10-15 years earlier in infertile compared to fertile men. Single-cell transcriptome profiling of testes of men with idiopathic germ cell aplasia revealed an immature testis with a somatic environment stuck at puberty with immaturity of Leydig cells associated with chronic tissue inflammation, fibrosis, and senescence phenotype of the somatic cells, as well markers of chronic inflammation in the blood. Compelling evidence indicate that defects in the regulatory arms of the immune system and metabolic status can be associated with the risk of developing numerous oncological and non-oncological diseases. Thus far, limited reliable data on the immunological status of infertile men are available. Preliminary data from the coordinating group of the project revealed increased pro-inflammatory cells and reduced T cells in peripheral blood of infertile men. Moreover, the few T cells observed in infertile men displayed an exhausted phenotype.
We proposed an innovative multidisciplinary approach to identify causal links between infertility as a disease and chronic inflammation and comorbidities. Moreover, we aim at identifying novel prognostic biomarkers/tools toward the development of tailored prevention strategies of severe comorbidities in infertile men. We will combine extensive clinical data of a unique and homogenous cohort of infertile men and controls with state-of-the art transcriptomic approaches, a comprehensive monitoring and functional characterization of adaptive and innate immune cells, and the endocrine-metabolomic signature of men with primary infertility. The success of our strategy will be instrumental for defining novel molecular causes of male infertility, along with the pathophysiological mechanism behind the higher burden of comorbid conditions. Results will provide information on cell compartments, and the senescent and endocrine status, mostly in men with primary idiopathic infertility, and new insights into the etiology responsible for the early onset of comorbidities in infertile men. This will represent a breakthrough in the infertility field and a major advancement towards better caring of male patients and healthy ageing in the real-life setting and will set the stage for the development of more tailored clinical approaches to prevent malignant and non-malignant comorbidities in infertile men. Thereof, the findings of the research project will have a significant rebound in terms of cost-effectiveness on the national health system.

In order to best review your application, do you agree that the above non-confidential proposal title and abstract can be used, without disclosing your identity, when contacting potential reviewers? Yes

2 - Participants & contacts

Project Code: PNRR-MAD-2022-12375994	**Call section:** Malattie Croniche non Trasmissibili (MCnT) ad alto impatto sui sistemi sanitari e
Applicant Institution: Ospedale San Raffaele - Milano	**Applicant/PI Coordinator:** Salonia Andrea

Operative Units

Institution that perform as UO	CF Institution	Department / Division / Laboratory	Role in the project	Southern Italy	SSN
1 - Ospedale San Raffaele - Milano	07636600962	Unit of Urology &San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	Coordinator and Project PI		X
2 - Institute of Biomedical and Genetic Research	80054330586	Department of Biomedicine - National Research Council (CNR)	Collaborator	X	
3 - Università Degli Studi di Sassari	00196350904	Dipartimento di Medicina Clinica e SperimentaleUnità Operativa di Chirurgia generale 2 ¿Clinica Chirurgica¿ Azienda Ospedaliero Universitaria di Sassari	Collaborator	X	X
4 - Università degli Studi di Messina	80004070837	Dipartimento di Patologia Umana dell'adulto e dell'età evolutiva "G. Barresi"	Collaborator	X	X

Principal Research Collaborators

Key Personnel Name	Operative Unit	Role in the project
1 - Gregori Silvia Adriana	Ospedale San Raffaele - Milano	Co-PI, responsible for immunological profiling (Aim1)
2 - ANGIUS ANDREA	Institute of Biomedical and Genetic Research	Principal collaborator responsible for the bio-informatic analysis (Aim1, Aim2, Aim3)
3 - Ginesu Giorgio Carlo	Università Degli Studi di Sassari	Principal collaborator responsible for recruitment of cancer patients and infertile men from Sardinia (Aim1, Aim2)
4 - FICARRA VINCENZO	Università degli Studi di Messina	Principal collaborator responsible for recruitment of cancer patients and infertile men from Sicily (Aim1, Aim2)
5 - MORTELLARO ALESSANDRA ROSA	Ospedale San Raffaele - Milano	Principal collaborator responsible for study the innate response (Aim2)
6 Under 40 - LOCATELLI IRENE	Ospedale San Raffaele - Milano	Data Manager and Research Associate (Aim2)
7 Under 40 - IAIA SILVIA	Ospedale San Raffaele - Milano	Research Associate (Aim1)

Key Personnel Name	Co-PI	Resp. CE	Resp. Animal	Birth Date	Gender
1 - Gregori Silvia Adriana	X			27/05/1969	F
2 - ANGIUS ANDREA				31/03/1964	M
3 - Ginesu Giorgio Carlo				02/06/1970	M
4 - FICARRA VINCENZO				02/10/1969	M
5 - MORTELLARO ALESSANDRA ROSA				25/04/1971	F
6 Under 40 - LOCATELLI IRENE				03/06/1985	F
7 Under 40 - IAIA SILVIA				06/12/1993	F

Additional research collaborators under 40 to hire

Key Personnel Name	Operative Unit	Birth Date	Gender	Role in the project	Degree	Actual Pos. and Inst.
0 - ONANO STEFANO	Ospedale San Raffaele - Milano	26/02/1986	M	Bioinformatician	PhD	PostDoctoral Fellow/Department of Biomedicine - National Research Council (CNR)
1 - Rallo Vincenzo	Institute of Biomedical and Genetic Research	04/10/1990	M	Bioinformatician	PhD	PostDoctoral Fellow/Department of Biomedicine - National Research Council (CNR)
2 - PAVIA CHIARA	Institute of Biomedical and Genetic Research	02/07/1997	F	Bioinformatician	Bachelor in bioinformatic	Ungraduated student/Universita degli Studi di Milano, Politecnico di Milano
3 - Tedde Matteo	Università Degli Studi di Sassari	27/02/1989	M	Researcher	Medicine	Resident/Cliniche San PietroOspedale Civile SS. Annunziata

2.1 Administrative data of participating

Operative Unit Number 1:

Address: Via Olgettina 60, 20132 Milan, Italy
PEC: dir.scientifica@hsr.postecert.it

Operative Unit Number 2:

Address: Cittadella Universitaria di Cagliari, 09042 Monserrato
PEC: protocollo.irgb@pec.cnr.it

Operative Unit Number 3:

Address: Viale San Pietro 43, 07100, Sassari, Italy
PEC: dip.scienze.mediche.chirurgiche.sperimentali@pec.uniss.it

Operative Unit Number 4:

Address: Via Consolare Valeria 1, 98100 ¿ Messina, Italy
PEC: protocollo@pec.unime.it

Operative Unit Number 5 (self financing):

Address: N/A
PEC: N/A

2.2 Principal Investigator (PI) Profile

Last Name: Salonia
First Name: Andrea
Last name at birth: Salonia
Gender: M

Title: Principal investigator
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 06/05/1971
Place of Birth: Como

Official H index (Scopus or Web of Science): 71.0

Scopus Author Id:7004134984 **ORCID ID:**0000-0002-0595-7165 **RESEARCH ID:**H-1025-2016

Contact address

Current organisation name: Ospedale San Raffaele - Milano
Current Department / Faculty / Institute / Laboratory name: Unit of Urology &San Raffaele Telethon Institute for Gene Therapy
(SR-Tiget)

Street: Via Olgettina 60
Postcode / Cedex: 20132
Town: Milano
Phone:+393355344263
Phone 2: 0226437286

Education / training

Educational institution and location	Degree	Field of study	From year	To year
URI-Urological Research Institute, Division of Experimental Oncology, Milan, Italy	Specialization / Specializzazione	Director	2014	2022
University Vita-Salute San Raffaele, Milan, Italy	Specialization / Specializzazione	Full Professor/Urology	2018	2022
University Magna Graecia Catanzaro, School of Medicine, Catanzaro, Italy	PhD	Clinical and Experimental Biotechnology in Urology	2010	2015
University Vita-Salute San Raffaele, School of Medicine, Milan, Italy	Specialization / Specializzazione	Residency in Endocrinology and Replacement Diseases/Endocrinology	2001	2007
University of Trieste, School of Medicine, Trieste, Italy	Specialization / Specializzazione	Recidency in Urology, Urology	1999	2001
University of Milan, School of Medicine, Milan, Italy	Master's Degree / Laurea Magistrale	Medicine	1990	1996

Personal Statement:

My research focused on male infertility as a proxy of somatic male health, specifically investigating comorbidities and studying micro- and macro-environments in idiopathic infertile vs. fertile men. I will directly supervise the project and the enrollment phase of patients and age-matched fertile controls, the comprehensive clinical characterization and tissues biobanking of both cohorts, and the final analyses to achieve novel biomarkers/tools.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University Vita-Salute San Raffaele,	International PhD Course in Clinical and Experimental Medicine	Milan, Italy	Co-Chairman	2018	2022
University Vita-Salute San Raffaele	International Medical Doctor Program	Milan, Italy	Dean of the Studies	2020	2022
University Vita-Salute San Raffaele (uniSR)	Urology (MED24)	Milan, Italy	Full professor	2018	2022
University Vita-Salute San Raffaele (uniSR)	Urology (MED24)	Milan, Italy	Associate professor	2015	2018
IRCCS Ospedale San Raffaele	Division Experimental Oncology	Milan, Italy	Director URI, Senior staff physician	2014	2019
IRCCS Ospedale San Raffaele	Dept. Urology	Milan, Italy	Senior staff physician (Assistant professor)	2008	2019
Weill-Cornell University	Dept. Urology/Reproductive Medicine Clinical/Research Unit	New York, NY State, USA	Translation research fellow	2008	2008
IRCCS Ospedale San Raffaele	Dept. Urology	Milan, Italy	Staff physician (Assistant professor)	2001	2008
Boston University	Dept. Urology/Sexual Medicine Research Laboratory	Boston, MA, USA	Translational research fellow	2001	2001

Other awards and honors

Prof. Salonia has been awarded with the award of Excellence, European Society for Sexual Medicine [ESSM] 2006 and the Matula Award of the Italian Society of Urology 2011, for the best research CV in Urology and Andrology.

Other CV informations

Prof. Salonia is: i) Chief Physician for Male sexual and reproductive medicine and ii) Director, Urological Research Institute, Division of Experimental Oncology, IRCCS Ospedale San Raffaele, Milan; iii) member Interfaculty Group on gender studies, UniSR; iv) member, Executive Committee European Association of Urology (EAU) Section of Andrological Urology (ESAU); v) Chairman, EAU guidelines panel on Male Sexual and Reproductive Health.

Selected peer-reviewed publications of the PI valid for minimum expertise level

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
One patient out of four with newly diagnosed erectile dysfunction is a young man-worrisome picture from the everyday clinical practice	Article	1833-1841	10	2013	10.1111/jsm.12179	23651423	83	C
Infertility as a proxy of general male health: Results of a cross-sectional survey	Article	48-55	104	2015	10.1016/j.fertnstert.2015.04.020	26006735	65	C
Low birth weight is associated with a decreased overall adult health status and reproductive capability - Results of a cross-sectional study in primary infertile patients	Article	1-14	11	2016	10.1371/journal.pone.0166728	27893825	14	C
Long-term recovery of normal sexual function in testicular cancer survivors	Article	85-89	18	2016	10.4103/1008-682X.149180	26112476	15	C

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Metabolic syndrome in White-European men presenting for secondary couple's infertility: An investigation of the clinical and reproductive burden	Article	NOT_FOUND	18	2016	10.4103/1008-682X.175783	27004539	19	C
Orgasmic Dysfunction After Robot-assisted Versus Open Radical Prostatectomy	Article	223-226	70	2016	10.1016/j.eururo.2015.10.046	26572706	29	C
When to Perform Karyotype Analysis in Infertile Men? Validation of the European Association of Urology Guidelines with the Proposal of a New Predictive Model	Article	920-923	70	2016	10.1016/j.eururo.2016.06.015	27343001	29	C
Metabolic syndrome in white European men presenting for primary couple's infertility: investigation of the clinical and reproductive burden	Article	944-951	4	2016	10.1111/andr.12232	27368157	40	C
Diagnosis and Treatment of Testosterone Deficiency: Recommendations From the Fourth International Consultation for Sexual Medicine (ICSM 2015)	Article	1787-1804	13	2016	10.1016/j.jsxm.2016.10.009	27914560	93	L
Sexual functioning mirrors overall men's health status, even irrespective of cardiovascular risk factors	Article	63-69	5	2017	10.1111/andr.12299	27989023	15	C
Primary, secondary and compensated hypogonadism: a novel risk stratification for infertile men	Article	505-510	5	2017	10.1111/andr.12335	28409903	25	C
Sexual Rehabilitation After Treatment For Prostate Cancer¿Part 2: Recommendations From the Fourth International Consultation for Sexual Medicine (ICSM 2015)	Article	297-315	14	2017	10.1016/j.jsxm.2016.11.324	28262100	51	C
The Microbiome of the Prostate Tumor Microenvironment [Figure presented]	Article	625-631	72	2017	10.1016/j.eururo.2017.03.029	28434677	93	C
Male infertility as a proxy of the overall male health status	Review	286-299	70	2018	10.23736/S0393-2249.18.03063-1	29595040	16	C
Testicular microbiome in azoospermic men'first evidence of the impact of an altered microenvironment	Article	1212-1217	33	2018	10.1093/humrep/dey116	29850857	29	C
Undiagnosed prediabetes is highly prevalent in primary infertile men ¿ results from a cross-sectional study	Article	1070-1077	123	2019	10.1111/bju.14558	30328251	12	C
Age at First Presentation for Erectile Dysfunction: Analysis of Changes over a 12-yr Period	Article	899-905	5	2019	10.1016/j.euf.2018.02.006	29506875	12	C
High Blood Pressure Is a Highly Prevalent but Unrecognised Condition in Primary Infertile Men: Results of a Cross-sectional Study	Article	178-183	6	2020	10.1016/j.euf.2018.07.030	30082228	16	C
European Association of Urology Guidelines on Male Sexual and Reproductive Health: 2021 Update on Male Infertility	Article	603-620	80	2021	10.1016/j.eururo.2021.08.014	34511305	23	C
European Association of Urology Guidelines on Sexual and Reproductive Health¿2021 Update: Male Sexual Dysfunction[Formula presented]	Article	333-357	80	2021	10.1016/j.eururo.2021.06.007	34183196	53	F

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Selected peer-reviewed publications of the PI for the evaluation CV

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**
High prevalence of vitamin D deficiency in infertile women referring for assisted reproduction	Article	9972-9984	7	2015	10.3390/nu7125516	26633484	39
Infertility as a proxy of general male health: Results of a cross-sectional survey	Article	48-55	104	2015	10.1016/j.fertnstert.2015.04.020	26006735	65
Sexual functioning mirrors overall men's health status, even irrespective of cardiovascular risk factors	Article	63-69	5	2017	10.1111/andr.12299	27989023	15
Male infertility as a proxy of the overall male health status	Article	286-299	70	2018	10.23736/S0393-2249.18.03063-1	29595040	16
Undiagnosed prediabetes is highly prevalent in primary infertile men ¿ results from a cross-sectional study	Article	1070-1077	123	2019	10.1111/bju.14558	30328251	12
Seminal plasma of men with severe asthenozoospermia contain exosomes that affect spermatozoa motility and capacitation	Article	897-908.e2	111	2019	10.1016/j.fertnstert.2019.01.030	31029245	37
Paediatric and adult-onset male hypogonadism	Article	1-21	5	2019	10.1038/s41572-019-0087-y	31147553	49
Aging, inflammation and DNA damage in the somatic testicular niche with idiopathic germ cell aplasia	Article	1-17	12	2021	10.1038/s41467-021-25544-0	34471128	2
The Association between Mortality and Male Infertility: Systematic Review and Meta-analysis	Article	148-157	154	2021	10.1016/j.urology.2021.02.041	33819517	5
Severely low testosterone in males with COVID-19: A case-control study	Article	1043-1052	9	2021	10.1111/andr.12993	33635589	35

** Autocertificated

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
The Italian Ministry of Health	Ospedale San Raffaele, Dpt of Urology	2021-2024	Novel System Mecidine approach to develop primary prevention strategies of comorbidities in infertile men.	Coordinator	240.000,00	The Italian Ministry of Health

2.3 CO-PI Profile

Last Name: Gregori
First Name: Silvia Adriana

Last name at birth: Gregori
Gender: F

Title: Co-PI, responsible for immunological profiling (Aim1)
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 27/05/1969
Place of Birth: Milano

Official H index (Scopus or Web of Science): 44.0

Scopus Author Id:7003330902 **ORCID ID:**0000-0002-3517-9683 **RESEARCH ID:**J-7718-2016

Contact address

Current organisation name: Ospedale San Raffaele - Milano
Current Department / Faculty / Institute / Laboratory name: Unit of Urology &San Raffaele Telethon Institute for Gene Therapy
(SR-Tiget)

Street: Via Indipendenza 5A
Postcode / Cedex: 20090
Town: Buccinasco
Phone:+393382966759
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
MIUR, National Scientific Certification	Specialization / Specializzazione	Full Professor 06/A2 Pathology and Clinical Pathology	2018	2026
MIUR, National Scientific Certification	Specialization / Specializzazione	Associate Professor05/E2 - Molecular Biology; 05/F1- Applied Biology	2014	2023
University of Milan, Milan, Italy	Specialization / Specializzazione	Biotechnology	1995	1999
Department of Pharmacology, School of Medicine, University of Milan, Milan Italy	Single-cycle master's degree / Laurea magistrale a ciclo unico	Biology, Immunology	1989	1994

Personal Statement:

My research has been focused on immunological tolerance, specifically i) in defining the mode of induction/function of IL-10-producing regulatory cells; ii) cellular/molecular mechanisms underlying tolerogenic dendritic cell induction and functions; iii) in defining the role of HLA-G and Il-10 in tolerance in healthy and pathological conditions.
I will directly supervise immunological studies focus on define phenotype models related to the high risk of developing clinically significant comorbidities in infertile men. Combining measurement of inflammation markers and gene signatures and deep andrological phenotyping of infertile men, will phenotypically define subsets of men at risk of developing preventable comorbidities.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
IRCCS Ospedale San Raffaele/San Raffaele Telethon institute for Gene Therapy (SR-Tiget)	Mechanisms of Peripheral Tolerance Unit	Milan, Italy	Group Leader	2014	2022
IRCCS San Raffaele Telethon Institute for Gene Therapy (HSRTiget)	Tolerogenic Dendritic Cells Unit	Milan, Italy	Group Leader	2006	2008
IRCCS Ospeale San Raffaele/San Raffaele Telethon institute for Gene Therapy (HSRTiget)	Immunological Tolerance Unit	Milan, Italy	Project Leader	2003	2006
San Raffaele Telethon institute for Gene Therapy (HSR-Tiget)	Immunological Tolerance Unit	Milan, Italy	Senjor Post-Doctoral Fellow	2001	2003
Roche	Roche Milano Ricerche	Milan, Italy	Post-Doctoral Fellow	1999	2001
Roche	Roche Milano Ricerche	Milan, Italy	PhD student	1995	1999
London Hospital	Department of Immunology	London UK	Resident Research Fellow	1994	1995
University of Milan	Department of Pharmacology, School of Medicine	Milan Italy	Ungraduated Student	1992	1994

Other awards and honors

Annual Meeting of the FOCIS, 18-21 June 2019, Boston MA, Travel Grant Recipient.
Annual Meeting of the FOCIS, 24-27 June 2015, Boston MA, Travel Grant Recipient.
Award for best presentation. XXII European Congress of Obstetrics and Gynaecology.

Other CV informations

Dr. Gregori is currentlly staff member of SR-TIGET, received as PI funding from Telethon Foundation, AIRC, JDRF, EFSD, and Italian Ministry of Health. She is inventor of 8 patents. She is peer reviewer for the following international Journals: Blood, Journal of Clinical Investigation, Science Translational Medicine, Immunity, Clinical and Experimental Immunology, Cancer Research, Frontiers in Immunology, Journal of Immunology. Ad hoc scientific evaluator for the following funding agencies: Dutch Arthritis Foundation, ERA-Net for Research Programmes on Rare Diseases, Immune Tolerance Network, AFM Telethon, JDRF She is member of the Scientific Committee of the European Federation of Immunogenetics, and of the Federation of Clinical Immunology Societies.

Selected peer-reviewed publications of the Co-PI valid for minimum expertise level

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
HLA-G expressing DC-10 and CD4+ T cells accumulate in human decidua during pregnancy	Article	406-411	74	2013	10.1016/j.humimm.2012.11.031	23238214	89	C
Mixed Chimerism Evolution is Associated with T Regulatory type 1 (Tr1) Cells in a ß-Thalassemic Patient After Haploidentical Haematopoietic Stem Cell Transplantation	Article	75-79	5	2014	10.1080/19381956.2015.1103423	26650878	13	C

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
New insights into HLA-G mediated tolerance	Review	255-263	84	2014	10.1111/tan.12427	25132109	57	C
HLA-G expression on blasts and tolerogenic cells in patients affected by acute myeloid leukemia	Article	1-10	N/A	2014	10.1155/2014/636292	24741612	22	C
HLA-G orchestrates the early interaction of human trophoblasts with the maternal niche	Review	1-8	6	2015	10.3389/fimmu.2015.00128	25870595	46	C
Hla-g expression levels influence the tolerogenic activity of human DC-10	Article	548-557	100	2015	10.3324/haematol.2014.113803	25661445	51	C
Association of genetic variants in the 3¿UTR of HLA-G with Recurrent Pregnancy Loss	Article	886-891	77	2016	10.1016/j.humimm.2016.06.020	27370685	23	C
Monitoring T-cell responses in translational studies: Optimization of dye-based proliferation assay for evaluation of antigen-specific responses	Review	1-15	8	2017	10.3389/fimmu.2017.01870	29312346	24	L
IL-10-Engineered Human CD4+ Tr1 Cells Eliminate Myeloid Leukemia in an HLA Class I-Dependent Mechanism	Article	2254-2269	25	2017	10.1016/j.ymthe.2017.06.029	28807569	23	C
Interleukin-10-producing DC-10 is a unique tool to promote tolerance via antigen-specific T regulatory type 1 cells	Review	1-8	9	2018	10.3389/fimmu.2018.00682	29686676	34	C
Engineered T regulatory type 1 cells for clinical application	Review	1-8	9	2018	10.3389/fimmu.2018.00233	29497421	44	C
Targeting a Pre-existing Anti-transgene T Cell Response for Effective Gene Therapy of MPS-I in the Mouse Model of the Disease	Article	1215-1227	27	2019	10.1016/j.ymthe.2019.04.014	31060789	11	C
Induction of Antigen-Specific Tolerance in T Cell Mediated Diseases	Review	1-14	11	2020	10.3389/fimmu.2020.02194	33133064	5	C
HLA-G Genotype/Expression/Disease Association Studies: Success, Hurdles, and Perspectives	Review	1-9	11	2020	10.3389/fimmu.2020.01178	32733439	12	C
Generation of Powerful Human Tolerogenic Dendritic Cells by Lentiviral-Mediated IL-10 Gene Transfer	Article	1-14	11	2020	10.3389/fimmu.2020.01260	32695103	6	C
Intrathymic delivery a new route for adenoviral-associated vector gene therapy	Article	499-501	145	2020	10.1016/j.jaci.2019.11.037	31830489	0	F
Coexpression of CD163 and CD141 identifies human circulating IL-10-producing dendritic cells (DC-10)	Article	95-107	17	2020	10.1038/s41423-019-0218-0	30842629	25	C
Altered Frequency and Phenotype of HLA-G-Expressing DC-10 in Type 1 Diabetes Patients at Onset and in Subjects at Risk to Develop the Disease	Article	1-11	12	2021	10.3389/fimmu.2021.750162	34659254	1	C
Tolerogenic dendritic cell-based approaches in autoimmunity	Review	1-21	22	2021	10.3390/ijms22168415	34445143	0	C

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
JDRF. 3-SRA-2021-1007-S-B	Ospedale San Raffaele, San Raffaele Telethon Institute for Gene Therapy	2021-2024	Personalized antigen-specific immunotherapy to halt autoimmunity in pre-symptomatic and symptomatic Type 1 Diabetic subjects.	Coordinator	555.000,00	JDRF
The Italian Ministry of Health	Ospedale San Raffaele, San Raffaele Telethon Institute for Gene Therapy	2021-2024	Novel System Mecidine approach to develop primary prevention strategies of comorbidities in infertile men.	Collaborator	210.000,00	The Italian Ministry of Health
Innovative Training Networks (ITN). Call: H2020-MSCA-ITN-2018	Ospadale San Raffaele, San Raffaele Telethon Institute for Gene Therapy	2020-2023	INnovative Training in Myeloid Regulatory Cell Therapy, INsTRuCT	Collaborator	233.899,00	European Commission
The Italian Ministry of Health	Ospedale San Raffaele, San Raffaele Telethon Institute for Gene Therapy	2018-2023	Innovative Regulatory Cell-Based Strategies to Cure Celiac Disease	Coordinator	432.000,00	The Italian Ministry of Health
Fondazione Telethon	Ospedale San Raffaele, San Raffaele Telethon Institute for Gene Therapy	2022-2025	Tolerogenic cells: biology and approaches for their application to prevent unwanted immune responses in protein/gene replacement therapies.	Coordinator	540.000,00	Fondazione Telethon

2.3 Research Collaborators n. 2

Last Name: ANGIUS
First Name: ANDREA

Last name at birth: Angius
Gender: M

Title: Principal collaborator responsible for the bio-informatic analysis (Aim1, Aim2, Aim3)
Nationality: Italiana
Date of birth: 31/03/1964

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Trieste

Official H index (Scopus or Web of Science): 38.0

Scopus Author Id:57204537218 **ORCID ID:**0000-0003-2596-6461 **RESEARCH ID:**P-9549-2015

Contact address

Current organisation name: Institute of Biomedical and Genetic Research
Current Department / Faculty / Institute / Laboratory name: Department of Biomedicine - National Research Council (CNR)
Street: cittadella universitaria
Postcode / Cedex: 09042
Town: Monserrato
Phone:+393384177680
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
ASN - Abilitazione Scientifica Nazionale - ANVUR	Specialization / Specializzazione	Qualified as Associate Professor in Medical Genetics for Italian Universities	2017	2026
ASN - Abilitazione Scientifica Nazionale - ANVUR	Specialization / Specializzazione	Qualified as Associate Professor in Genetics and Microbiology for Italian Universities	2014	2023
Adjunct Associate Professor in Medical Genetic	Specialization / Specializzazione	djunct Associate Professor in Biotechnologies	2011	2022
Adjunct Associate Professor	Specialization / Specializzazione	Adjunct Associate Professor in Medical Genetic	2010	2019
University of Sassari	PhD	Genetic medicine	1997	1999
University of Cagliari	Single-cycle master's degree / Laurea magistrale a ciclo unico	Biology	1988	1992

Personal Statement:

Dr. Angius expertise derives from his involvement in international massive sequencing projects and management at the molecular and bioinformatics levels of advanced research laboratories. Dr. Angius' recent scientific interests have focused on multi-omics approach (genomics, transcriptomics, miRNAs, etc.) using innovative massive sequencing approaches in various fields ranging from rare diseases, cancers and complex diseases and/or traits. The results of these activities have enabled the sequencing of the exome of hundreds of individuals with complex autoimmune diseases, intellectual disability, rare diseases and syndromic forms, and the transcriptome sequencing of thousands of individuals and cell subpopulations to ascertain expression profiles associated with complex diseases.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Institute of Biomedical and Genetic Research	Department of Biomedicine, National Research Council (CNR)	Monserrato, Cagliari, Italy	Lead Researcher	2021	2022
University of Sassari	Department L.240/2010 Veterinary Medicine	Sassari, Italy	Adjunct Associate Professor in	2011	2022
Institute of Biomedical and Genetic Research	Department of Biomedicine, National Research Council (CNR),	Monserrato, Cagliari, Italy	Researcher	2011	2020
University of Sassari	Department L.240/2010 Biomedical Science	Sassari, Italy	Adjunct Associate Professor in Medical	2010	2019
Centro di ricerca, sviluppo e studi superiori in Sardegna (CRS4)	N/A	Pula (Cagliari), Italy	Head and Scientific Coordinator of the Next Generation Sequencing Core	2010	2014
Institute of Population Genetics	Department of Life Science, National Research Council (CNR)	Alghero, Sassari, Italy	Researcher	2001	2011
¿Polaris¿ Scientific and Technological Park of Sardinia	Sardegna Ricerche	Pula (Cagliari), Italy	Head and Scientific Coordinator of the Genotyping Laboratory	2006	2010
Soc. SHARDNA Life Sciences	N/A	Cagliari, Italy	Lab Manager Genotyping	2002	2006
University of Sassari	Department L.240/2010 Biomedical Science	Sassari (Italy)	Adjunct Associate Professor in Genetics	2003	2004
Institute of Molecular Genetics	Department of Life Science, National Research Council (CNR)	Alghero (Italy)	Fixed-term Researcher	2000	2001

Other awards and honors

N/A

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Ministero dell'Istruzione, dell'Università e della Ricerca. FIRB Laboratori 2003	Department of Biomedicine - National Research Council (CNR)	2005-2011	Identificazione di geni-malattia mediante genotipizzazione ad alta densità di popolazioni	Coordinator	6.637.000,00	FIRB Laboratori 2003
Telethon Foundation	Department of Biomedicine - National Research Council (CNR)	2013-2015	Post GWAS functional characterization of BCL11A locus toward the development of a treatment for ß-thalassemia	Coordinator	262.800,00	Grant number: GGP13246

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Fondo di Beneficenza, Intesa Sanpaolo S.p.A	Department of Biomedicine - National Research Council (CNR)	2020-2022	PREDICT) PREcision meDicine In ColorecTal cancer: new clinical-genomic network for expanding tailored oncologic care	Coordinator	258.950,00	GRANT_NUMBER: B/2020/0094
Fondazione Sardegna	Department of Biomedicine - National Research Council (CNR)	2021-2022	Sviluppo di un panello di marcatori immunoistochimici ed epigenetici a scopo predittivo nella terapia del carcinoma mammario 'Triplo Negativo'	Coordinator	10.000,00	GRANT_NUMBER: 2021.0494
MIUR	Department of Biomedicine - National Research Council (CNR)	2022-2025	Unveiling the hidden side of NEUrodevelopmental DIsorder Genetics (NEUDIG): a multidisciplinary pathway to new molecular diagnoses by integrating genomic, transcriptomic, and functional analyses	Coordinator	837.106,00	GRANT NUMBER: 20203P8C3X

2.4 Research Collaborators n. 3

Last Name: Ginesu
First Name: Giorgio Carlo

Last name at birth:
Gender: M

Title: Principal collaborator responsible for recruitment of cancer patients and infertile men from Sardinia (Aim1, Aim2)

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Sassari

Nationality: italiana
Date of birth: 02/06/1970

Official H index (Scopus or Web of Science): 11.0

Scopus Author Id:6507560482 **ORCID ID:**0000-0001-9073-6721 **RESEARCH ID:**N/A

Contact address

Current organisation name: Università Degli Studi di Sassari

Current Department / Faculty / Institute / Laboratory name: Dipartimento di Medicina Clinica e SperimentaleUnità Operativa di Chirurgia generale 2 ¿Clinica Chirurgica¿ Azienda Ospedaliero Universitaria di Sassari

Street: viale San Pietro 43

Postcode / Cedex: 07100 **Town:** Sassari

Phone:+393291710164 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Università degli Studi di Sassari	Single-cycle master's degree / Laurea magistrale a ciclo unico	Medicine	1989	1994
Università degli Studi di Sassari	Specialization / Specializzazione	General Surgery	1994	1999

Personal Statement:

Dr. Ginesu has long lasting surgical expertise, which includes thoracic surgery (lung neoplasms, interstitial lung diseases, mediastinal neoplasms), General Surgery, Oncologic Surgery, Vascolar surgery, Urogenital surgery, Endocrine surgery, Geriatric Surgery. This expertise allows him to study patients with different pathology and age. Dr. Ginesu will provide biological samples from patients affected by chest and pancreatic cancer, which will represent a critical and important controls for the definition of specific biomarkers associated with high-risk of developing comorbidities in infertile men. Dr. Ginesu will provide also all the clinical information for the data base generation and bioinformatic analysis.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Università degli Studi di Sassari	Department general surgery	Sassari, Italy	Researcher	2000	2022
Università degli Studi di Sassari	Department general surgery	Sassari, Italy	Resident	1994	1999

Other awards and honors

N/A

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/A	N/A	N/A	Coordinator	0,00	N/A

2.5 Research Collaborators n. 4

Last Name: FICARRA
First Name: VINCENZO

Last name at birth:
Gender: M

Title: Principal collaborator responsible for recruitment of cancer patients and infertile men from Sicily (Aim1, Aim2)

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Messina

Nationality: Italiana
Date of birth: 02/10/1969

Official H index (Scopus or Web of Science): 73.0

Scopus Author Id:7006254052 **ORCID ID:**0000-0002-2447-5196 **RESEARCH ID:**N/A

Contact address

Current organisation name: Università degli Studi di Messina
Current Department / Faculty / Institute / Laboratory name: Dipartimento di Patologia Umana dell'adulto e dell'età evolutiva "G. Barresi"

Street: via Consolare Valeria
Postcode / Cedex: 98100 **Town:** Messina
Phone:+393475252918 **Phone 2:** 0432552931

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Messina	Specialization / Specializzazione	Full professo in Urology	2017	2022
University of Udine	Specialization / Specializzazione	Professor of Urology	2013	2017
University of Padua	Specialization / Specializzazione	Associate Professor in Urology	2006	2013
University of Verona	Specialization / Specializzazione	Assistant professor in Urology	1998	2006
University of Verona	Specialization / Specializzazione	Urology	1993	1998
University of Verona	Single-cycle master's degree / Laurea magistrale a ciclo unico	Medicine	1988	1993

Personal Statement:

Prof. Ficarra has long lasting expertise in the conservative and radical robotic treatment of kidney tumors and in the classification of renal neoplasms. Moreover, he has experience in the robotic treatment of prostate tumors with Retzius sparing posterior approach, and experience in reconstructive treatment after radical cystectomy and in ureteral pathologies. Prof. Ficarra will be responsible of the enrollment of infertile and fertile man from Sicily and men affected by bladder and renal cancer. Prof. Ficarra will provide all the clinical information for the data base generation and for the bioinformatic analysis.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Messina	Residency Program in Urology	Messina, Italy	Director	2021	2022
University of Messina	Department of Urology	Messina, Italy	Director	2017	2022
University of Udine	Department of Urology	Udine, Italy	Professor of Uroloy	2013	2017
OLV Robotic Surgery Institute	OLV Robotic Surgery Institute	Aalst, Belgium	Scientific Director	2011	2013
University of Padua	Department of Oncological and Surgical Sciences, Urology Clinic	Padova, Italy	Associate Professor of Urology	2006	2013
University of Messina	Department of Urology	Verona, Italy	Assistant professor	1998	2006

Other awards and honors

2008, winner of the Crystal Matula Award. 2010, the winner for the best paper published in European Urology journal. 2012 received a mention from Polish Urologic Society for his role in the diffusion of robotic surgery in urology. 2019 received a mention from Ordine dei Medici di Messina as Innovator in the field of Robotic Surgery. 2020, winner of the 'Premio Bracci' by Italian Society of Urology, for the best Italian paper published in a International Journal in the 2019

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/a	N/a	N/A	Coordinator	0,00	N/A

2.6 Research Collaborators n. 5

Last Name: MORTELLARO
First Name: ALESSANDRA ROSA

Last name at birth:
Gender: F

Title: Principal collaborator responsible for study the innate response (Aim2)
Nationality: Italiana
Date of birth: 25/04/1971

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Milano

Official H index (Scopus or Web of Science): 27.0

Scopus Author Id:6506432509 **ORCID ID:**0000-0003-1744-2430 **RESEARCH ID:**AAN-4678-2020

Contact address

Current organisation name: Ospedale San Raffaele - Milano
Current Department / Faculty / Institute / Laboratory name: Unit of Urology &San Raffaele Telethon Institute for Gene Therapy
(SR-Tiget)

Street: Via Olgettina 60
Postcode / Cedex: 20132 **Town:** Milano
Phone:+393493504178 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Open University of London (UK) & Vita-Salute San Raffaele University (Italy)	PhD	Cellular and Molecular Biology	2000	2005
University of Milan	Single-cycle master's degree / Laurea magistrale a ciclo unico	Biological	1992	1998

Personal Statement:

Dr. Mortellaro is an experienced scientist with more than ten years of experience managing a successful research group. Her research interests lie in understanding innate mechanisms responsible for the inflammatory process in response to microbial and danger triggers and their role in pathological conditions, such as autoinflammatory disorders. She is a renewed leader in the biology of the inflammasome, a cytosolic multiprotein complex of the innate immune system responsible for the activation of inflammatory responses. Dr. Mortellaro will be responsible for supervising all the activity dissecting the inflammasome activation in infertile men and controls.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Ospedale San Raffaele	San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	Milan, Italy	Group Leader	2021	2022
Ospedale San Raffaele	San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	Milan, Italy	Project Leader	2017	2021
Agency for Science, Technology and Research (A*STAR)	Singapore Immunology Network (SIgN)	Singapore	Principal Investigator	2011	2017
Agency for Science, Technology and Research (A*STAR)	Singapore Immunology Network (SIgN)	Singapore	Senior Research Scientist	2010	2011
Agency for Science, Technology and Research (A*STAR)	Singapore Immunology Network (SIgN)	Singapore	Research Scientist	2008	2010
University of Milano-Bicocca	Department of Biotechnology and Biosciences	Milan, Italy	Postdoctoral Fellow	2006	2007
Ospedale San Raffaele	San Raffaele Telethon Institute for Gene Therapy (SR-Tiget),	Milan, Italy	Ph.D. Student	2000	2005
Ospedale San Raffaele	San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	Milan, Italy	Postgraduate Fellow	1998	2000

Other awards and honors

2011 Young Investigator Award from the League Associations of Rheumatology, Taiwan

2019 Recipient of the prestigious Marie Skodowska-Curie Individual Fellowship funded under the Horizon 2020 program by the European Commission.

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Italian Ministry of Health	Ospedale San Raffaele, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	2021-2024	Expanding the spectrum of adenosine deaminase 2 (ADA2) deficiency: towards a gene therapy approach	Collaborator	540.000,00	Italian Ministry of Health
Jeffrey Modell Foundation (USA)	Ospedale San Raffaele, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	2019-2021	Preclinical development of a hematopoietic stem cell gene therapy for adenosine deaminase 2 deficiency	Collaborator	190.000,00	Jeffrey Modell Foundation (USA)
European Commission, Horizon 2020 program, Marie Sk¿odowska-Curie Individual Fellowship	Ospedale San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	2019-2021	Development of gene therapy and genome editing strategies to treat adenosine deaminase 2 deficiency	Coordinator	180.000,00	European Commission
National Healthcare Group, Singapore	Singapore Immunology Network (SIgN)	2016-2018	Targeting the inflammasome pathway for therapeutic treatment of atopic dermatitis	Coordinator	150.000,00	N/A

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
National Medical Research Council, Singapore	Singapore Immunology Network (SIgN)	2014-2015	Role of NLRP3 Inflammasome activation in the pathogenesis of gout and correlation with disease severity	Collaborator	135.000,00	N/A
Agency for Science, Technology and Research (A*STAR) Joint Council Office, Singapore	Singapore Immunology Network (SIgN)	2014-2015	The role of electrostatic charge in particulate-mediated activation of the NLRP3 Inflammasome	Coordinator	135.000,00	N/A
Agency for Science, Technology and Research (A*STAR) Joint Council Office, Singapore	Singapore Immunology Network (SIgN)	2014	Suppressing NLRP3 inflammasome activity in immune cells by high affinity antagonistic peptides	Coordinator	130.000,00	N/A

2.7 Research Collaborators n. 6 - Under 40

Last Name: LOCATELLI
First Name: IRENE
Last name at birth:
Gender: F

Title: Data Manager and Research Associate (Aim2)
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 03/06/1985
Place of Birth: Novara

Official H index (Scopus or Web of Science): 12.0

Scopus Author Id:57207513242 **ORCID ID:**0000-0002-8467-2132 **RESEARCH ID:**ABH-4738-2020

Contact address

Current organisation name: Ospedale San Raffaele - Milano
Current Department / Faculty / Institute / Laboratory name: Unit of Urology &San Raffaele Telethon Institute for Gene Therapy
(SR-Tiget)

Street: via Olgettina 60
Postcode / Cedex: 20132
Town: Milano
Phone:+393487402670
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Eastern Piedmont Amedeo Avogadro, Novara	PhD	Molecular Medicine	2010	2014
University of Eastern Piedmont Amedeo Avogadro, Novara	Master's Degree / Laurea Magistrale	Medical and Pharmaceutical Biotechnologies	2007	2009
University of Eastern Piedmont Amedeo Avogadro, Novara	Bachelor Degree / Laurea Triennale	Biotechnologies	2004	2007

Personal Statement:

Dr. Locatelli is a senior researcher with several years of experience in tissue culture and molecular biology. During the last years, she focused her efforts on studying cellular and molecular mechanisms involved in male infertility obtaining excellent results published on peer-reviewed journals. In particular, her experience concerns citofluorimetry analysis, Real Time PCR and Digital Droplet PCR and single-cell analysis that permit to find new pathways and markers involved to male infertility. She will be involved in the characterization of the senescence signature of peripheral blood cells and seminal fluids of infertile men and controls under the supervision of Prof. Salonia.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
IRCCS Ospedale San Raffaele	Urological Research Institute (URI)	Milan, Italy	Research Assistant	2015	2022
IRCCS Ospedale San Raffaele	Urological Research Institute (URI)	Milan, Italy	Data Manager	2014	2015
University of Eastern Piedmont ¿A.	General Pathology Laboratory at the Department of Health Sciences	Novara, Italy	PhD student	2010	2014
Queen Mary University	William Harvey Research Institute, Barts and the London School of Medicine and Dentistry	London, UK	Trainee	2012	2013
IFOM (Institute FIRC for Molecular Oncology)	N/A	Milan, Italy	Fellow	2010	2010
University of Eastern Piedmont ¿A. Avogadro¿	Biochemistry Laboratory, Department of Translational Medicine	Novara, Italy	Graduate student	2007	2009

Other awards and honors

N/A

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/A	N/A	N/A	Collaborator	0,00	N/A

2.8 Research Collaborators n. 7 - Under 40

Last Name: IAIA
First Name: SILVIA
Last name at birth:
Gender: F

Title: Research Associate (Aim1)
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 06/12/1993
Place of Birth: Mesagne

Official H index (Scopus or Web of Science): 0.0

Scopus Author Id:N/A **ORCID ID:**0000-0002-0527-6845 **RESEARCH ID:**AHE-8578-2022

Contact address

Current organisation name: Ospedale San Raffaele - Milano
Current Department / Faculty / Institute / Laboratory name: Unit of Urology &San Raffaele Telethon Institute for Gene Therapy
(SR-Tiget)

Street: Via Olgettina 58
Postcode / Cedex: 20132 **Town:** Milano
Phone:+393497720536 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Università degli studi di Verona	PhD	Study of molecular mechanisms involved in inflammation of autoimmune and neurodegenerative disorders in vivo and in vitro.	2017	2020
Università degli studi di Parma	Master's Degree / Laurea Magistrale	Molecular Biology	2015	2017

Personal Statement:

Dr.Iaia is a senior researcher with experience in tissue culture and immunology, her experience concerns citofluorimetry analysis, Real Time PCR and Digital Droplet PCR and functional characterization on immune responses. She will perform the immunophenotyping of immune cells, and relative functional analysis, and the analysis of the inflammassome activation in cells isolated from peripheral blood of infertile men and controls under the supervision of Dr. Gregori and Dr. Mortellaro, respectively. Dr. Iaia will also delineate the cytokine and chemokines profile on plasma and seminal fluids of enrolled patients.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
IRCCS Ospedale San Raffaele	San Raffaele Telethon Institute for Gene Therapy (SR-TIGET)	Milan, Italy	Research Assistant	2021	2022
Ospedale G.B.Rossi	Department of Anatomy Pathology	Verona, Italy	Post-doctoral fellow	2020	2021
Università degli studi di Verona	Department of Medicine, Section of General Pathology	Verona,Italy	PhD student	2017	2020

Other awards and honors

Dr. Iaia does not have pubblication.
ORCID ID: 0000-0002-0527-6845
RESEARCHER ID:AHE-8578-2022

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/A	N/A	N/A	Collaborator	0,00	N/A

2.9 Additional Research Collaborators n. 2 - Under 40 to hire

Last Name: ONANO
First Name: STEFANO
Last name at birth:
Gender: M

Title: Bioinformatician
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 26/02/1986
Place of Birth: Cagliari

Official H index (Scopus or Web of Science): 5.0

Scopus Author Id:57191412925 **ORCID ID:**0000-0002-8478-5137 **RESEARCH ID:**AHE-13415-2022

Contact address

Current organisation name: Ospedale San Raffaele - Milano
Current Department / Faculty / Institute / Laboratory name: Unit of Urology &San Raffaele Telethon Institute for Gene Therapy
(SR-Tiget)

Street: c/o S.S 554 bivio per Sestu Km 4,500 Cittadella Universitaria di Cagliari
Postcode / Cedex: 09042
Town: Monserrato
Phone:+393201885266
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Sassari	PhD	Biomedical Sciences, Medical Genetics	2015	2018
University of Cagliari	Master's Degree / Laurea Magistrale	Biology	2009	2011
University of Cagliari	Bachelor Degree / Laurea Triennale	Biology	2005	2008

Personal Statement:

Dr. Onano's scientific activity includes several years of experience in the analysis of large genomic and transcriptomic datasets focused on understanding by GWAS analysis of variants correlated/predisposing to complex diseases. Recently, he has focused specifically on gene expression analysis in complex traits (eQTLs). He also had research experiences at the Biostatistics Faculty of the University of Michigan and at CRS4 bioinformatics laboratories, during which he was involved in NGS data analysis and development of Galaxy Platform.He will prepare samples for single cell RNAseq analysi under the supervision of Dr. Gregori. Dr. Onano will be also involved in transcriptomic analysis under the supervision of Dr. Angius.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
CNR - IRGB	Bioinformatics -Trascriptomic team	Monserrato, Cagliari, Italy	Postdoc Fellow	2019	2022
University of Michigan	Department of Biostatistics, Prof. Abecasis Team	Ann Arbor (MI ¿ USA)	Visiting researcher	2018	2018
CNR - IRGB	Bioinformatics -Trascriptomic team	Monserrato, Cagliari, Italy	PhD student	2015	2018
CNR-IRGB and CRS4	Exome team, bioinformatic lab	Cagliari, Italy	Professional Training	2015	2014
Bambino Gesù Children Hospital and CRS4	Genetic expression and microarray lab, bioinformatic lab	Rome, Italy	Collaborator	2013	2014
CRS4	Bioinformatic lab	Pula (CA), Italy	Internship	2013	2013

Other awards and honors

N/A

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/A	N/A	N/A	Collaborator	0,00	N/A

2.10 Additional Research Collaborators n. 3 - Under 40 to hire

Last Name: Rallo
First Name: Vincenzo
Last name at birth: Rallo
Gender: M

Title: Bioinformatician
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 04/10/1990
Place of Birth: Mazara del Vallo

Official H index (Scopus or Web of Science): 2.0

Scopus Author Id:57211314479 **ORCID ID:**0000-0002-8938-5650 **RESEARCH ID:**AHE-8124-2022

Contact address

Current organisation name: Institute of Biomedical and Genetic Research
Current Department / Faculty / Institute / Laboratory name: Department of Biomedicine - National Research Council (CNR)
Street: C.N.R. - Istituto di Ricerca Genetica e Biomedica - Cittadella Universitaria di Cagliari - SS 554 Km 4,500, 09042 Monserrato CA
Postcode / Cedex: 09042 **Town:** Monserrato
Phone:+393336406495 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Sassari	PhD	Biomedical Sciences, Human	2018	2021
University of Sassari	Master's Degree / Laurea Magistrale	Human and animal health biotechnologies	2015	2017
University of Sassari	Bachelor Degree / Laurea Triennale	Biotechnology	2009	2014

Personal Statement:

Dr. Rallo's expertise focuses on the analysis of genomic and transcriptomic data (WGS, WES, RNA seq and Single cell Seq). In recent years, he has structured and validated bioinformatics pipelines including database/dataset querying and optimization and automation of complex workflows for the identification of causal and/or predisposing variants to Mendelian disorders, complex diseases and related traits. Recently, Dr. Rallo's scientific interests have focused on multi-omics approaches and statistical analysis of colocalizations on large datasets. Dr. Rallo will be responsible for performing the computation analysis to identify signature associated to chronic inflammation and senescence in infertile men. He will work under the supervision of Dr. Angius.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Institute for Genetic and Biomedical Research (IRGB)	Department of Biomedicine, National Research Council (CNR)	Monserrato, Cagliari, Italy	Postdoctoral Fellow	2021	2022
Institute for Genetic and Biomedical Research (IRGB)	Department of Biomedicine, National Research Council (CNR)	Monserrato, Cagliari, Italy	PhD Student	2018	2021
Institute for Genetic and Biomedical Research (IRGB)	Department of Biomedicine, National Research Council (CNR)	Monserrato, Cagliari, Italy	Professional Training Internship	2017	2018
Institute for Genetic and Biomedical Research (IRGB)	Department of Biomedicine, National Research Council (CNR)	Monserrato, Cagliari, Italy	Training Internship	2017	2017
University of Sassari	Department of Biomedical Sciences	Sassari, Italy	Undergraduate Training Internship	2014	2014

Other awards and honors

N/A

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/A	N/A	N/A	Collaborator	0,00	N/A

2.11 Additional Research Collaborators n. 4 - Under 40 to hire

Last Name: PAVIA
First Name: CHIARA
Last name at birth:
Gender: F

Title: Bioinformatician
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 02/07/1997
Place of Birth: Brescia

Official H index (Scopus or Web of Science): 0.0

Scopus Author Id:N/A **ORCID ID:**N/A **RESEARCH ID:**N/A

Contact address

Current organisation name: Institute of Biomedical and Genetic Research
Current Department / Faculty / Institute / Laboratory name: Department of Biomedicine - National Research Council (CNR)
Street: SR-TIGET
Postcode / Cedex: 20100
Town: Milano
Phone:+393317610642
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Universita degli Studi di Milano, Politecnico di Milano	Master's Degree / Laurea Magistrale	Bioinformatics for computational genomis	2020	2022
Universita degli Studi di Verona	Bachelor Degree / Laurea Triennale	Bioinformatics	2016	2020

Personal Statement:

Dr. Pavia is a research fellow in Bioinformatics, currently she is finalizing her theses on defining approaches for filtering aberratn events in sequency library. She has expertise in data base development and she will be dedicated to geenrate the data base for the bioinformatic analysis and she will be primarily responsible for the multivariant unsupervise bioinformatic analysis of the immunological and senescence data collected within the project.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Universita degli Studi di Milano, Politecnico di Milano	San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	Milan, Italy	Ungraduated student	2021	2022
Universita degli Studi di Verona	Functional genomic laboratory	Verona, Italy	Ungraduated Fellow	2019	2022

Other awards and honors

Expertise in developing variant frequency database. Master degree on identifcation and filtering of aberrant events in

sequence library. Thesis defence will be on October 2022.
GA level 2 certificate in ESOL international (Classix C1) 24/11/2020.

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/A	N/A	N/A	Collaborator	0,00	N/A

2.12 Additional Research Collaborators n. 5 - Under 40 to hire

Last Name: Tedde
First Name: Matteo

Last name at birth: Tedde
Gender: M

Title: Researcher
Nationality: Italiana
Date of birth: 27/02/1989

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Sassari

Official H index (Scopus or Web of Science): 5.0

Scopus Author Id:57225165013 **ORCID ID:**0000-0002-3252-5587 **RESEARCH ID:**N/A

Contact address

Current organisation name: Università Degli Studi di Sassari

Current Department / Faculty / Institute / Laboratory name: Dipartimento di Medicina Clinica e SperimentaleUnità Operativa di Chirurgia generale 2 ¿Clinica Chirurgica¿ Azienda Ospedaliero Universitaria di Sassari

Street: Via Lago di Baratz 13
Postcode / Cedex: 07100
Town: Sassari
Phone:+393473927026
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Università degli Studi di Sassari	Specialization / Specializzazione	Urology	2018	2022
Università degli Studi di Sassari	Master's Degree / Laurea Magistrale	Medicina e Chirurgia	2010	2016

Personal Statement:
Dr. Tedde is resident fellow in urology. He will be responsible for recruiting infertile and fertile men from Sardinia and provide all clinical data from these patients for the data base and the analysis.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Università degli Studi di Sassari	UOC di Urologia (AOU Sassari)	Sassari, Italy	Resident	2018	2022

Other awards and honors
N/A

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
N/A	N/A	N/A	N/A	Collaborator	0,00	N/A

2.17 Expertise Research Collaborators

Selected peer-reviewed publications of the Research Group / Collaborators

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
LOCATELLI IRENE	NF-¿B1 deficiency stimulates the progression of non-alcoholic steatohepatitis (NASH) in mice by promoting NKT-cell-mediated responses	Article	279-287	124	2013	10.1042/CS20120289	22970906	39	F
Gregori Silvia Adriana	Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells	Article	739-746	19	2013	10.1038/nm.3179	23624599	506	O
MORTELLARO ALESSANDRA ROSA	Cutting edge: The NLRP3 inflammasome links complement-mediated inflammation and IL-1b release	Article	1006-1010	191	2013	10.4049/jimmunol.1300489	23817414	118	L
MORTELLARO ALESSANDRA ROSA	Caspase-11: the driving factor for non-canonical inflammasomes	Review	2240-2245	43	2013	10.1002/eji.201343800	24037676	59	C
ANGIUS ANDREA	Genetic variants regulating immune cell levels in health and disease	Article	242-156	155	2013	10.1016/j.cell.2013.08.041	24074872	200	C
FICARRA VINCENZO	A multicentre matched-pair analysis comparing robot-assisted versus open partial nephrectomy	Article	936-941	113	2014	10.1111/bju.12570	24219227	71	F
FICARRA VINCENZO	Understanding pathologic variants of renal cell carcinoma: Distilling therapeutic opportunities from biologic complexity	Review	85-97	67	2015	10.1016/j.eururo.2014.04.029	24857407	286	C
Gregori Silvia Adriana	Hla-g expression levels influence the tolerogenic activity of human DC-10	Article	548-557	100	2015	10.3324/haematol.2014.113803	25661445	51	C
FICARRA VINCENZO	What is the optimal definition of misclassification in patients with very low-risk prostate cancer eligible for active surveillance? Results from a multi-institutional series	Article	164.e1¿164.e9	33	2015	10.1016/j.urolonc.2014.12.011	25620154	31	O
LOCATELLI IRENE	Is there a role for adaptive immunity in nonalcoholic steatohepatitis?	Article	1725-1729	7	2015	10.4254/wjh.v7.i13.1725	26167244	10	O
MORTELLARO ALESSANDRA ROSA	A unique role for p53 in the regulation of M2 macrophage polarization	Article	1081-1083	22	2015	10.1038/cdd.2014.212	25526089	83	O

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
MORTELLARO ALESSANDRA ROSA	Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes	Article	1-13	6	2015	10.1038/ncomms9761	26508369	83	C
ANGIUS ANDREA	Genome sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers	Article	1272-1281	47	2015	10.1038/ng.3368	26366553	44	O
FICARRA VINCENZO	Complications and quality of life in elderly patients with several comorbidities undergoing cutaneous ureterostomy with single stoma or ileal conduit after radical cystectomy	Article	521-526	118	2016	10.1111/bju.13462	26935245	45	O
MORTELLARO ALESSANDRA ROSA	Inflammasome-dependent IL-1ß release depends upon membrane permeabilization	Article	1219-1231	23	2016	10.1038/cdd.2015.176	26868913	110	O
Ginesu Giorgio Carlo	Conservative approach to Hepatic Portal Venous Gas: A case report	Article	183-185	30	2017	10.1016/j.ijscr.2016.12.006	28024211	10	F
FICARRA VINCENZO	Renal cell carcinoma	Review	1-19	3	2017	10.1038/nrdp.2017.9	28276433	971	C
Ginesu Giorgio Carlo	Cost and morbidity analysis of chest port insertion in adults: Outpatient clinic versus operating room placement	Article	81-84	21	2017	10.1016/j.amsu.2017.07.056	28794870	4	O
LOCATELLI IRENE	Anti-Mullerian Hormone-to-Testosterone Ratio is Predictive of Positive Sperm Retrieval in Men with Idiopathic Non-Obstructive Azoospermia	Article	1-9	7	2017	10.1038/s41598-017-17420-z	29247212	21	O
Ginesu Giorgio Carlo	Inflammatory cell indexes as preoperative predictors of hospital stay in open elective thoracic surgery	Article	616-620	88	2018	10.1111/ans.14557	29687547	9	O
LOCATELLI IRENE	Testicular microbiome in azoospermic men'first evidence of the impact of an altered microenvironment	Article	1212-1217	33	2019	10.1093/humrep/dey116	29850857	29	O
ANGIUS ANDREA	The changing landscape of naive T cell receptor repertoire with human aging	Article	1-12	9	2018	10.3389/fimmu.2018.01618	30087674	53	O
Gregori Silvia Adriana	The Biology of T Regulatory Type 1 Cells and Their Therapeutic Application in Immune-Mediated Diseases	Review	1004-1019	49	2018	10.1016/j.immuni.2018.12.001	30566879	105	O

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Gregori Silvia Adriana	Role of myeloid regulatory cells (MRCs) in maintaining tissue homeostasis and promoting tolerance in autoimmunity, inflammatory disease and transplantation	Review	661-672	68	2019	10.1007/s00262-018-2264-3	30357490	32	C
LOCATELLI IRENE	Impaired testicular signaling of vitamin A and vitamin K contributes to the aberrant composition of the extracellular matrix in idiopathic germ cell aplasia	Article	687-698	111	2019	10.1016/j.fertnstert.2018.12.002	30929729	9	O
ANGIUS ANDREA	Integrated Analysis of miRNA and mRNA Endorses a Twenty miRNAs Signature for Colorectal Carcinoma	Article	1-16	20	2019	10.3390/ijms20164067	31434359	19	F
ANGIUS ANDREA	MicroRNA-425-5p Expression Affects BRAF/RAS/MAPK Pathways In Colorectal Cancers	Article	1480-1491	11	2019	10.7150/ijms.35269	31673240	20	F
Gregori Silvia Adriana	Coexpression of CD163 and CD141 identifies human circulating IL-10-producing dendritic cells (DC-10)	Article	95-107	17	2020	10.1038/s41423-019-0218-0	30842629	25	C
ONANO STEFANO	Complex genetic signatures in immune cells underlie autoimmunity and inform therapy	Article	1036-1045	52	2020	10.1038/s41588-020-0684-4	32929287	15	O
Ginesu Giorgio Carlo	Elective Cancer Surgery in COVID-19-Free Surgical Pathways During the SARS-CoV-2 Pandemic: An International, Multicenter, Comparative Cohort Study	Article	66-78	39	2021	10.1200/JCO.20.01933	33021869	88	O
Rallo Vincenzo	Portrait of cancer stem cells on colorectal cancer: Molecular biomarkers, signaling pathways and mirnaome	Article	2-35	22	2021	10.3390/ijms22041603	33562604	2	O
Ginesu Giorgio Carlo	Metformin and Vitamin D Modulate Inflammation and Autophagy during Adipose-Derived Stem Cell Differentiation	Article	1-12	22	2021	10.3390/ijms22136686	34206506	2	O
Tedde Matteo	Effect of COVID-19 pandemic lockdowns on planned cancer surgery for 15 tumour types in 61 countries: an international, prospective, cohort study	Article	1507-1517	22	2021	10.1016/S1470-2045(21)00493-9	34624250	17	O

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

3 - Ethics

1. HUMAN EMBRYOS/FOETUSES	
Does your research involve Human Embryonic Stem Cells (hESCs)?	No
Does your research involve the use of human embryos?	No
Does your research involve the use of human foetal tissues / cells?	No
2. HUMANS	
Does your research involve human participants?	Yes
Does your research involve physical interventions on the study participants?	Yes
3. HUMAN CELLS / TISSUES	
Does your research involve human cells or tissues (other than from Human Embryos/ Foetuses?	Yes
4. PERSONAL DATA	
Does your research involve personal data collection and/or processing?	Yes
Does your research involve further processing of previously collected personal data (secondary use)?	Yes
5. ANIMALS	
Does your research involve animals?	No
6. ENVIRONMENT & HEALTH and SAFETY	
Does your research involve the use of elements that may cause harm to the environment, to animals or plants?	No
Does your research deal with endangered fauna and/or flora and/or protected areas?	No
Does your research involve the use of elements that may cause harm to humans, including research staff?	No
7. DUAL USE	
Does your research involve dual-use items in the sense of Regulation 428/2009, or other items for which an	No
8. EXCLUSIVE FOCUS ON CIVIL APPLICATIONS	
Could your research raise concerns regarding the exclusive focus on civil applications?	No
9. MISUSE	
Does your research have the potential for misuse of research results?	No
10. OTHER ETHICS ISSUES	
Are there any other ethics issues that should be taken into consideration? Please specify	No

I confirm that I have taken into account all ethics issues described above and that, if any ethics issues apply, I will complete the ethics self-assessment and attach the required documents. [X]

4 - Call-specific questions

Eligibility	
I acknowledge that I am aware of the eligibility requirements for applying as specified in the Call-PNRRXXXX_M6/C2, and certify that, to the best of my knowledge my application is in compliance with all these requirements. I understand that my proposal may be declared ineligible at any point during the evaluation or granting process if it is found not to be compliant with these eligibility criteria.	[X]
I confirm that the proposal that I am about to submit draws substantially don't repeat on an existing or recently finished GRANT funded.	[X]
Data-Related Questions and Data Protection (Consent to any question below is entirely voluntary. A positive or negative answer will not affect the evaluation of your project proposal in any form and will not be communicated to the evaluators of your project.)	
For communication purposes only, the MoH asks for your permission to publish,in whatever form and medium, your name, the proposal title, the proposal acronym, the panel, and host institution, should your proposal be retained for funding.	[X]
Some national and regional public research funding authorities run schemes to fund MoH applicants that score highly in the MoH's evaluation but which can not be funded by the MoH due to its limited budget. In case your proposal could not be selected for funding by the MoH do you consent to allow the MoH to disclose the results of your evaluation (score and ranking range) together with your name, non- confidential proposal title and abstract, proposal acronym, host institution and your contact details to such authorities?	[X]
The MoH is sometimes contacted for lists of MoH funded researchers by institutions that are awarding prizes to excellent researchers. Do you consent to allow the MoH to disclose your name, non-confidential proposal title and abstract, proposal acronym, host institution and your contact details to such institutions?	[X]
The Ministry of Health occasionally could contacts Principal Investigators of funded proposals for various purposes such as communication campaigns, pitching events, presentation of their project's evolution or outcomes to the public, invitations to represent the Ministry of Health in national and international forums, studies etc. Should your proposal be funded, do you consent to the Ministry of Health staff contacting you for such purposes?	[X]
For purposes related to monitoring, study and evaluating implementation of MoH actions, the MoH may need that submitted proposals and their respective evaluation data be processed by external parties. Any processing will be conducted in compliance with the requirements of Regulation 45/2001.	

5 – Description Project

Summary description

Infertility is a disease of nearly endemic proportions, affecting up to 15% of couples of reproductive age. Overall, a pure male factor infertility underlies the problem in at least 30% of cases. Growing clinical data shows that infertile men are at increased risk of developing unfavorable age-related comorbidities in almost 10% of cases. Despite the common ground

fostering overall male health status and infertility is not understood yet, preliminary evidence indicates chronic inflammation and early senescence are the biopathological link between male infertility and comorbid conditions. The overarching aim of the project is to delineate key mechanisms that lead to increases in inflammation, unhealthy aging, and early comorbidities in infertile men. Via a multifaceted approach, we aim to discover effective biomarkers and personalized signatures identifying infertile men at actual risk to develop comorbidities, with a rebound on the preventive strategies of the national health system.

Background / State of the art

Infertility is a disease that affects up to 15% of couples worldwide (1). A pure male factor infertility underlies the problem in at least 30% of infertile couples, and the etiology of male infertility remains unknown in about 30% of those cases (2). Infertility per se is now considered not only an epiphenomenon of diseases but a disease, with relevant clinical implications and health-related outcomes (3,4). Indeed, it has become evident that infertile men are less healthy than age-comparable fertile men5. Associations have been described between infertility and non-malignant chronic diseases(4,5). Moreover, growing evidence supports a link between male infertility and the risk of developing malignant diseases(6-9). These findings are in accordance with large population-based studies suggesting a higher overall mortality risk for infertile men(10), and quantitative measures of the overall health status have been recently linked to alterations, mostly quantitative, of sperm parameters (11,12). Despite these robust epidemiological studies, the pathogenetic link between infertility and overall male health status is still elusive, and the mechanisms fostering the two need to be elucidated. However, a recent study led by the coordinator of the team revealed that at least 10% of infertile men are at high risk for premature development of comorbidities, possibly sharing one central unifying mechanism, namely increased inflammation(13).
See References below.

Description and distribution of activities of each operating unit

The Coordinating unit (UO1) will be responsible of supervising the whole project. We foresee to enroll 100 infertile and 100 fertile men (OSR). Notably, UO1 has collected clinical data and biological material (e.g., samples of semen, stool, urine, blood) of a cohort of 200 italian infertile men from 2006, with a median 12-14-year follow-up in 2022, along with 142 age-comparable fertile men from 2018, thus guarantee the enrollment plan. To select and validate signatures associated with infertility we plan to enroll additional 50 infertile and 50 fertile men from Sardinia (UO3) and Sicily (UO4) and 50 oncologic patients (e.g. kidney, bladder, chest, and pancreas) (UO3 and UO4).
The collaborating units will collect clinical variables for each patient: date of birth; ethnicity; complete medical history, including a compilation of the Charlson Comorbidity Index (CCI); history of undescended testis/testes; measured Body Mass Index (BMI); waist circumference; presence of varicocele; testicular volume; hormonal parameters; genetic profile; semen parameters; partner¿s age; and, partner¿s health status.
UO1 will define the relative frequencies of lymphoid and myeloid cells by multi-color flow cytometry. The presence of pro-/anti-inflammatory cytokines, chemokines, and biomarkers of inflammation in sera and/or plasma, and sperm will be assessed by multiplex bead arrays. Moreover, UO1 will prepare biological samples for single cell RNAseq analysis. To this end, FACS-sorted T cells from peripheral blood and CD45 cells from seminal fluids will be isolated from intertile [mostly men with oligoasthenoteratozoospermia (OAT) or idiopathic non-obstructive azoospermia (iNOA)] and age-matched fertile men.
UO1 in collaboration with UO2 will perfom wet RNAseq activities and UO2 will be responsible to carry out all the computational studies in order to identify the signatures, and the biomarkers of chronic inflammation and/or senescence that will be available for future validation.

5.4 Specific Aims and Experimental Design

Specific aim 1

Characterization of early exhaustion in peripheral blood and seminal fluid cell composition in infertile men.
To characterize in depth T cell compartments in peripheral blood of infertile men (mostly OAT and iNOA) we will perform single cell RNAseq analysis of purified T cells by FACS-sorting. The choice of this methodology is aimed at targeted

processing of an adequate number of T cells to obtain significant statistical evidence. In parallel, we will perform immuno-phenotyping and functional characterization of effector/memory (e.g., CD45RA, CD45RO, CD62L, CCR7), activated (e.g., CD69, CD25, HLA-DR, CD71) and exhausted (e.g., PD-1, CTLA-4, ICOS, TIGIT, TIM3, LAG3, eomes, t-bet) T cells and regulatory (e.g., FOXP3 Tregs and IL-10-producing Tr1 cells) cells in peripheral blood by multi-color flow cytometry. In addition, fertile controls (age-comparable and older individuals) will be recruited. Moreover, to select and validate signature associated with male infertility, chronic inflammation and T-cell exhaustion we will characterize peripheral blood cells of infertile and fertile men from Sardinia and Sicily and patients with cancer (e.g., kidney, bladder, chest, and pancreas) by flow cytometry. Unsupervised "t-stochastic neighbor embedding method (t-SNE)" analysis will be performed.
To define the signature and composition of immune cells in seminal fluids of infertile men, single-cell RNAseq analysis will be performed. Samples from infertile men (mostly OAT and iNOA) will be compared to those of fertile men. The experiments will be carried out in two phases: "Single Cell Capture" and NGS data generation. Once the cell count and viability have been determined, the cell suspensions will be loaded onto the Chromium Single-Cell instrument (10x Genomics) to generate Gel Bead-in-Emulsions (GEM). Single cell RNAseq libraries will be prepared with Chromium SingleCell 3 Library & Gel Bead Kit to generate NGS data and will be sequenced on Illumina instruments. We will use the Cell Ranger software suite to perform sample "demultiplexing", molecular code identification, and single cell gene counts. The number of reads that provide meaningful information is calculated as the product of four parameters: 1) valid molecular codes; 2) valid UMI code; 3) association to a unique cellular code; and 4) quality of mapping to exons. We will perform Principal Component Analysis (PCA) for the quality metrics listed above to identify outliers for rejection. Normalization and identification of highly variable genes (HVGs) associated with biological variance will be performed. HVGs will be used to identify new cellular subpopulations by applying t-SNE from normalized expression values of HVGs. Cells separated into different clusters, in combination with the biological properties of the more specific HVGs, will be considered as belonging to different cellular subpopulations. Differentially expressed genes between these cell subpopulations will be identified to determine subpopulation-specific biomarkers. These will be evaluated for functional significance, effect size, absolute expression level, and subcellular compartment localization.

Specific aim 2

Inflammatory/senescence signature in peripheral blood and seminal fluids.
To identify the inflammatory profile at a systemic level, we will perform a multidimensional flow cytometric analysis of myeloid cell populations [e.g., monocytes, neutrophils, and dendritic cell - (DC)] in the blood of infertile men. Within the DC compartment we will define the presence and phenotype of classical and tolerogenic DC (e.g., IL-10-producing DC-10). Moreover, the activation of the inflammasome, a crucial platform in the inflammatory pathway, will also be determined in monocytes of infertile men (mostly OAT and iNOA), as IL-1ß/IL-18 release, caspase-1 activation, and cell death induction. The inflammatory mediators of plasma and seminal fluid will be analysed with a multicytokine assay. Results will identify at the molecular level the link between inflammation and inflammasome activation in infertile men paving the way for developing preventive anti-inflammatory treatments.
Analysis of senescence markers (e.g., CDKN2A/p16, CDKN1A/p21, LMNA/B1/B2, telomere-associated DNA damage foci including, for example, gH2AX, 53BP1) will be performed. In parallel, mutations in a well-defined set of genes (i.e., DNMT3A, TET2, ASXL1, TP53, PPM1D, JAK2, CBL, SF3B1, U2AF1) associated with clonal haematopoiesis in normal ageing will be defined in peripheral blood mononuclear cells. To evaluate markers of senescence from the transcriptome point of view, we will perform a bulk RNAseq on peripheral blood. RNA libraries will be generated using the Illumina Stranded Total RNA Library Prep Kit and sequenced on Illumina instruments. High-quality fastq files will align to the human genome by using STAR. Reads will be trimmed for low-quality ends with TrimGalore and transcript abundance will be analyzed with Kallisto. Gene read counts will be calculated with featureCounts. Normalized expression data and differentially expressed (DE) genes will be identified using DeSeq2. Transcriptomes will be further analyzed by both univariate filtering and multivariate supervised analysis for feature subset selection from expression data. The performance of classifiers will be evaluated in terms of area under the Receiver Operating Characteristic (ROC) curve after leave-one-out or k-fold cross validation. The resulting signature will be functionally analyzed by ORA and conventional expression

based GSEA. To identify the pathways likely involved we will use three strategies: 1) enrichment of pathways among DEGs; 2) enrichment of gene-sets by using summary statistics from Deseq2; and, 3) identification of co-expression networks that will reveal correlation of expression levels between genes in the transcriptome across different samples in functionally relevant modules. The co-expression network will be computed with WGCNA on variance stabilized levels obtained with DESeq2. WGNA modules will be correlated with the sample groups to identify co-expression modules related to disease status. gProfiler will be used to determine whether gene modules are enriched for biological functions or pathways. Furthermore, knowing the genes involved in the module and identifying those that are more strongly interconnected in the module as well as determining modules more related to the biological condition of interest, will allow us to formulate hypotheses on the mechanisms involved and prioritize the genes for functional studies.

Specific aim 3

Identification of trajectory signatures predictive of comorbidities in infertile men.
A comprehensive database containing clinical parameters, immunological, and senescence variables will be generated. The immunological parameters associated with exhaustion, senescence, and inflammasome activation will be correlated with clinical parameters to select a panel of potential biomarkers/tools for subsequent translational applications. An integrated bioinformatic analysis of single cell RNAseq, transcriptome, and whole genome data will help and confirm to prioritize candidate disease-causing genes. We shall focus on cases of specific transcript expression in seminal fluid and integrate it with data found in whole blood sample. Recent data have reported that the integration of omics approaches including single cell RNAseq, RNA-seq and WGS genome data analysis allow to produce high detection rates compared to the use of a single approach, with higher rates when analyzing the target tissue of disease. We will focus primarily to analyze the interaction between genomics and transcriptomics, which should reveal clonal cell expansion and mutations that lead to differences in gene expression. The most promising variants identified will be further characterized from a functional point of view by performing ad hoc experiments. An in-silico model of the intracellular signaling cascade will also be developed and fine-tuned using 'omics' data obtained from the previous steps along with information from relevant databases of pathways and interaction networks. Further analyses will be conducted on quantitative and qualitative alterations of mRNA, analysis of promoters, enhancers, 5'UTR and 3' UTR. Likewise, a further analysis of publicly available databases that represent a good resource to leverage public expression data from patients for comparison and integration with our results will be also performed. This may increase the significance of our findings. Furthermore, using databases such as GTEx, their similarity to our data can introduce and confirm signals. Clinical data, parameters, and omics variables identified will be further integrated using analyses specific to the type of data and categories to be tested. Correlation strengths of the omics variables will be quantified using parametric and nonparametric methods, and the results will be represented with graphs such as dendrograms and heatmaps. Hierarchical clustering analyses will be performed using specific levels of categories and traits and using distance formulas as appropriate. Multiple test correction will be performed specifically for the set of correlation analyses displayed in each of the heatmaps using, for example, the Benjamini-Hochberg method, and significant correlations (False discovery rate < 5%) will be marked.

Experimental design aim 1

During the grant period, we will enroll 100 idiopathic infertile OAT or iNOA and 100 age-matched fertile men from Milan, and additional 50 infertile and 50 fertile men from Sardinia and Sicily and 25 chest, and pancreas patients from Sardinia and 25 kidney and bladder patients from Sicily. Sample collection: seminal fluid samples will be stored at -80°C until use. Cells will be centrifuged and used for subsequent scRNA sequencing. Whole blood samples from enrolled subjects: an aliquot will be taken from the whole blood and used for extraction of nucleic acids that will be subjected to NGS; the remaining amount will be separated into peripheral blood mononuclear cells and plasma and stored at -80° C until used. Protocols for cryopreservation and thawing methods are still being optimized. Immunophenotyping of peripheral blood T cells: we will define by multicolor flow cytometry the relative frequencies of several effector and regulatory cell populations, using the indicated markers. Functional T cell characterization: we will define the functionality of the T cell compartment in peripheral blood by analyzing the proliferation in response to anti-CD3 mAbs by flow cytometry, and cytokine profile by multiplex bead array. T cell exhaustion characterization: to determine the T cell-related immune signature, scRNAseq and bulk RNAseq

analysis on total T cells isolated from peripheral blood of patients and control will be performed. We will analyze in total 40 subjects (10 infertile OAT, 10 infertile iNOS, 10 fertile controls, and 10 cancer patient control). scRNAseq: single-cell capture protocol starts when cell suspensions will be loaded onto a Chromium Single-Cell Instrument to generate single-cell Gel Bead-in-Emulsions. Libraries will be prepared using the Chromium Single-Cell 3¿ Library, producing a full-length barcoded cDNA from poly-adenylated mRNA. An amplification reaction will generate sufficient material to construct a 3' cDNA library. Quantification of the constructed libraries will be evaluated using QubitdsDNA HS Assay Kit and Bioanalyzer High Sensitivity Kit. Libraries can be sequenced on Illumina instruments, using a 2x100 bp paired-end sequencing protocol. The first steps of the analysis will be represented by alignment, barcode assignment and Unique Molecular Identifiers (UMI) code counting, and subsequently by gene expression quantification. Principal Component Analysis (PCA) and Highly Variable Gene (HVG) identification will be performed to identify cell subpopulations. Genes differently expressed in each sub-population will be analyzed with the aim to identify specific biomarkers to be evaluated to achieve functional significance, effect entity and level of absolute expression. Bulk RNAseq on peripheral blood: RNA libraries will be prepared with Illumina Stranded Total RNA Prep and sequenced in paired-end mode (2×100 bp; with at least 50M reads each) on the Illumina NovaSeq6000. Fastq files will align to the human genome by using STAR. Reads will be trimmed for low-quality ends with TrimGalore and transcript abundance will be estimated with Kallisto. Gene read counts will be calculated with featureCounts. Normalized expression data and differentially expressed (DE) genes will be identified with DeSeq2. If present, fusion transcripts will be identified with FusionCatcher and validated by Sanger sequencing. The transcriptomes will be further analyzed by univariate filtering and supervised multivariate analysis for feature subset selection from the expression data. This will lead to a reduction in the dimensionality of the experimental data sets, which can be further improved by recursive feature elimination. The performance of the classifiers will be evaluated in terms of area under the Receiver Operating Characteristic (ROC) curve after leave-one-out or k-fold cross-validation. The resulting signature will be functionally analyzed by ORA and conventional expression-based GSEA.

Experimental design aim 2

IImmunophenotyping of myeloid cells in peripheral blood: we will define by multicolor flow cytometry the relative frequencies of conventional and regulatory antigen-presenting cell compartments will be characterized based on the detection of the following cell surface markers: CD11c, CD11b, CD163, BCDA-1, BDCA-2, BDCA-3, CD14, CD16, CD103. Myeloid DC and plasmacytoid DC will be identified as BCDA-1+CD11c+, and CD11c-CD123+BDCA-2+. DC-10 will be assessed as CD14+CD16+CD141+CD163+. HLA-G, ILT4 and costimulatory molecules (CD40, CD80, CD86) will be also investigated. As an internal control, the frequency of CD14lowCD16+ and CD14+CD16- monocytes will be defined.
Inflammasome activation in peripheral blood: to measure caspase-1 activation and inflammasome-related gene expression upon specific stimulation of NLRP3, NLRC4, and inflammasomes we will use a low-volume human whole blood assay (WBA). In brief, total blood will be collected from patients and healthy volunteers. For NLRP3, blood samples will be incubated in a 96-well plate with lipopolysaccharide (LPS) for 5.5 hours on a shaker in a humidified incubator with 37°C, 5% CO2, and NLRP3 will be activated by 30-min treatment with ATP. AIM2 will be activated by transfection of double-stranded DNA (poly(dA:dT) in the presence of Lipofectamine 2000 for either 6 h or 24 h. The NLRC4 inflammasome will be stimulated with Salmonella typhimurium for 2 hours at an MOI of 10/leukocyte. At the end of all stimulations, the plates will be centrifuged and the supernatants will be collected for the quantification of IL-1ß/IL-18 by ELISA, cell death by a colorimetric assay measuring lactate dehydrogenase release (Promega), and caspase-1 activation by the Caspase-Glo®1 Inflammasome Assay (Promega). Following erythrocyte lysis, total RNA will be purified from the leukocyte pellets, and cDNA will be generated using the Sensiscript RT kit (Qiagen). The expression of CASP1, NLRP3, AIM2, NLRC4, ASC, and IL1B genes will be determined by qRT-PCR.The results will be expressed as mean ± SD.
Senescence signatures: analysis of senescence markers (CDKN2A/p16, CDKN1A/p21, LMNA/B1/B2, telomere-associated DNA damage foci including, for example, gH2AX, 53BP1) will be performed. In parallel, mutations in a well-defined set of genes (DNMT3A, TET2, ASXL1, TP53, PPM1D, JAK2, CBL, SF3B1, U2AF1) associated with clonal hematopoiesis in normal aging will be defined in peripheral blood mononuclear cells. From the transcriptional point of view, RNAseq analysis on total PBMC will be performed as described in Aim1.

Experimental design aim 3

Starting from integrated bioinformatics analysis of RNAseq of single cells and the bulk transcriptome, we will confirm and prioritize candidate genes causing infertility and focus our attention on specific variants altering transcript expression in seminal fluid compared with data found in peripheral blood. Co-expression network analysis will be used to detect functionally related expression pathways and identify hub genes. The identified genes/variants will contribute to a refined understanding of disease pathophysiology by showing functional roles and convergent expression patterns: genes that are co-expressed, interact, or are regulated by known genes could shed light on convergent or auxiliary functions related to disease risk. We will use in silico network analyses to discover additional risk genes (gene co-expression data, enrichment analysis using protein-protein interaction (PPI) networks) and chromatin and immunoprecipitation assays to evaluate regulatory networks. We will generate a PPI network of proteins involved in infertility using Cytoscape and retrieving protein interaction data from the International Molecular Exchange Consortium (IMEx) database. The PPI network will be generated from genes in the international databases (DECIPHER) to identify potential biological links between specific proteins, in which we have identified variants in our cases and known proteins involved in infertility. This network will include edges describing direct or additional gene-mediated interactions and will be segmented into communities using the GLay algorithm. Variant-carrying genes from this study will be mapped onto this network and a functional enrichment analysis will be performed on each community by over-representation analysis (ORA) using Gene Ontology and Reactome/Panther/KEGG as pathway databases. The resulting network will be analyzed from a topological point of view to find the most central genes in terms of centrality measures (closeness centrality, betweenness centrality, clustering coefficient). The new candidate genes emerging from this analysis will require further evidence to prove, conclusively, that they contribute to the disease, which will be validated by functional assays (e.g., T cell proliferation and cytokine production profile)
A comprehensive database containing clinical parameters, and immunological and senescence variables will be generated. The immunological and senescent parameters identified as well as the transcriptomic profile identified above will be included and correlated with a comprehensive set of clinical parameters to select a panel of potential biomarkers/tools associated with malignant and non-malignant diseases. The database will contain clinical variables: partner¿s age; partner¿s health status; date of birth; race; ethnicity; complete medical history, also including a compilation of the Charlson Comorbidity Index (CCI); history of undescended testis/testes; measured Body Mass Index (BMI); waist circumference; the presence of varicocele; testicular volume; hormonal parameters (at least, total testosterone, FSH, LH, AMH, InhB, prolactin, TSHr, Osteocalcin); genetic profile (e.g., karyotype, Y chromosome microdeletions, CFTR mutations (both for <5x106 sperm/ml) according to EAU guidelines on male infertility); semen parameters, according to WHO 2010 standard references.

Picture to support preliminary data

PRELIMINARY DATA SALONIA.pdf

Hypothesis and significance

At present times, up to 60% of infertile men receive a diagnosis during the routinary diagnostic work-up. Of them, almost 15% are positive for some known genetic abnormalities (e.g., karyotype abnormalities, Y chromosome microdeletions, or CFTR mutations) (14). Moreover, it has been reported in a 9-year most comprehensive long-term, prospective monocentric study for the causes of male factor infertility that a reduced total sperm counts in 60% of cases (15). Thus, the etiology of male infertility remains unknown in no less than 30% of cases (named idiopathic infertility cases). From the preclinical standpoint, it was reported that infertile men show: i) an immature testis with a somatic environment stuck at puberty, characterized by low testis volume, altered expression of sexual hormones 16 and paternally-imprinted genes (16), along with immature Sertoli and Leydig cells (17); ii) prematurely-aged phenotype of testicular somatic cell populations and misshapen nuclei associated with a mild genetic splice variant of LAMIN A (LMNA) gene unbalancing LMNA isoforms (16); iii) a chronic pro-inflammatory environment, including accumulation of testicular resident cytotoxic T cells, fibrosis of the seminiferous tubules, DNA damage and cytoplasmic localization of HMGB1 in the local somatic cells, and overexpression of the chemokine CCL4 at the system level 16; iv) a testicular microbiome dysbiosis 18, as a further potential association

with early aging. Overall, testicular somatic cells of infertile men do not mature at puberty and are embedded in a chronic pro-inflammatory environment associated with DNA damage, cellular senescence, and Senescence-Activated Secretory Phenotype (SASP) (16). Compelling evidence indicates that defects in the regulatory arms and/or hyperactivation of the proinflammatory arm of the immune system can be associated with the risk of developing numerous non-oncological and oncological diseases. To the best of our knowledge, little is known about the immunological status of infertile men and its correlation with their poorer overall health status compared to age-matched fertile individuals. The group coordinating the project performed a multi-parameter analysis on immune mediators and immune cell compartments in peripheral blood and semen of severe infertile men patients and age-matched fertile men. Despite the low frequency of T cells, a significantly higher proportion of T cells exhibiting an exhausted phenotype has been found in the peripheral blood of patients with OAT and iNOA compared to T cells of fertile and iNOA men (preliminary data). Notably, dysregulated tolerance, lower frequency of IL-10-regulatory cells, and lower circulating IL-10 were observed in OAT patients (data not shown). Based on these premises and on clinical evidence that male fertility is an early life proxy of male health, the leading hypothesis of the present proposal is that chronic inflammation is the functional link between male infertility and comorbidities. We propose to identify clinical, molecular, and immunological characteristics of a sub-cohort of infertile men suffering from infertility-associated comorbidities. This knowledge will be adopted to develop robust biomarkers of comorbidity prediction and develop effective tailored prevention strategies and measures of comorbidities development and progression.

References

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5.5 Methodologies and statistical analyses

Methods of data collection

We will collect (personal) data during the runtime of the project. Proper data management is of critical importance. Therefore, a Data Protection Officer (DPO) from OSR will be involved throughout the preparation of the documentation, also taking care of the retrieved data, along with ethical aspects, GDPR compliance, and the organization processes. The DPO will guarantee that partners apply the national laws protecting data and the procedure's compliance. For this purpose, specific meetings with the Consortium will be organized at critical points if they occur. All issues related to data management will be considered (Data Types, Formats, Standards and Capture Methods, Ethics and Intellectual Property, Access, Data Sharing and Reuse, Resourcing, Deposit and Long-Term Preservation, Short-Term Storage). OSR will produce General Data Management Plan in Month 6, describing all data management principles and processes in details. Moreover, each partner will have to sign a processing data agreement. The GDMP will also cover the Data Management aspects related to Personal Data collection and Research Data. How this will be organized will be discussed and decided

by all the partners during the design of the GDMP. The final solution will be taken based on being compliant with the GDPR and privacy-by-design foundational principles.

Statistic plan

Statistical analysis to establish the statistical power based on sample size, effect size and statistical significance required has been performed. Considering that the project will involve continuous phenotypes, we applied a sensitivity test based on a t-test and normal distribution to compute the effect size we could obtain at a statistical significance level (p-value), a predefined statistical power and the expected available sample size. We used G-power version 3.1, a tool to compute statistical power analyses for many different tests. Considering our bulk RNAseq data to reach a statistical significance of p-value < 0.001 and a discovery power of 0.8, we will expect to see differences between cases and matched controls influencing our phenotype with an effect size of 0.29. At a more stringent p-value < 0.0001, we will have a power of 0.8 to observe an effect size of 0.34 that allow even small effects on the phenotype to be identified. We can also identify variables with less biological effect by reducing the power. In addition, a priori t-test was carried out to compute sample size given p-value, power and effect size. In this case, we estimated the sample size over a range of effect sizes (0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 2.0, 2.5, 3.0) to calculate how many samples are needed to reach a certain significance. Moreover, we expect to obtain higher discovery power than our estimates, considering the data from single-cell RNAseq analysis on 40 samples, that allow us to dissect cellular heterogeneity by identifying subpopulations in detail. Deconvolution analyses on bulk RNA seq data, on these same samples, will allow an initial comparison/validation of the characteristics of the populations (heterogeneity, abundance, etc.) that can be extended on the large sample data (350 samples). Specific bioinformatics analyses (GSEA, WGCNA) will lead to the identification of molecular pathways and interaction networks whose alteration leads to the clinical-pathological phenotype. All these integrated approaches will allow us to reach a higher level of resolution.

Statistical analysis

Correlation of the immunological parameters with disease outcomes will be performed with Classification and Regression Tree (CART) procedures (binary recursive partitioning that allows merging data of mixed nature, continuous and categorical, in a multivariate framework). Mann-Whitney test will be used to determine the statistical significance of the data. Statistical calculations will be performed with GraphPad Prism Software. For the analysis of RNA sequencing data, several differential analysis methods are available by which transcriptomes will be analyzed in detail by univariate filtering and supervised multivariate analysis for feature subset selection from the expression data. The dimensionality reduction of the experimental data sets can thus be further improved by recursive feature elimination. The performance of the classifiers will be evaluated in terms of area under the ROC curve after leave-one-out or k-fold cross-validation. The resulting signature will be functionally analyzed by ORA and conventional expression-based GSEA. Firstly, quantitative variables will be described by median and interquartile range (IQR) to consider possible non¿normal distribution of variables, whereas absolute and relative percentages will be used for qualitative variables. Mann-Whitney test will be used to estimate the difference between the median. Spearman¿s correlations between fertile and infertile men with expression levels will be computed. After evaluating quantitative transcriptomic variables which follow a Gaussian distribution using the Shapiro-Wilk and the Kolmogorov-Smirnov test, a t-test will be used to estimate the difference in the means between infertile patients (OAT and iNOS) and fertile controls. Linear and/or logistic regression analysis, when appropriate, will be performed to evaluate the association between cell counts, DEG expression, pathological phenotype subtypes, clinic-pathological features, and molecular variables. Statistical differences for qualitative variables will be evaluated using Chi2 or Fisher¿s exact tests, when appropriate. All these statistical analyses will be carried out using R software. Additionally, we want to apply bulk RNASeq deconvolution to our cohort to identify cell-specific expression profiles and compare them with scRNAseq data. Since 2015, several methods have been developed to apply Pseudotime (PT) approaches to single-cell sequencing data, and recently some of them have been refined specifically for bulk RNA-seq data. Cell deconvolution and pathway analysis will be used to find cell-specific molecular pathways. Our goal is to confirm the ability of the deconvolution approach to detect undetectable molecular processes using the entire list of differentially expressed genes, highlighting a clear contribution of cell-specific genes. We will generate PT trajectories on patients using the phenoPath model, identifying

genes whose expression is significantly linearly correlated with the trajectory. The co-expression network will be computed with WGCNA on variance stabilized levels obtained with DESeq2. WGNA modules will be correlated with the sample groups to identify co-expression modules related to disease status. Profiler will be used to determine whether gene modules are enriched for biological functions or pathways.

Timing of analysis data

We plan to enroll patients from OSR starting from month 0, while from OU3 and OU4 starting from month 6. Enrollment will last till month 18.

5.6 Expected outcomes

Infertility per se is now considered not only an epiphenomenon of diseases but a disease, with relevant clinical implications and health outcomes (3,5). the last few years, it has become evident that infertile men are less healthy than age-matched fertile men (6). In this context, associations have been described between infertility and non-malignant and malignant diseases. Given the existing lack of research on the immunological as well as unhealthy aging status over men¿s fertility potential, we will further investigate the molecular causes of idiopathic male infertility and the pathophysiological mechanisms behind the higher burden of clinically relevant comorbidities observed in infertile men we will address a clinically relevant question and will deliver for the first-time information to define infertile men at actual risk of developing non-malignant and malignant comorbidities. According to the almost endemic dramatic prevalence of infertility, this is an unparalleled opportunity to identify the etiologies of chronic disease in these men and develop tailored health prevention strategies with a significant rebound toward the national health systems.

5.7 Risk analysis, possible problems and solutions

The rationale for this proposal is based on robust preliminary data and the application of innovative technologies that will produce reliable results that will advance basic biomedical research and create new precision medicine techniques. The novelty of the proposed studies makes it difficult to predict the specific impact of each aspect of the project. External factors that could influence activities and goals include, for example, the number of patients enrolled, clinical characteristics, and identification of cell subtypes, although preliminary data support our estimates. Our own risk analysis, however, identified some important points and their possible solution.
To limit potential biases associated with causal heterogeneity of infertile men will be included in the study, if they are aged 18-50 years, white Caucasians, have a diagnosis of primary couples' infertility, with pure male infertility, have complete clinical data available at time of inclusion, have given informed consent to use their clinical data and pathological specimen for further studies.
The main limitation of this proposal is the volume of seminal fluids collected, and the number of CD45+ hematopoietic cells that can be retrieved. According to preliminary data, we will perform transcriptomic analysis when the total number of CD45+ cells will be >5000. In the case of fertile men in case the number of CD45+ cells will be <5000 we will pool different donors. Our preliminary data support the good success and replicability of our experiments even with lower numbers, but it will be important to maintain uniformity and normalization of the data by additional quality analysis. Bioinformatic analyses will still be able to point out potential biases.
For the measurement of inflammasome activation, the following potential risks have been identified and possible solutions proposed as follows: i) availability of patients' blood: increase blood volume. ii) suboptimal inflammasome activation: perform the WBA with more blood volume/cell number and test higher concentrations of inflammasome stimuli; iii) problems in data harmonization across different batches: process blood in larger batches from multiple patients and include the same reference samples in each batch.

5.8 Significance and Innovation

The project will address the relationship between male infertility and potential further comorbidities. It is known that

inflammation induces molecular phenotypes of premature aging, thus implying that testicular inflammation associated with infertility status could promote inflammation and premature tissue aging in peripheral organs. The project is based on the unique combination of preclinical and clinical data showing a chronic pro-inflammatory environment both in the testis and at the systemic level, along with an exhaustion profile within the immune compartment, and the onset of age-related comorbidities in infertile men. The project advances beyond the state of the art, being able for the first time to refute or confirm the leading hypothesis the high burden of comorbidities in infertile men is associated with chronic inflammation and early senescence. Results will allow developing tailored and cost-effective preventive strategies in this increasingly large subset of population.

5.9 Bibliography

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5.10 Timeline / Deliverables / Payable Milestones

The final goal of the proposal is to identify immune and senescence biomarkers for tailoring personalized prevention strategies of comorbidities in infertile men. The immune and senescence characterization of cells in peripheral blood and seminal fluid of males with infertility (OAT and iNOA) and controls will cover the entire grant period. Identification of the predictive biomarkers/signatures will be performed in the second grant period (18-24 months).
D1) To define T cell exhaustion in PB of infertile men;
D2) To characterize the inflammasome status in monocytes of infertile men;
D3) To define at single cell levels the signature of immune cells in seminal fluid of infertile men;
D4) To identify potential prognostic biomarkers/signatures of T cell exhaustion and senescence in infertile men.

Milestones 12 month

M1. General Data Management Plan completed and approved by participants (month 6)
M2. To set up a database including clinical immunological, senescence and transcriptional parameters (month 6).
M3. Testing the WBA for inflammasome activation in a cohort of healthy volunteers (month 6)
M4. Enrollment of 30% half of the patients and controls (month 12)
M5. Immunophenotyping of seminal fluid cells in 30% of patients and controls (month 12)
M6. Inflammasome activation in 30% of patients and controls (month 12)
M7. scRNA and Bulk RNASeq sequencing of T cells in 20 patients and 20 controls (month 12)

M8. Quality control and Bioinformatic analysis (month 12)

Milestones 24 month

M9. Complete the enrollment of the patients and controls (month 20)
M10. Complete immunophenotyping of peripheral blood cells and seminal fliuds of infertile men (month 21)
M11. Complete WBA for inflammasome activation in enrolled patients (month 21)
M12. To complete bioinformatic analysis (month 22)
M13. Integration of omics approaches (month 24)

Gantt chart

Gantt chart_PNRR_SALONIA.pdf

5.11 Equipment and resources available

Facilities Available

UO1. OSR units are San Raffaele Urological Research Institute (URI) and SR-Tiget institute. URI consists of more than 500m2, Dr. Gregori's and Dr. Mortellaro's laboratory at SR-TIGET consists of more than 70m2 and 40m2 fully equipped with instrumentation for preclinical and clinical research. SR-TIGET laboratories are equipped with instruments for cellular and molecular biology studies; automated sequencing device, Real Time PCRs, ELISA and ELISPOT readers, a MAGPIX system (BioRad) for magnetic bead multi-analytes , and AutoMacs. Access to the Core facilities of OSR: Flow cytometry Resource, Advanced Cytometry Technical Applications Laboratory (FRACTAL) UO2. IRGB CNR site covers an area of 1000m2 and is staffed by about 50 personnel. It includes laboratories well equipped to perform molecular biology, genomics and proteomics studies, cell culture rooms, rooms for storage and cryopreservation of biological samples. The IRGB has a data-intensive computational infrastructure that provides specialized capabilities to track data and the origin of biological data, managing complex analysis workflows and results. In addition, the IRGB has access to the University Service Center for Research (CeSAR), located in the University Campus, which houses equipment of the highest technological level . UO3. UOC General Surgery 2 and SSD Thoracic Surgery consists of more than 1500m2 equipped with instrumentation for preclinical and clinical hospitalization. It features: 5 surgeries (30m2 each), 3 operating room (40m2 each) equipped with 1 Da Vinci Xi robot, 3 endoscopy columns. UO4. Urologic unit of Azienda Ospedaliera Universitaria G. Martino is staffed by urologists, residents in urology, a PhD student and a data manager. The unit is equipped with all instruments and technologies (included Xi Da Vinci robot) for the management of uro-oncologic tumors and male infertile patients. It includes several laboratories well equipped to perform molecular, biology, genomics and proteomics analyses.

Subcontract

Based on the number of patients, the number of sc and bulk RNAseq s to be performed the instrument currently on the market that allows a better ratio of data quantity/quality at a lower cost is the NovaSeq 6000 system, which cost for maintenance about € 1,100,000/year. The preparation of the sample, libraries, and quality checks will be done in house. The raw data will be transferred to our servers and bioinformatics analysis will be done by the research group.

5.12 Desc. of the complementarity and sinergy of secondary collab. researchers

This proposal foresees a joint effort of collaborating units, whose complementary expertise will be essential for achieving the expected results. The Coordinating unit is a worldwide leader group in demographic and comorbidities studies of infertile men and has performed pioneer works in revealing that infertile men are less healthy than age-comparable fertile male. Moreover, UO1 recently characterized testicular extracellular matrix and microbiome in iNOA patients, which is known to be linked to a worse overall prognosis. Dr. Gregori and primary collaborators in UO1 (Dr. Mortellaro, Dr. Iaia, and Dr. Locatelli) have long expertise in human adaptive immune responses, tolerance, inflammasome and in cellular and molecular mechanisms involved in male infertility. Dr. Gregori and Dr. Mortellaro have been involved in studying the association of dysregulated immune response and activation of inflammasome with pathological conditons such as

autoimmune and autoinflammatory diseases and overall in chronic inflammation. The immunological analysis will be performed by Dr. Iaia under the supervision of Dr. Gregori and Dr. Mortellaro, and the identification of biomarkers of senescence with be carried out by Dr. Locatelli under the supersivion of Prof. Salonia. The unique opportunity to investigate immunological and inflammatory traits in infertile men in Sardinia and Sicily will be possible thank to the collaboration with Dr. Ginesu and Dr. Tedde (UO3), and Prof. Ficarra (UO4), thus promoting a better definition of inflammation associated to infertility. Moreover, the long lasting expertise of Dr. Ginesu and Prof. Ficarra in studying patients affected by kidney, bladder, chest, and pancreas cancer, will support the validation and the subsequent selection of the most suitable signature to identify and monitor infertile men at higher risk of developing comorbidities. The main activities of UO2 will be the bioinformatics analysis of data from single cell experiments both from semen and peripheral blood along with the analysis of total RNAseq and WGS data that will be analyzed separately and jointly through standardized pipelines and workflows and software optimized by the research group. Methodologies for preparing and analyzing libraries for RNASeq experiments on very small amounts of biological material, that could result from the separation of subpopulations, has already been implemented and optimized on various cell subtypes for several hundred individuals for various projects at the institute. UO2 now have decades of experience in both laboratory methodologies and protocol and pipeline development for NGS data analysis, having participated extensively in their development within international consortia and/or world-leading research groups in these topics. Dr. Angius will supervise the bioinformatic teams comprased by Dr. Onano, who will perform both wet and computational analysis in UO1, Dr. Rallo, who will perform computational analysis and Dr. Pavia, who will generate the database and perfom unsupervised analysis of immunological parameters. The research team covers all the expertise and technical know-how required for the fulfilment of this project: isolation and manipulation of immune cells, handling of samples with limited number of cells, immunological functional assays, innovative bioinformatic approaches for transcriptional profiles, and multi-omics results together with clinical expertise in male infertility and cancer patients.

5.13 Translational relevance and impact for the national health system (SSN)

What is already know about this topic?

Clinical data showed that primary infertile men are less healthy than fertile men (1), and the coexistence of specific diseases and sperm alterations may lead to a decreased lifespan and development of specific comorbidities. However, the identification of effective risk predictors for health status worsening in infertile men is not yet possible. Finding biomarkers, which may help and personalize preventive strategies for those infertile men who are at higher risk of developing chronic diseases, emerged to be clinically relevant. Based on published and preliminary data in infertile men, a dysfunctional spermatogenesis causes a general stress scenario, leading to local and systemic inflammation promoting early-onset age-related comorbidities(2), such as neoplastic, dysmetabolic, cardiovascular, and neurodegenerative diseases, with all possible subsequent long-term sequelae. Ref: 1. Boeri L. Andrology 2022. 2. Alfano M, Nat Commun 2021

Details on what is already know about this topic

It has become evident that infertile men are less healthy than age-matched fertile men (5,13). Despite several epidemiological studies, the pathogenetic link between infertility and male health status is still unclear. A recent study led by the coordination team revealed that at least 10% of infertile men are at high risk for premature development of comorbidities. Infertile men show: i) an immature testis along with immature Sertoli (16) and Leydig cells (17). ii) prematurely aged phenotype of testicular somatic cell populations (16). iii) a chronic pro-inflammatory environment includes the accumulation of testicular resident cytotoxic T cells, fibrosis of the seminiferous tubules, DNA damage and of CCL4 at the system level (16).

What this reasearch adds?

This project will help to explain the pathobiology behind the observed lower overall health status of infertile men based on cohorts of infertile men for easily retrievable clinical data, along with chronic inflammation and early unhealthy ageing parameters. Given the lack of knowledged on these parameters over men's fertility potential, we will investigate the molecular causes of male infertility and the pathophysiological mechanisms behind the higher burden of comorbidities. We

shall address a clinically relevant question, while delivering for the first-time information to define infertile men at actual risk of developing non-malignant and malignant comorbidities. According to the almost endemic dramatic prevalence of infertility, this is an unparalleled opportunity to identify the etiologies of chronic disease in these men and develop tailored health prevention strategies, with a significant rebound toward the national health systems.

Details on what this reasearch adds

The project will address a clinically relevant question and will deliver for the first-time information to better investigate idiopathic and unplaced infertility cases, along with the definition of the subset of infertile men at actual risk of developing non-malignant and malignant comorbidities. According to the almost endemic dramatic prevalence of infertility, this is an unparalleled opportunity to identify the aetiologies of chronic disease in these men and develop tailored health prevention strategies with a significant rebound toward the national health systems.

What are the implications for public health, clinical practice, patient care?

We will leverage further development of effective strategies of patients monitoring and personalized treatments to prevent chronic comorbidities in infertile men. Addressing infertility has also several important social implications. Indeed, individuals and couples have the right to decide number and timing of their children, and infertility can negate the realisation of these human rights. The proposed project long-sought in-depth research on male infertility will provide results that will ultimately help equalize the treatment burden to improve treatment options, enable natural conception, and decrease infertility related morbidity in men. Therefore, results will have a huge sociodemographic impact in terms of both personalized- and community-medicine, with a significant rebound toward the national health systems.

Details on what are the implications for public health, clinical practice, patient care

Being a consortium of experts on male infertility, genetics, and bio-pathological mechanisms of diseases, our project will aim to effectively investigate the cause of early development of comorbidities; thereof, findings will be potentially used to identify patients at risk to develop comorbidities, together with senescence/chronic inflammatory markers, with a huge sociodemographic repercussion in terms of personalized and community-medicine via the national health systems. Health researchers and clinicians will have access to new markers for early detection and prevention of comorbidities, but also with scientific tools, to evaluate the links and assess the risk of developing comorbidities. Moreover, the promotion of real-world use cases will allow and present its scientific outputs to policymakers (e.g., National Health Systems) and the public via use cases to increase realism when communicating science.

6 - Budget

Total proposed budget (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	0,00	-0,00	not permitted	0,00
2 Researchers' Contracts	380.000,00	0,00	380.000,00	38,00
3a.1 Equipment (Leasing -	0,00	0,00	0,00	0,00
3a.2 Equipment (buying)	0,00	0,00	0,00	0,00
3b Supplies	482.320,00	0,00	482.320,00	48,23
3c Model Costs	0,00	0,00	0,00	0,00
4 Subcontracts *	9.000,00	0,00	9.000,00	0,90
5 Patient Costs	0,00	0,00	0,00	0,00
6 IT Services and Data Bases	0,00	0,00	0,00	0,00
7 Travels	29.680,00	0,00	29.680,00	2,97
8 Publication Costs	9.630,00	0,00	9.630,00	0,96
9 Dissemination	0,00	0,00	0,00	0,00
10 Overheads *	69.370,00	0,00	69.370,00	6,94
11 Coordination Costs	20.000,00	0,00	20.000,00	2,00
Total	1.000.000,00	0,00	1.000.000,00	100,00

* percentage calculated as average value between all the Operating Units.

Report the Co-Funding Contributor:

N/A

Budget Justification

1 Staff Salary	N/A
2 Researchers' Contracts	Salary for one Bioinformatician for 2 years, and for one data manager for 2 years UO1; Salary for one Bioinformatician for 2 years OU2; Salary for one clinician research fellow for 2 years OU3, Salary for one clinician research fellow for 2 years OU4
3a.1 Equipment (Leasing - Rent)	N/A
3a.2 Equipment (buying)	N/A

3b Supplies	Monoclonal antibodies for immunomonitoring, and for detecting cytokines by ELISA and by multi beads arrays, Culture medium for T cell cultures, human serum, Plastic ware, Reagent for total and scRNAseq, costs for sample shipping to the collaborating units
3c Model Costs	N/A
4 Subcontracts	Costs for runs and NGS data generation
5 Patient Costs	N/A
6 IT Services and Data Bases	N/A
7 Travels	Travels cost for the personnel involved in the research project for kick of and final meetings
8 Publication Costs	Publication costs of manuscript related to the project
9 Dissemination	N/A
10 Overheads	7%
11 Coordination Costs	Cost for kick of and final meetings, preparation of the protocol for the collection of patients¿ material for the projects

Proposed total budget UO1 Institution: Ospedale San Raffaele - Milano (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	0,00	-0,00	not permitted	0,00
2 Researchers' Contracts	140.000,00	0,00	140.000,00	33,02
3a.1 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a.2 Equipment (buying)	0,00	0,00	0,00	0,00
3b Supplies	224.320,00	0,00	224.320,00	52,91
3c Model Costs	0,00	0,00	0,00	0,00
4 Subcontracts	0,00	0,00	0,00	0,00
5 Patient Costs	0,00	0,00	0,00	0,00
6 IT Services and Data Bases	0,00	0,00	0,00	0,00
7 Travels	5.000,00	0,00	5.000,00	1,18
8 Publication Costs	5.000,00	0,00	5.000,00	1,18
9 Dissemination	0,00	0,00	0,00	0,00
10 Overheads	29.680,00	0,00	29.680,00	7,00
11 Coordination Costs	20.000,00	0,00	20.000,00	4,72
Total	424.000,00	0,00	424.000,00	100,00

Budget Justification	
1 Staff Salary	N/A
2 Researchers' Contracts	Salary for one Bioinformatician for 2 years and for one data manager for 2 years
3a.1 Equipment (Leasing - Rent)	N/A
3a.2 Equipment (buying)	N/A
3b Supplies	Monoclonal antibodies for immunomonitoring, and for detecting cytokines by ELISA and by multi beads arrays, Culture medium for T cell cultures, human serum, Plastic ware
3c Model Costs	N/A
4 Subcontracts	N/A
5 Patient Costs	N/A
6 IT Services and Data Bases	N/A
7 Travels	Travels cost for the personnel involved in the research project for kick of and final meetings
8 Publication Costs	Publication costs of manuscript related to the project
9 Dissemination	N/A
10 Overheads	7%
11 Coordination Costs	Costs for kick off and final meetings, preparation of the protocol for the collection of patients¿ material for the projects

Proposed total budget UO2 Institution: Institute of Biomedical and Genetic Research (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	0,00	-0,00	not permitted	0,00
2 Researchers' Contracts	80.000,00	0,00	80.000,00	26,67
3a.1 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a.2 Equipment (buying)	0,00	0,00	0,00	0,00
3b Supplies	178.000,00	0,00	178.000,00	59,33
3c Model Costs	0,00	0,00	0,00	0,00
4 Subcontracts	9.000,00	0,00	9.000,00	3,00
5 Patient Costs	0,00	0,00	0,00	0,00
6 IT Services and Data Bases	0,00	0,00	0,00	0,00
7 Travels	8.000,00	0,00	8.000,00	2,67
8 Publication Costs	4.630,00	0,00	4.630,00	1,54
9 Dissemination	0,00	0,00	0,00	0,00
10 Overheads	20.370,00	0,00	20.370,00	6,79
11 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	300.000,00	0,00	300.000,00	100,00

Budget Justification	
1 Staff Salary	N/A
2 Researchers' Contracts	Salary for one Bioinformatician for 2 years
3a.1 Equipment (Leasing - Rent)	N/A
3a.2 Equipment (buying)	N/A
3b Supplies	Total RNASeq reagents for libraries and data generation, single cell reagents for capture and NGS data generation, reagents for storing and/or processing of biological samples for several DNA applications, Plastic ware
3c Model Costs	N/A
4 Subcontracts	Costs for runs and NGS data generation
5 Patient Costs	N/A
6 IT Services and Data Bases	N/A
7 Travels	Travels cost for the personnel involved in the research project for kick of and final meetings
8 Publication Costs	Publication costs of manuscript related to the project
9 Dissemination	N/A
10 Overheads	7%
11 Coordination Costs	N/A

Proposed total budget UO3 Institution: Università Degli Studi di Sassari (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	0,00	-0,00	not permitted	0,00
2 Researchers' Contracts	80.000,00	0,00	80.000,00	57,97
3a.1 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a.2 Equipment (buying)	0,00	0,00	0,00	0,00
3b Supplies	40.000,00	0,00	40.000,00	28,99
3c Model Costs	0,00	0,00	0,00	0,00
4 Subcontracts	0,00	0,00	0,00	0,00
5 Patient Costs	0,00	0,00	0,00	0,00
6 IT Services and Data Bases	0,00	0,00	0,00	0,00
7 Travels	8.340,00	0,00	8.340,00	6,04
8 Publication Costs	0,00	0,00	0,00	0,00
9 Dissemination	0,00	0,00	0,00	0,00
10 Overheads	9.660,00	0,00	9.660,00	7,00
11 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	138.000,00	0,00	138.000,00	100,00

Budget Justification	
1 Staff Salary	N/A
2 Researchers' Contracts	Salary for one clinician research fellow for 2 years
3a.1 Equipment (Leasing - Rent)	N/A
3a.2 Equipment (buying)	N/A
3b Supplies	Sample collection and biobanking, Reagents for storing and/or processing of biological samples for several DNA applications, Plastic ware, costs for sample shipping to the collaborating units
3c Model Costs	N/A
4 Subcontracts	N/A
5 Patient Costs	N/A
6 IT Services and Data Bases	N/A
7 Travels	Travels cost for the personnel involved in the research project for kick of and final meetings
8 Publication Costs	N/A
9 Dissemination	N/A
10 Overheads	7%
11 Coordination Costs	N/A

Proposed total budget UO4 Institution: Università degli Studi di Messina (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	0,00	-0,00	not permitted	0,00
2 Researchers' Contracts	80.000,00	0,00	80.000,00	57,97
3a.1 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a.2 Equipment (buying)	0,00	0,00	0,00	0,00
3b Supplies	40.000,00	0,00	40.000,00	28,99
3c Model Costs	0,00	0,00	0,00	0,00
4 Subcontracts	0,00	0,00	0,00	0,00
5 Patient Costs	0,00	0,00	0,00	0,00
6 IT Services and Data Bases	0,00	0,00	0,00	0,00
7 Travels	8.340,00	0,00	8.340,00	6,04
8 Publication Costs	0,00	0,00	0,00	0,00
9 Dissemination	0,00	0,00	0,00	0,00
10 Overheads	9.660,00	0,00	9.660,00	7,00
11 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	138.000,00	0,00	138.000,00	100,00

Budget Justification	
1 Staff Salary	N/A
2 Researchers' Contracts	Salary for one clinician research fellow for 2 years
3a.1 Equipment (Leasing - Rent)	N/A
3a.2 Equipment (buying)	N/A
3b Supplies	Sample collection and biobanking, Reagents for processing of biological samples, Plastic ware, costs for sample shipping to the collaborating units
3c Model Costs	N/A
4 Subcontracts	N/A
5 Patient Costs	N/A
6 IT Services and Data Bases	N/A
7 Travels	Travels cost for the personnel involved in the research project for kick of and final meetings
8 Publication Costs	N/A
9 Dissemination	N/A
10 Overheads	7%
11 Coordination Costs	N/A

Principal Investigator Data

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Istituzione: IRCCS Ospedale San Raffaele
Datore/ente di lavoro? Yes
Datore/ente di lavoro SSN? No
Nome datore/ente di lavoro non SSN: Università Vita-Salute San Raffaele
Nome istituzione SSN: IRCCS Ospedale San Raffaele
Tipo contratto: Professore Ordinario distaccato presso IRCCS/IZS/ISS/Ente SSN (convenzione di clinicizzazione e/o ricerca)

Con l'invio della presente proposta si dichiara che la stessa o parti significative di essa non sono oggetto di altri finanziamenti pubblici o privati e che di conseguenza vi è assenza del c.d. doppio finanziamento ai sensi dell'art. 9 del Regolamento (UE) 2021/241, ossia che non ci sia una duplicazione del finanziamento degli stessi costi da parte di altri programmi dell'Unione, nonché con risorse ordinarie da Bilancio statale.

By submitting this proposal, I declare that no significant part or parts of it are recipient of any other public or private funding and that consequently there isn't any so-called double financing pursuant to art. 9 of Regulation (EU) 2021/241, i.e. that there is no duplication in the financing of the same costs by other Euopean Union programs or any other ordinary resources from the State budget.

Project validation result