Ministero della Salute

Direzione Generale della Ricerca e dell' Innovazione in Sanità

ABSTRACT DEI PROGETTI FINANZIATI NEL2015 CON IL BANDO RICERCA FINALIZZATA - FINANZIAMENTO 2013 NELLA SEZIONE

Progetti con Ricercatore Italiano all'Estero

INDICE PROGETTI

CODICE
PE-2013-02354871

Destinario Istituzionale
RIZZOLI

TITOLO PROGETTO
Mesenchymal Stem Cells and photoactivable Nanoparticles: a novel Anticancer Phototherapy System for High grade Osteosarcoma Treatment (SNAPSHOT)

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Oncology 2 - Translational Clinical	Biomedica	Oncology	369.020,00

CODICE
PE-2013-02354961

Destinario Istituzionale
Istituto Superiore di Sanita'

TITOLO PROGETTO
Role of lipid rafts in the immune response to fungal infections.

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Immunology	Biomedica	Infectious and immunological diseases	285.200,00

CODICE
PE-2013-02355126

Destinario Istituzionale
Associazione Oasi Maria SS

TITOLO PROGETTO
Homer-mGlu5 scaffold as common abnormal mechanism and therapeutic target for Intellectual Disability (ID) and Autism Spectrum Disorders (ASD)

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Molecular, Cellular, and Developmental Neuroscience	Biomedica	Neurologic diseases	427.007,00

CODICE
PE-2013-02355206

Destinario Istituzionale
SAN RAFFAELE MILANO

TITOLO PROGETTO
Novel pathways in PNS myelination and remyelination

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Molecular, Cellular, and Developmental Neuroscience	Biomedica	Neurologic diseases	427.007,00

ABSTRACT DEI PROGETTI FINANZIATI NEL2015 CON IL BANDO RICERCA FINALIZZATA - FINANZIAMENTO 2013 NELLA SEZIONE

Progetti con Ricercatore Italiano all'Estero

INDICE PROGETTI

CODICE

PE-2013-02355271

Destinario Istituzionale

OSPEDALE BAMBINO GESU'

TITOLO PROGETTO

Targeting oncogenic epigenetic factors as an innovative anticancer strategy in Rhabdomyosarcoma

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Oncology 1 - Basic Translational	Biomedica	Oncology	381.362,00

CODICE

PE-2013-02355346

Destinario Istituzionale

Toscana

TITOLO PROGETTO

Hematopoietic stem cell transplantation for malignant forms of Multiple Sclerosis.

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	382.008,00

CODICE

PE-2013-02355372

Destinario Istituzionale

SANTA LUCIA

TITOLO PROGETTO

Brain Mechanisms of Chronic Subjective Dizziness

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Integrative, Functional, and Cognitive Neuroscience	Biomedica	Neurologic diseases	382.008,00

CODICE

PE-2013-02355484

Destinario Istituzionale

SAN RAFFAELE MILANO

TITOLO PROGETTO

Impact of carotid endarterectomy and stenting on hemodynamics, fluid-structure interaction, autonomic modulation, and cognitive brain function.

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Surgical Sciences, Biomedical Imaging, and Bioengineering	Clinico-assistenziale	Metabolic and cardiovascular diseases	341.507,00

ABSTRACT DEI PROGETTI FINANZIATI NEL2015 CON IL BANDO RICERCA FINALIZZATA - FINANZIAMENTO 2013 NELLA SEZIONE

Progetti con Ricercatore Italiano all'Estero

INDICE PROGETTI

CODICE
PE-2013-02355948

Destinario Istituzionale
AUXOLOGICO

TITOLO PROGETTO
High-end and Low-End Virtual Reality Systems for the Rehabilitation of Friality in the Elderly

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Emerging Technologies and Training in Neurosciences	Clinico-assistenziale	Neurologic diseases	335.545,00

CODICE
PE-2013-02356465

Destinario Istituzionale
Lombardia

TITOLO PROGETTO
Evaluation of the diagnostic accuracy of three memory tests for early Alzheimer's disease

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	296.508,00

CODICE
PE-2013-02356613

Destinario Istituzionale
GALEAZZI

TITOLO PROGETTO
Microfluidic organotypic model of monocyte transendothelial migration to the joint for the screening of promising therapeutic strategies in obese osteoarthritic patients

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Musculoskeletal, Oral and Skin Sciences	Biomedica	Innovative biotechnologies	382.008,00

CODICE
PE-2013-02356818

Destinario Istituzionale
HUMANITAS

TITOLO PROGETTO
Circulating microRNAs as biomarkers of chemotherapy-related cardiotoxicity and myocardial inflammation

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Cardiovascular and Respiratory Sciences	Biomedica	Metabolic and cardiovascular diseases	450.000,00

ABSTRACT DEI PROGETTI FINANZIATI NEL2015 CON IL BANDO RICERCA FINALIZZATA - FINANZIAMENTO 2013 NELLA SEZIONE

Progetti con Ricercatore Italiano all'Estero

INDICE PROGETTI

CODICE
PE-2013-02357094

Destinario Istituzionale
Lombardia

TITOLO PROGETTO
Etiology and prevention of type 1 diabetes

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Endocrinology, Metabolism, Nutrition and Reproductive Sciences	Clinico-assistenziale	Metabolic and cardiovascular diseases	318.258,00

CODICE
PE-2013-02357476

Destinario Istituzionale
MONZINO

TITOLO PROGETTO
Circulating cell-derived microvesicles in coronary artery disease: molecular signature, functional properties, and predictive value in coronary artery bypass graft patency

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Vascular and Hematology	Clinico-assistenziale	Metabolic and cardiovascular diseases	321.174,00

CODICE
PE-2013-02357669

Destinario Istituzionale
Toscana

TITOLO PROGETTO
The oncogenic potential of the AID/APOBECs: involvement in tissue transformation and oncogenesis - new tools to better model cancer

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Oncology 1 - Basic Translational	Biomedica	Oncology	382.008,00

CODICE
PE-2013-02357745

Destinario Istituzionale
REGINA ELENA

TITOLO PROGETTO
Coagulation/complement activation and cerebral hypoperfusion in relapsing-remitting multiple sclerosis

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Biomedica	Neurologic diseases	419.902,00

ABSTRACT DEI PROGETTI FINANZIATI NEL2015 CON IL BANDO RICERCA FINALIZZATA - FINANZIAMENTO 2013 NELLA SEZIONE

Progetti con Ricercatore Italiano all'Estero

INDICE PROGETTI

CODICE
PE-2013-02357826

Destinario Istituzionale
I.N.R.C.A.

TITOLO PROGETTO
Chronic Kidney Disease as a Dysmetabolic Determinant of Disability among Older People (CKD-3D)

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Population Sciences and Epidemiology	Clinico-assistenziale	Metabolic and cardiovascular diseases	356.508,00

CODICE
PE-2013-02357936

Destinario Istituzionale
SPALLANZANI

TITOLO PROGETTO
A registry of severe malaria in returning travellers from endemic countries: clinical outcome and adverse events including haematological and pharmacokinetic data. NetwOrk for severe MALaria treatment - NOMAL study

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Infectious Diseases and Microbiology	Clinico-assistenziale	Infectious and immunological diseases	322.015,00

CODICE
PE-2013-02357974

Destinario Istituzionale
Toscana

TITOLO PROGETTO
Virtual and Augmented Reality Support for Transcatheter Valve Implantation by usingCardiovascular MRI

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Surgical Sciences, Biomedical Imaging, and Bioengineering	Biomedica	Metabolic and cardiovascular diseases	427.700,00

CODICE
PE-2013-02357980

Destinario Istituzionale
NEUROLESI

TITOLO PROGETTO
Does intensive exercise induce plasticity-related changes in Parkinson's Disease?

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	232.705,00

ABSTRACT DEI PROGETTI FINANZIATI NEL2015 CON IL BANDO RICERCA FINALIZZATA - FINANZIAMENTO 2013 NELLA SEZIONE

Progetti con Ricercatore Italiano all'Estero

INDICE PROGETTI

CODICE
PE-2013-02358099

Destinario Istituzionale
PASCALE

TITOLO PROGETTO
Reduction of breast cancer recurrence in women: lifestyle strategies and microRNA expression

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Oncology 2 - Translational Clinical	Clinico-assistenziale	Oncology	375.208,00

CODICE
PE-2013-02358145

Destinario Istituzionale
MAUGERI

TITOLO PROGETTO
Clinical, structural and functional markers for recovery of consciousness

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	317.508,00

CODICE
PE-2013-02358393

Destinario Istituzionale
Istituto Superiore di Sanita'

TITOLO PROGETTO
PREDICTING THE FUTURE HEALTH CARE NEEDS OF CANCER SURVIVORS IN AGEING POPULATIONS (FORECARE)

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Population Sciences and Epidemiology	Clinico-assistenziale	Methodological, epidemiological, socio-economic, organizational, managerial emerging public health issues related to the above reported areas	187.495,00

CODICE
PE-2013-02358887

Destinario Istituzionale
SAN MATTEO

TITOLO PROGETTO
Pancreatic ductal adenocarcinoma (PDAC): development of a new communication platform between radiologists, surgeons and pathologists based on virtual and 3D printed reconstructions of the pancreas and the tumor mass.

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Surgical Sciences, Biomedical Imaging, and Bioengineering	Biomedica	Innovative biotechnologies	380.000,00

ABSTRACT DEI PROGETTI FINANZIATI NEL2015 CON IL BANDO RICERCA FINALIZZATA - FINANZIAMENTO 2013 NELLA SEZIONE

Progetti con Ricercatore Italiano all'Estero

INDICE PROGETTI

CODICE: **PE-2013-02359028**

Destinario Istituzionale: MAUGERI

TITOLO PROGETTO: **Sensory and autonomic markers in early diagnosis of parkinsonism. A new strategy to predict clinical evolution toward different neruodegenerative disorders.**

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	339.299,00

CODICE: **PE-2013-02359172**

Destinario Istituzionale: Piemonte

TITOLO PROGETTO: **Development and pre-clinical validation of a soft-tethered endoscopic robot to replace colonoscopy as a screening tool for colorectal cancer**

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Surgical Sciences, Biomedical Imaging, and Bioengineering	Biomedica	Innovative biotechnologies	274.534,00

CODICE: **PE-2013-02359287**

Destinario Istituzionale: CARLO BESTA

TITOLO PROGETTO: **Structural biomarkers of awareness and wakefulness in disorders of consciousness**

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	239.508,00

CODICE: **PE-2013-02359574**

Destinario Istituzionale: Toscana

TITOLO PROGETTO: **In vivo assessment of the role of Locus Coeruleus in the development of Alzheimer's Disease and other types of Dementia.**

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	337.008,00

SEGUONO ABSTRACT PROGETTI FINANZIATI

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Mesenchymal Stem Cells and photoactivable Nanoparticles: a novel Anticancer Phototherapy System for High grade Osteosarcoma Treatment (SNAPSHOT)
Project Code: PE-2013-02354871	Principal Investigator: DONATI DAVIDE MARIA
Research Type: Biomedical/Biomedica	Applicant Institution: Istituto Ortopedico Rizzoli
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Oncology	
Project Classification IRG:	Oncology 2 - Translational Clinical
Project Classification SS:	Developmental Therapeutics - DT
Project Keyword 1:	Evaluation of drug-delivery strategies (including nanoparticles, liposomes and other delivery vehicles) and gene therapy approaches involving non-immunologic targets for the treatment of cancer.
Project Keyword 2:	Mesenchymal Stem Cells (MSC)
Project Keyword 3:	Osteosarcoma (OS)
Project Request:	Animals: [X] Humans: [X] Clinical trial: [ ]
The project has already been presented: [ ]	Project code reference:
[ ] I declare that the object/s of this application is/are under patent copyright	

Italian Researcher Abroad – Foreign Operative Unit	
Name and Surname:	Mauro Ferrari
Foreign Institution:	Houston Methodist Research Institute
Department/Division/Laboratory:	Nanomedicine
City-State and Country:	Houston, Texas, USA
Years of Residence Abroad:	20
Email: mferrari@houston.methodist.org	Phone: +17134418439

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Ortopedico Rizzoli	Laboratorio di Patologia Ortopedica e Rigenerazione Tissutale Osteoarticolare	Project coordinator. Operative Unit
2	Houston Methodist Research Institute	Nanomedicine	Operative Unit
3	National Research Council (CNR)	Istituto per la Sintesi organica e la Fotoreattività	Operative Unit

Project Title:
Mesenchymal Stem Cells and photoactivable Nanoparticles: a novel Anticancer Phototherapy System for High grade Osteosarcoma Treatment (SNAPSHOT)

Project Code: PE-2013-02354871

Principal Investigator: DONATI DAVIDE MARIA

Research Type: Biomedical/Biomedica

Applicant Institution: Istituto Ortopedico Rizzoli

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	SOTGIU Giovanna	National Research Council (CNR)	Coordinator operative unit.Production of fotoactivable nanoparticles.	04/10/1970
2	Lucarelli Enrico	Istituto Ortopedico Rizzoli Dirigente Biologo	In vitro studies,development of animal model and in vivo efficacy of the strategy.	29/12/1962
3	Ferrari Mauro	Houston Methodist Research Institute President and CEO	Coordinator operative unit.Supervise the fotoactivable nanoparticles production.	05/07/1959

Background and Significance

Osteosarcoma (OS) is a malignant tumor most common in children and adolescents typically developing in the long bones, resulting in rapid spreading in the surrounding tissues and metastasis in the lung. Currently OS is treated with neo-adjuvant chemotherapy and surgery. Chemotherapy induces OS necrosis and tumor shrinking, allowing for defined margins and facilitating surgical resection. Only in 10% of patients necrosis rate is 100%, leaving 90% of the patients with malignant cells. Surgery should remove those cells, but if the margins are not adequate the remaining OS cells may metastasize. Currently there are no tools/treatments that enable the surgeon to identify surviving tumor cells. The successful management of OS would greatly benefit from novel techniques able to selectively kill tumor cells left in the patients. Given the tumor tropism of Mesenchymal Stem Cells (MSC) they may be used as "Trojan horses" to shuttle photoactivable agents to the tumor. Photodynamic therapy (PDT) is a minimally invasive procedure in which cells are destroyed by reactive oxygen species generated by excitation of photosensitizers via wavelength-specific light. In this project we propose the association of MSC and PDT to kill OS cells in vivo. This treatment if proved successful would enable: (i) surgeons to kill remaining OS cells after chemotherapy and during surgery decreasing the rate of recurrence/metastasis (ii) surgeons to be more conservative to improve patient's quality of life.

Specific aims

Aim 1: Innovative strategies are urgently needed for the effective treatment of cancer. Emerging techniques combining cell therapy and nanomedicine hold substantial promise in providing the development of tools to delineate tumor margins and reduce tumor recurrence. The objective of this study is to extend the in vitro proof of concept obtained by the PI group and refine the platform to test if photoactivation of MSC+NP is effective in vivo. This will allow to translate our therapeutic approach into the clinic.
AIM 1: Expand the repertoire of photoactivable-NPs in the near infrared (NIR) spectrum to allow sufficient laser light penetration compatible with OS location.

Aim 2: Tailor the biodegradability, biocompatibility, and physiochemical (size, shape, porosity and pore size) features of particles to control the dose of photosensitizer.

Aim 3: Demonstrate the efficacy of the therapeutic approach to achieve 50% tumor size reduction in orthotopic model of OS.

Hypothesis: Given that MSC loaded with photoactivable-NPs are able to kill OS cells in vitro and that gene engineered

Project Title:
Mesenchymal Stem Cells and photoactivable Nanoparticles: a novel Anticancer Phototherapy System for High grade Osteosarcoma Treatment (SNAPSHOT)

Project Code: PE-2013-02354871

Principal Investigator: DONATI DAVIDE MARIA

Research Type: Biomedical/Biomedica

Applicant Institution: Istituto Ortopedico Rizzoli

Project Type: PE- ITALIAN RESEARCHER ABROAD

MSC are currently used in clinical trials to decrease tumor volume in vivo, in this proposal we hypothesized that MSC can reach the tumor site and reduce tumor volume in vivo when photoactivated.

Preliminary data: The project takes advantage from a published proof of concept and from the experience in photoactivable compounds and micro-nano particles of the involved groups. The CNR unit demonstrated that fluorescent-PMMA-nanoparticles (FNPs) were able to efficiently load the photosensitizer porphyrin (TPPS) through electrostatic binding and to be stable inside the MSC for several days. The study validated that OS cells were eliminated in a rapid and highly controlled manner upon photoactivation of TPPS@FNP+MSC in co-culture assays. In addition, our preliminary data show that other NIR photostimulating agents (Aluminium Phtalocianine, PtI) can be easily electrostatically bound to FNP (PtI@FNP) and are able to induce 90% of both vehicle MSC and OS cells upon 680 nm excitation in vitro. Furthermore, in our PC3 xenograft tumor model, PtI@FNP+MSC are able to reduce tumor size upon photoactivation with 680 nm light. Doctor Ferrari's group has extensive knowledge on the fabrication, characterization, biodegradation, biocompatibility, and manipulation of physiochemical features of multistage Nanoporous Silicon Particles (NSP). Using these NSP, his group has shown successful loading of various therapeutic agents. For example, they demonstrated that upon loading NSP with gold NP and allowing internalization, MSC+NSP retained the ability to be excited with laser resulting in the selective death of MSC. In vivo results demonstrated that after internalization of NSP, MSC preserved the ability to target primary/metastatic breast cancer and successfully allowed NSP to avoid accumulation within the mononuclear phagocyte system.

Materials and Methods

NSP and FNP will be coated with PtI and then loaded into MSC, isolated and expanded by UNIT1, using a non-toxic concentration measured through cytotoxicity and flow cytometry assays. TIRF microscopy will allow to observe the kinetic of internalization of NSP and FNP. MSC ability to migrate and release sufficient toxic ROS to destroy OS tumor cells and MSC will be monitored by time-lapse confocal microscopy. Photoactivation will be performed with LED/LASER at 680 nm. OS cell line and MSC will be stably transfected with luciferase to enable bioluminescence imaging (BLI). OS bearing mice will be induced by bilateral intratibial injection of OS cells. Tumor growth will be monitored weekly by BLI. Different MSC dosage, time of administration, and PDT parameters will be tested. Photoactivation will be performed on the tumor area with LED/LASER. The corresponding reduction in tumor growth will be measured by BLI and histological analysis.

Impact and Translational Implications

The data obtained from the work proposed here will represent the preclinical basis to request an authorization for testing in humans. If found to be effective, the translation of our strategy can be implemented into clinical trials at the IOR, the world's leading institution for treatment of OS. Furthermore as MSC display tumor tropism toward several types of cancer and inflammation in general, this approach may be useful for the treatment of various cancers and other inflammatory disorders.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Role of lipid rafts in the immune response to fungal infections.
Project Code: PE-2013-02354961	Principal Investigator: Gagliardi Maria Cristina
Research Type: Biomedical/Biomedica	Applicant Institution: Istituto Superiore di Sanita'

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Infectious and immunological diseases
Project Classification IRG: Immunology
Project Classification SS: Immunity and Host Defense - IHD
Project Keyword 1: host-microbe interactions: innate and acquired host immune responses to specific pathogenic organisms including viruses, bacteria, fungi and parasites; host responses to commensal microbes; influence of host factors, including genetic predisposition or resistance to infection.
Project Keyword 2: fungal infection
Project Keyword 3: lipid rafts
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Federica Sallusto
Foreign Institution: Institute for Research in Biomedicine
Department/Division/Laboratory: Cellular Immunology Laboratory
City-State and Country: Bellinzona, Switzerland
Years of Residence Abroad: 18
Email: federica.sallusto@irb.usi.ch Phone: 0041-91-8200315

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Superiore di Sanita'	Dipartimento Malattie Infettive, Parassitarie e Immunomediate	PI. Supervision and coordination of all the research activities of the project
2	Institute for Research in Biomedicine	Cellular Immunology Laboratory	Coordination and supervision of immunological investigations on adaptive T cell response
3	Istituto Italiano di Tecnologia	Center for Life Nanoscience,IIT@Sapienza	Coordination and supervision of confocal and time lapse fluorescence microscopy studies

Project Title:
Role of lipid rafts in the immune response to fungal infections.

Project Code: PE-2013-02354961

Principal Investigator: Gagliardi Maria Cristina

Research Type: Biomedical/Biomedica

Applicant Institution: Istituto Superiore di Sanita'

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	de Turris Valeria	Istituto Italiano di Tecnologia	Coordinator of UO3. Supervision of confocal and time lapse fluorescence microscopy studies	18/03/1977
2	Latorre Daniela	Institute for Research in Biomedicine, post-doc	functional studies on T cells	02/12/1984
3	teloni raffaela	Istituto Superiore di Sanità, tecnician	functional studies on phagocytes	22/06/1972

Background and Significance

Recurrent and invasive fungal infections remain a significant health problem: disseminated candidiasis and invasive pulmonary aspergillosis can result in 30-80% mortality in spite of treatments and are 100% fatal if not diagnosed correctly. Our team has previously shown that human monocytes treated with ß-glucan of Candida albicans cell wall differentiated into mature dendritic cells (DCs) with altered phenotype and functional behavior (Nisini et al, J Leuk Biol 2007) and that ß-glucan induces production of prostaglandin E2 by DCs that in turn enhances Th17 polarization by increasing IL-23 production (Gagliardi et al, J Leuk Biol 2010). We have also shown that memory T cells from healthy donors produce large amounts of IL-17 (Acosta-Rodriguez et al, Nat Immunol 2007), suggesting that polarized Th17 cell responses play a protective role in vivo. These findings have been subsequently corroborated by the observation that patients with defects in the Th17 axis due to mutations in STAT3, IL17F, or IL17RA suffer from recurrent C. albicans infections (Puel et al, Curr Opin Immunol 2010). Understanding how C. albicans-host cell interactions regulate innate and adaptive immunity is therefore of both fundamental and clinical relevance for the development of novel and more effective treatments against Candida albicans and other fungal infections.

Specific aims

Aim 1: Based on preliminary data we are proposing in this project:
To investigate the role of lipid rafts in fungi (Candida albicans and Aspergillus fumigatus) recognition and cytokine/signaling response by human phagocytes.

Aim 2: To define the impact of lipid raft-mediated fungal recognition in the induction of antifungal specific T cell responses.

Aim 3: To evaluate in vitro the effects of polyene antibiotics on lipid raft functions in antifungal innate and adaptive immune response

Hypothesis: Lipid rafts correspond to membrane areas stabilized by the presence of cholesterol within a liquid-ordered phase and have been suggested to be involved in a great variety of cellular functions and biological events. By recruiting receptors and molecules important for the formation of the immune synapse in T or B cells and for pathogen immune recogniton by phagocytes lipid rafts constitute a signal transduction platform for activation of both adaptive and innate immune responses. The role of lipid rafts in fungal infections has been poorly investigated. Recently it has been shown that the C-type lectin receptor Dectin-1, which recognizes fungal ß-glucan, localizes into lipid rafts upon ligand binding (Shengli et al, J. Biol. Chem 2009). Other receptors involved in pathogen recognition, such as CR3, TLR2, and CD14 have been shown to translocate into lipid rafts upon binding by specific agonists.

Project Title:
Role of lipid rafts in the immune response to fungal infections.

Project Code: PE-2013-02354961

Principal Investigator: Gagliardi Maria Cristina

Research Type: Biomedical/Biomedica

Applicant Institution: Istituto Superiore di Sanita'

Project Type: PE- ITALIAN RESEARCHER ABROAD

Our working hypothesis is that C. albicans, as well as other fungi such as Aspergillus fumigatus, engages specific pathogen recognition receptors (PRRs) into lipid rafts of human phagocytes to orchestrate innate immune response. We also hypothesize that lipid rafts on professional antigen presenting cells, such as monocytes and DCs, participate to the induction of specific antifungal T cell response, influencing antigen presentation and T cell polarization. In particular, we hypothesized that lipid rafts have a crucial role in antifungal
Th17 immune response triggered via dectin-1.

Preliminary data: Our preliminary data by time lapse fluorescence microscopy show that the dynamic raft microdomains are involved in C.albicans phagocytosis by human monocytes. We also found that ß-methyl cyclodextrin, a drug that disrupts lipid raft architecture by extracting cholesterol from cell membrane and the cholesterol binding amphotericin B, one of the most effective antifugal drugs, strongly inhibited fungus uptake. Both drugs also impair the uptake of ß-glucan from C. albicans by monocytes, suggesting a role of lipid rafts in Dectin-1-mediated phagocytosis of C. albicans. Lastly both drugs inhibit T cell proliferation in response to C. albicans but not in response to mitogenic stimuli, suggesting a defective antigen presentation.

Materials and Methods

For phagocytosis studies human monocytes, DCs and neutrophils will be incubated with FITC- labeled C. albicans and A. fumigatus in the presence or absence of lipid raft disrupting agents and analyzed by flow cytometry and confocal microscopy both in fixed and live cells. Fraction of cellular membranes enriched in lipid rafts will be obtained by preparation of detergent resistant membranes by sucrose density gradient centrifugation: analysis of the content of signaling molecules and PRRs and in this fraction will be determined by immunoblotting.
Cytokine production by human phagocytes and T cells will be measured by Multiplex Assay. ROS production in neutrophils will be detected using CellROX reagents fluorogenic probes to measure oxidative stress by flow cytometry or Fluoroskan
The analysis of the specific T cell responses will be performed by an antigen specific T cell priming approach using naive T cells, APCs and whole microbes as recently described (Zielinsky et al, Nature 2012).

Impact and Translational Implications

The increase in the incidence of life-threatening fungal infections and in drug resistance have made these infections a major therapeutic challenge. Dissection of the mechanisms by which fungi exploit lipid rafts could provide new therapeutic insights for prevention and/or treatment. In particular the study of the effects of cholesterol-binding polyene antibiotics, such as amphotericin B, on lipid raft functions could reveal new mode of action for the immunomodulatory effects of this drug.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Homer-mGlu5 scaffold as common abnormal mechanism and therapeutic target for Intellectual Disability (ID) and Autism Spectrum Disorders (ASD)
Project Code: PE-2013-02355126	Principal Investigator: Catania Maria Vincenza
Research Type: Biomedical/Biomedica	Applicant Institution: Associazione Oasi Maria SS

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases
Project Classification IRG: Molecular, Cellular, and Developmental Neuroscience
Project Classification SS: Molecular Neuropharmacology and Signaling - MNPS
Project Keyword 1: Pharmacological and neurochemical studies of receptor activation, G-protein coupling and signal transduction cascades of G-protein coupled receptors; studies of receptor agonists and antagonists; studies of receptor modulation by interacting proteins.
Project Keyword 2: neurodevelopmental disorders, Fragile X, Angelman , Tuberous sclerosis comples
Project Keyword 3: mGlu5, Homer
Project Request: Animals: [X] Humans: [ ] Clinical trial: [ ]
The project has already been presented: [ ] Project code reference:
[ ] I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Eleonora Aronica
Foreign Institution: Academisch Medisch Centrum
Department/Division/Laboratory: Dep. (Neuro) Pathology
City-State and Country: Amsterdam The Netherland
Years of Residence Abroad: 23
Email: e.aronica@amc.uva.nl Phone: 31-20-5662943

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Associazione Oasi Maria SS	Neurobiology	DI - evaluation of mechanisms underlying abnormal mGlu5-Homer scaffold in Fragile X mice (Fmr1 -/-)
2	Academisch Medisch Centrum	Neuropathology	expression of mGlu5 and Homer isoforms in human tissue from patients affected by different ASDs.
3	Neuromed	Neuropharmacology	evaluation of mechanisms underlying abnormal mGlu5-Homer scaffold in Angelman mice (Ube3a +/-)

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Homer-mGlu5 scaffold as common abnormal mechanism and therapeutic target for Intellectual Disability (ID) and Autism Spectrum Disorders (ASD)
Project Code: PE-2013-02355126	Principal Investigator: Catania Maria Vincenza
Research Type: Biomedical/Biomedica	Applicant Institution: Associazione Oasi Maria SS

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Nicoletti Ferdinando	Neuromed	coordinator of unit 3 - experiments on Angelman models	12/09/1959
2	Bonaccorso Carmela Maria Giovanna	Associazione Oasi Maria SS	experiments on FXS mice	19/10/1971

Background and Significance

Intellectual disability (ID) and autism spectrum disorder (ASD) are the most common developmental disorders and are often combined in the same individual. Many genetic syndromes exhibit ID and autism at higher frequency than in the general population, and account for more than 10% of all ASD cases. Among these, tuberous sclerosis complex (TSC), fragile X (FXS), and Angelman (AS) syndromes are frequent monogenic causes of autism and ID. Gene discovery-based studies on animal models laid the groundwork for the understanding the pathophysiology of syndromic forms of ID/ASD. From these studies the concept that the dysregulation of converging signalling pathways may be common to different forms of ID/ASD has recently emerged, suggesting that drugs targeting similar pathways might be used for different form of ID/ASD. In this context, much work has focused on dysregulation of mRNA translation at synapses as common pathogenetic causes in syndromic forms of ID and autism, including FXS, TSC and AS. We have found that the interaction between metabotropic glutamate receptor subtype 5 (mGlu5) and its scaffolding protein Homer is abnormal in mouse models of FXS and AS, with ensuing receptor dysfunction and phenotypes independent of signalling to protein synthesis. Rescue of mGlu5/Homer interaction has been found to correct some but not all phenotypic features in the FXS mouse model. Mechanistic studies suggest that ERK and CDK signalling are crucial for mGlu5/Homer interactions.

Specific aims

Aim 1: To identify the neurotransmitters/receptors and activated pathways involved in modulation of mGlu5/Homer interaction. We will first consider neurotransmitters known to activate proline-directed kinases, such as ERK and CKD5, which play a crucial role in mGlu5/Homer interaction (group-I metabotropic glutamate, dopamine D1 and D2, 5HT1A/5HT7, muscarinic and beta-adrenergic receptors). Modulation of mGlu5/Homer interaction by trophic factor will also be considered.

Aim 2: To decipher mechanisms underlying the alteration of mGlu5/Homer in Fragile X and Angelman and evaluate mGlu5/Homer interaction in TSC mouse models. We will consider phosphorylation of mGlu5 receptors and expression of multidomain scaffolding proteins and its interaction with mGlu5 and Homer in the different models under basal and stimulated conditions. We will also examine the consequences of mGlu5/Homer altered scaffolds focusing on the modulation of NMDA receptor function, receptor localization at synapses and dendritic spine morphology, and tentatively correct mGlu5/Homer dependent phenotypes by modulation of identified pathways as in aim 1.

Aim 3: To study the expression and cellular distribution of mGlu5 and Homer isoforms in human tissue from neurodevelopmental disorders including FXS, AS, TSC, and others i.e. Down syndrome, and validate the hypothesis of an alteration of mGlu5/Homer interaction in human tissue.

Hypothesis: We hypothesise that an abnormal mGlu5/Homer scaffold is common to different neurodevelopmental disorders and that the modulation of mGlu5/Homer interaction is a possible therapeutic target in different developmental disorders associated with ID and autism.

Preliminary data:

We have previously reported that mGlu5/Homer interaction is reduced in FXS mouse model (Giuffrida et al., J Neurosci. 2005). We have then studied several properties and functions of mGlu5 receptors in FXS mice, including regional expression during development, surface expression and axonal/dendritic targeting, homologous desensitization, surface dynamics and mGlu5 receptor-mediated modulation of NMDAR function in the Fmr1 KO mouse. We have found that mGlu5 receptors are dysregulated in the absence of FMRP in a complex manner, which includes functional consequences of mGlu5/Homer disruption. We have also evidence that surface dynamics of mGlu5 receptors and mGlu5 modulation of NMDA function and plasticity are altered as a consequence of mGlu5/Homer disruption in the FXS model. Recently we have found that in a mouse model of AS Homer/mGlu5 receptor interaction is increased (Pignatelli et al., J Neurosci 2014). We have also evidence for a strong expression of mGlu5 in dysmorphic neurons within cortical tubers of TSC patients, pointing to the role of this receptor in epileptogenesis, and in TSC-related comorbidities. However, information regarding the expression and cellular distribution of Homer isoforms and mGlu5/Homer interaction, and the correlation with clinical features are still lacking.

Materials and Methods

mGlu5/Homer interaction will be studied by immuno-precipitation (IP) in brain regions from Fmr1-/-, TSC1+/- and TSC2+/-, and Ube3a +/- mice before/after pharmacological treatment with agonists, such as. glutamate, DHPG, SKF81297, 5-HT, 8-OHDPAT (5HT1A/7 receptors), isoproterenol, carbachol. After an initial screening with mixed agonists, specific antagonists will be used to identify the neurotransmitter system and receptors involved. Expression of proteins and phosphorylation of receptors will be studied by Western blotting. Immunohistochemistry, Western blotting and IP will be used in human tissue. NMDA receptor mediated excitatory post-synaptic currents will be recorded using patch-clamp techniques. Synaptic co-localization of receptors/proteins will be studied by multiple labelling immunocytochemistry and confocal microscopy. Dendritic spine morphology will be studied in brain slices by software-assisted analysis of confocal images of neurons labelled with the lipophilic tracer DiI.

Impact and Translational Implications

Epidemiological evidence suggests a substantial increase in the prevalence of both ID and ASD in the last decade with important social and economic consequences for affected families. Unravelling the molecular pathways common to different forms of ID/ASD will be extremely important for the treatment of ID/ASD because drugs targeting similar pathways might be used for different disorders, most of which are singularly rare and, therefore, do not attract pharmaceutical research.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Novel pathways in PNS myelination and remyelination
Project Code: PE-2013-02355206	Principal Investigator: Taveggia Carla
Research Type: Biomedical/Biomedica	Applicant Institution: Ospedale San Raffaele - Milano

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in
Neurologic diseases

Project Classification IRG: Molecular, Cellular, and Developmental Neuroscience

Project Classification SS: Cellular and Molecular Biology of Glia - CMBG

Project Keyword 1: Inductive signals for the initiation, synthesis, regulation, maintenance, and degradation of myelin; mechanisms involved in demyelinating and dysmyelinating diseases and remyelination processes.

Project Keyword 2: Myelin

Project Keyword 3: Schwann cells

Project Request: Animals: [X] Humans: [ ] Clinical trial: [ ]

The project has already been presented: [X] Project code reference: RF-2011-02348486

[X] I declare that the object/s of this application is/are under patent copyright

Patent owner: Carla Taveggia and Amelia Trimarco Patent number: PCT/EP2014/063995

Italian Researcher Abroad – Foreign Operative Unit

Name and Surname: Maria Laura Feltri

Foreign Institution: Hunter James Kelly Research Institute and University at Buffalo

Department/Division/Laboratory: Department of Biochemistry

City-State and Country: Buffalo, New York, US

Years of Residence Abroad: 3

Email: mlfeltri@buffalo.edu Phone: 001 (716) 881-8969

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Ospedale San Raffaele - Milano	Division of Neuroscience/Axo-Glial Interaction Unit	PI
2	Hunter James Kelly Research Institute and University at Buffalo	Department of Biochemistry	Collaborator
3	Ospedale San Raffaele	Division of Immunology, Transplantation, and Infectious Diseases/Biocrystallography Unit	Collaborator

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Degano Massimo	Ospedale San Raffaele	Collaborator	02/04/1968

Background and Significance

Myelin derives from the spiral wrapping of glial cells and is essential for efficient and rapid propagation of action potentials along neurites. Communication between glial cells and neurons is crucial for nerve development, yet their reciprocal interactions have remained elusive. In the absence of glial cells neurons die; similarly, neurons promote proliferation, survival of glial cells and formation of myelin.
Neuregulin 1 (NRG1) type III is a key molecule controlling all aspects of PNS myelination. We now show that NRG1 operates as a bidirectional molecule, as it is cleaved also by gamma-secretase. The generated intracellular fragment activates the expression of the L-PGDS gene that produces the PGD2 prostanoid, a ligand of the G protein-coupled receptor GPR44. In the nervous system PGD2 modifies calcium, cAMP and phosphoinositol concentrations. Treatment with 15d-PGJ2, the final non-enzymatic derivative of PGD2, increases neuronal survival and reduces demyelination in a model of spinal cord injury.
These studies aim at developing therapies for demyelinating disorders in which disability is correlated to myelin and axonal loss. To facilitate the design of novel therapeutical approaches, we will perform biochemical and structural studies to elucidate the structure-function relationship in GPR44. The crystal structure of GPR44 will allow the design of molecules endowed with agonist or antagonist activity that may lead to new therapeutic treatments for demyelinating diseases.

Specific aims

Aim 1: Modulation of L-PGDS activity in vitro
To confirm the role of L-PGDS and GPR44 and develop a suitable model to modulate their activity, we will test commercially available agonists and antagonists for L-PGDS and GPR44 in vitro in a Schwann cell (SC) myelinating coculture system. These studies will i) confirm the role of L-PGDS and GPR44 in myelin formation and ii) constitute the basis for pharmacological treatment.

Aim 2: Structural studies on GPR44 binding to agonist and antagonist ligands
To understand the properties of GPR44, we will crystallize and determine its structure in presence of natural ligands, or synthetic agonists or antagonists. We will also perform virtual screening of compound libraries using a homology model of GPR44 as the receptor in the docking procedure. These studies are instrumental to identify and design novel compounds to treat demyelinating neuropathies.

Aim 3: Determine the role of L-PGDS and GPR44 in remyelination
To assess if L-PGDS and GPR44 are important in PNS regeneration, we will analyze remyelination and regeneration in L-PGDS null and GPR44 null crushed sciatic nerves. These studies will clarify the role of this pathway i) after injury and ii) if it is important to prolong neuronal function and survival.

Hypothesis: The interaction between SC and axons is essential in nervous system development. We previously showed that NRG1 forward signaling is key for myelination. We now show that NRG1 activates the L-PGDS-GPR44 pathway, modulating PNS myelination via a backward mechanism. Modulation of this novel pharmacologically accessible pathway could enhance PNS myelination in demyelinating disorders.

Preliminary data: We previously showed that NRG1 activity is modulated by competitive extracellular cleavage between BACE1 and TACE. NRG1 is also processed by gamma-secretases (Fig.1). We recently

found that the latter cleavage generates a fragment, NRG1 ICD that translocates into the nucleus and upregulates the expression of the prostaglandin D2 synthase (L-PGDS) (Fig.2). L-PGDS converts prostaglandin H2 (PGH2) to prostaglandin D2 (PGD2). By HPLC MS/MS analyses we found PGD2 in conditioned media of DRG neurons expressing NRG1 ICD, suggesting that L-PGDS is enzymatically active (Fig.3). Further, sciatic nerves of L-PGDS null mice are hypomyelinated, confirming that L-PGDS activity modulates myelination (Fig.4). We also found that PGD2 activates the G protein coupled receptor GPR44 on SC. Accordingly, glial specific knock down of GPR44 impairs in vitro myelination and GPR44 null mice are hypomyelinated (Fig.5). Finally, L-PGDS expression is upregulated after injury (Fig.6).
We recently established a production pipeline for G protein-coupled receptors (GPCRs) in insect cells using the baculovirus technology and obtained milligram quantities of the sphingosine 1-phosphate receptors for structural studies. We will apply the same methodology to GPR44. Meanwhile, we already produced a homology model of GPR44 based on the adenosine receptor crystal structure (Fig 7).

Materials and Methods

Tissue Culture: Primary rat SC, DRG neurons and myelinating SC-neuronal coculture will be established and analyzed as described (Taveggia et al., 2005). We will determine L-PGDS and GPR44 modulators toxicity on rat SC and DRG neurons (Tunel and BrDU assays).
Nerve injury: Mouse sciatic nerves will be exposed and crushed distal to the sciatic notch for 30 seconds. 10, 21 and 45 days post injury mice will be sacrificed and the extent of remyelination/regeneration determined by morphological analyses 3 and 10 mm distal to the lesion site. Contralateral uncrushed nerves will serve as control.
Crystal structure: We will express recombinant GPR44 in baculovirus-infected insect cells. Constructs retaining binding activity but more amenable for purification and crystallization (fused to T4 lysozyme or cytochrome b562) will also be produced. Proteins will be purified by affinity chromatography and proceed to the crystallization and structure determination by X-ray diffraction.

Impact and Translational Implications

These studies are part of a broad effort to understand the mechanisms regulating PNS myelination and remyelination. Our data combined with the results of this grant application will provide proof of principle that modulation of L-PGDS and GPR44 is a valid approach for the treatment of demyelinating diseases. Modeling GPR44 activity will facilitate the designing of novel compounds that could be tested in our in vitro and in vivo models, facilitating the translation from research to clinics.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Targeting oncogenic epigenetic factors as an innovative anticancer strategy in Rhabdomyosarcoma
Project Code: PE-2013-02355271	Principal Investigator: Rota Rossella
Research Type: Biomedical/Biomedica	Applicant Institution: Ospedale pediatrico Bambino Gesù

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Oncology
Project Classification IRG: Oncology 1 - Basic Translational
Project Classification SS: Cancer Molecular Pathobiology - CAMP
Project Keyword 1: Gene regulation including chromatin structure and remodeling, transcription, RNA processing and stability, and translation relevant to oncogenesis
Project Keyword 2: EZH2
Project Keyword 3: epigenetic-targeted therapy
Project Request: Animals: [X] Humans: [X] Clinical trial: [ ]
The project has already been presented: [ ] Project code reference:
[ ] I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Ernesto Guccione
Foreign Institution: Institute of Molecular and Cell Biology (IMCB)
Department/Division/Laboratory: Discovery Research Division/Cancer Genetics and Therapeutics/Methyltransferases in Development and Disease Lab
City-State and Country: Singapore, Singapore
Years of Residence Abroad: 6
Email: eguccione@imcb.a-star.edu.sg Phone: +65 65869844

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Ospedale pediatrico Bambino Gesù	Oncoematologia/Laboratorio di Angiogenesi	PI (Coordinator of Operative Unit 1 (OU1)): Development and validation of epigenetic approaches
2	Institute of Molecular and Cell Biology (IMCB)	Discovery Research Division/Cancer Genetics and Therapeutics/Methyltransferases in Development and Disease Lab	Foreign Operative Unit (OU2): support on the validation and interpretation of results
3	Sapienza Università di Roma	Chimica e Tecnologia del Farmaco/Chimica Farmaceutica	Operative Unit 3 (OU3): development of new epi-inhibitory molecules

Project Title:
Targeting oncogenic epigenetic factors as an innovative anticancer strategy in Rhabdomyosarcoma

Project Code: PE-2013-02355271

Principal Investigator: Rota Rossella

Research Type: Biomedical/Biomedica

Applicant Institution: Ospedale pediatrico Bambino Gesù

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Mai Antonello	Sapienza Università di Roma	Coordinator of Operative Unit 3 (OU3)	14/11/1962

Background and Significance

Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma in children. The alveolar RMS (ARMS) subtype often expresses the PAX3-FOXO1 (P3F) fusion protein, which functions as an oncogenic transcriptional activator, while the embryonal RMS (ERMS) is fusion-negative.
For metastatic and P3F ARMS, survival rates are still around 20%. Therefore high risk RMS patients would benefit from novel targeted therapeutics. RMS cells resemble developing skeletal muscle perturbed in their ability to terminally differentiate.
Chromatin regulators control myogenesis but little is known about their role in RMS.
Some histone modifiers, such as EZH2, a core subunit of the Polycomb Repressor 2 (PRC2) that represses differentiation by trimethylating histone H3 on Lys27, have been shown by us and others implicated in RMS pathogenesis.
Due to the key role of chromatin remodeling in myogenesis, it is arguable that many more chromatin factors could participate in RMS, but have yet to be identified. Recently, the inhibition of chromatin-associated BET proteins (BRD2-4 and BRDT) has been shown to block MYC and MYCN oncogenic functions in some pediatric cancers. These oncogenes are deregulated in high risk RMS. Therefore, BET proteins inhibition could have an anti-tumor value in RMS, as also testified by our preliminary results.
Thus, investigation on epigenetic regulators appears an appealing strategy to identify useful markers of poorer progression-free survival and novel drug targets in RMS.

Specific aims

Aim 1: To dissect both EZH2- and BET proteins-dependent tumorigenic pathways in RMS

Aim 2: To identify histone modifiers and bromodomains-containing factors selectively required for RMS cell survival and proliferation

Aim 3: To develop novel epi-inhibitors targeting the identified epigenetic tumor drivers in RMS

Hypothesis: We believe that dissecting pathways dependent on EZH2 and on BET proteins, will unveil nodal points involved in RMS. Since epigenetic pathways are interconnected and a little number of epigenetic regulators have been evaluated in RMS, we also propose loss-of-function (LOF) genetic screens using pooled shRNA libraries and, in parallel, siRNA pools, to discover novel oncogenic epigenetic regulators in RMS, as successfully reported in other cancers.
Since some histone modifiers are, or modulate, P3F target genes, their inhibition could be exploited to hamper the P3F functions needed for ARMS cell survival. Because chromatin remodelers are crucial for stem cell maintenance, inhibitory strategies can be useful also to target the cancer stem cell compartment specific of the ERMS subtype.
The parallel validation in the RMS setting of epigenetic inhibitors already in clinical trials for other tumors, could provide compelling evidence to accelerate the progression to clinical trials for high risk RMS. The design and development of new inhibitors interfering with the oncogenic epi-interactions, will result in epi-drugs potentially useful for combinatorial/adjuvant therapeutic strategies.

Preliminary data: We have recently demonstrated that genetic or pharmacological inhibition of EZH2 using a catalytic

inhibitor from Mai lab (OU3) hampers RMS cell survival in vitro and in vivo (Ciarapica et al 2013; Ciarapica et al, 2014). Here we report that BRD2-4 and MED1 are overexpressed in RMS. A P3F ARMS and an ERMS cell line showed cell cycle arrest and apoptosis when treated with JQ1, an inhibitor of BET proteins. Both cell lines up-regulated p21Cip1 and down-regulated MYC, MYCN and EZH2. Therefore, EZH2 and BET proteins seem to be required for RMS cell survival and their potential crosstalk could be exploited in combinatorial approaches.
The Guccione lab (UO2) identified histone methyltransferases that bypass Ras-induced senescence (Phalke et al, 2012; Bezzi et al, 2013 and unpublished results). They are currently performing LOF pooled screens using a pCW-Cas9 Puro Dox-inducible vector in combination with a specific pLX-sgRNABlast vector to target over 150 epigenetic regulators. They also are screening in 384 well format, in collaboration with an IMCB facility (Chia et al, 2010), a custom library of epigenetic modifiers on a variety of cancer lines.
The Mai lab (OU3) identified some compounds able to selectively inhibit EZH2 (Valente et al, Biochimie 2012), validated in RMS (see the above references). The new MC2055 compound displayed increased potency against EZH2 and was more effective than GSK126 against medulloblastoma stem cells (manuscript in preparation).

Materials and Methods

Different EZH2 and BRDs commercially available inhibitors will be used on several RMS cell lines. Ablation and overexpression of EZH2 and components of PRC2, and each other BRD proteins will be also done.
We will use in vitro an already published inducible-shRNA library targeting histone modifiers and bromodomain-containing proteins. A constitutive shRNA library and a 384 well siRNA pool screen will be used in parallel. Deep sequencing of shRNA guide strands will be used to identify depleted genes. Selected epigenetic genes will be validated in independent cell lines and vector systems.
Genome-wide transcriptional profiling, RNAseq and ChIPseq will be done on cells specifically silenced for the selected genes. They will be investigated in RMS xenograft models, patient-derived xenografts and RMS samples.
Novel more potent and selective inhibitors against EZH2 as well as the identified oncogenic epigenetic molecules will be designed using molecular modelling studies and produced.

Impact and Translational Implications

The identification of new diagnostic markers and therapeutic targets in RMS, paralleled by the evaluation of existing cancer epi-inhibitors in phase I-II clinical trials and the development of novel epi-drugs for combinatorial approaches, will be useful to improve drug response reducing long-term morbidity. Results from this proposal will shed light on epigenetic modifications in RMS and will provide a proof-of-concept to accelerate the progression to clinical trials for high risk RMS.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Hematopoietic stem cell transplantation for malignant forms of Multiple Sclerosis.
Project Code: PE-2013-02355346	Principal Investigator: riccardo saccardi
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases	
Project Classification IRG:	Brain Disorders and Clinical Neuroscience
Project Classification SS:	Clinical Neuroimmunology and Brain Tumors - CNBT
Project Keyword 1:	The relevant diseases are multiple sclerosis, myasthenia gravis, inflammatory neuropathies and myopathies, infectious diseases of the nervous system, prion disease and nervous system tumors.
Project Keyword 2:	hematopoietic stem cell transplantation
Project Keyword 3:	immunomodulation
Project Request: Animals: [ ]	Humans: [X] Clinical trial: [X]
The project has already been presented: [X]	Project code reference: PE-2011-02352266
[ ] I declare that the object/s of this application is/are under patent copyright	

Italian Researcher Abroad – Foreign Operative Unit	
Name and Surname:	Paolo Muraro
Foreign Institution:	Imperial College London
Department/Division/Laboratory:	Department of Medicine, division of Experimental Medicine, Centre for Neurosciences
City-State and Country:	London-England
Years of Residence Abroad:	12
Email: p.muraro@imperial.ac.uk	Phone: +44(0)2075946670

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Toscana	SODc Ematologia, SOD Banca del Cordone Ombelicale, AOU Careggi, Firenze	Study coordination, performing transplants, biological sample collection and storage, managing of clinical database
2	Imperial College London	Department of Medicine, Division of Experimental Medicine, Centre for Neurosciences	Neuroimmunological study coordination, data analysis
3	IRCCS, Azienda Ospedaliera Universitaria San Martino, IST	Dipartimento di Neuroscienze, riabilitazione, oftalmologia, genetica e scienze materno-infantili, Università di Genova	Performing transplant and MRI analysis, biological sample collection

Project Title:
Hematopoietic stem cell transplantation for malignant forms of Multiple Sclerosis.

Project Code: PE-2013-02355346

Principal Investigator: riccardo saccardi

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	mancardi giovanni	IRCCS, Azienda Ospedaliera Universitaria San Martino, IST	Neurological study design and monitoring, MRI design and monitoring	15/12/1947

Background and Significance

Hematopoietic Stem Cell Transplantation (HSCT) has been tried in the last 15 years as a therapeutic option in patients with a poor prognosis autoimmune diseases not responding to conventional treatments. To date about 1000 multiple sclerosis (MS) patients have been treated with HSCT worldwide. Malignant forms of MS are uncommon (8-12% of MS cases), characterized by an unfavorable clinical evolution (walking aid needed within 5 years from onset) and by a high number and volume of inflammatory brain lesions. It is expected that these forms display an enhanced pathogenic immune activity, thus providing a window of opportunity to collect useful information about immunopathology of MS. In recent studies, the neurological outcome of HSCT has been reported to be especially favorable in patients transplanted in the Relapsing Remitting (RR) phase and particularly in malignant forms. Most importantly, immunological mechanisms of HSCT have been extensively studied in either low or high intensity immunosuppressive conditioning regimens. Intermediate intensity conditioning regimens, such as BEAM/ATG, are poorly investigated, despite they are the most frequently used in Europe. Therefore, a study that focuses on HSCT using BEAM/ATG would provide solid clinical data on this procedure and offer a therapeutic option to these patients. Clinical, MRI and immunological data would be prospectively collected and analysed to elucidate the treatment effect and mode of action in such set of patients.

Specific aims

Aim 1: To establish clinical efficacy of autologous HSCT using an intermediate intensity conditioning regimen (BEAM/ATG), in malignant forms of MS by an accepted set of clinical parameters, such as time to failure, defined as:
1) The occurrence of a relapse
2) The occurrence of sustained EDSS worsening
The occurrence of sustained EDSS improvement will also be monitored

Aim 2: To elucidate the immunological mechanisms underlying the durable remission of inflammatory disease activity after autologous HSCT (AHSCT) with BEAM/ATG through:
1) Characterizing the proliferation, cyto- and chemokine secretion and cytotoxic activity of T cells in response to myelin and EBV proteins before and after AHSCT
2) Analyzing the immunological reconstitution through monitoring of relevant pro-inflammatory and regulatory lymphocytic subsets and recovery of immunocompetence towards de novo and recall antigens
3) Analyzing the bone marrow microenvironment before and after transplantation, focusing on the characteristics of Mesenchymal Stromal Cells (MSC)
4) To create a comprehensive repository of biosamples for prospectively planned and future mechanistic studies

Aim 3: To establish the impact of AHSCT in malignant forms of MS with regards to a conventional and advanced set of MRI parameters such as:
1. New or enlarging T2 lesions, gadolinium enhancing lesions to evaluate the impact of HSCT on inflammation
2. Whole brain volume changes, cortical and deep gray matter volume changes, white matter volume changes,

changes in cortical thickness to evaluate the impact of HSCT on neurodegeneration
3. Changes in the number of cortical lesions as detected by double inversion recovery and phase sensitive inversion recovery
4. Changes in diffusion tensor imaging (DTI) derived metrics (mean diffusivity, fractional anisotropy, radial diffusivity and axial diffusivity) within T2 visible lesions and in the normal appearing white matter to evaluate tissue repair in response to HSCT

Hypothesis: HSCT is able to restore an immunocompetent but non-inflammatory immune system in patients diagnosed with malignant form of MS, resulting in a prolonged time free of clinical and MRI signs of flare and free of any immunosuppressive therapy.
The disability of patients with malignant MS forms may significantly improve, from 6 months after HSCT.

Preliminary data: Our group previously published:
1. the outcome of both prospective and registry clinical trials, showing a promising clinical impact of HSCT in advanced forms of MS
2. The efficacy of HSCT on MRI-detected activity, mostly in patients in secondary progressive MS phase
3. An altered pattern of cytokine production in MSC derived from bone marrow of MS patients
4. HSCT with a low-intensity protocol depletes circulating CD161highCD8 proinflammatory cells, a cell subset that resides in the gut mucosa but was also detected in MS brain lesions
5. AHSCT with a high-intensity protocol induces a massive regeneration of circulating T cells in the patients post-treatment

Materials and Methods

Ten patients with malignant MS forms showing a rapid deterioration of EDSS disability score in the two years prior to enrollment and evidence of inflammatory activity (Gd+ lesion and/or new T2 lesions), despite the administration of immunomodulating and immunosuppressive therapies, will be recruited and transplanted in Unit 1 and 3. Peripheral Blood Stem Cells will be mobilized by Cyclophosphamide and G-CSF and patients conditioned with the association of BEAM and ATG. No immunosuppressive treatments will be administered post HSCT. MRI will be acquired at baseline and 12, 24 and 36 months after transplantation. Bone marrow biopsies will be performed at baseline and at 24 months after HSCT; morphological analysis and MSC expansion will be carried out. An apheresis of peripheral mononuclear cells will be performed at baseline, 6 and 24 months after HSCT: cells will be stored in liquid nitrogen vapor until use in the mechanistic immunological assays outlined in specific Aim 2.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Hematopoietic stem cell transplantation for malignant forms of Multiple Sclerosis.
Project Code: PE-2013-02355346	Principal Investigator: riccardo saccardi
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

Impact and Translational Implications

This study will provide an evidence-based outlook of the therapeutic potential of HSCT using intermediate conditioning regimen in malignant forms of MS. The peculiarity of HSCT that induces a clinical remission, free of immunosuppression, will allow the collection of biosamples at baseline and in the sustained remission phase, to provide new mechanistic data on the MS pathogenesis as well as new information on the mechanism of action of this therapeutic approach.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Brain Mechanisms of Chronic Subjective Dizziness
Project Code: PE-2013-02355372	Principal Investigator: Indovina Iole
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Santa Lucia
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases	
Project Classification IRG:	Integrative, Functional, and Cognitive Neuroscience
Project Classification SS:	Auditory System - AUD
Project Keyword 1:	Vestibular system/end organ: anatomy, physiology, pharmacology, development, maturation, plasticity, and neuro-otological disorders using approaches ranging from molecular/cellular to systems/whole organism.
Project Keyword 2:	CSD
Project Keyword 3:	fMRI
Project Request: Animals: ☐	Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐	Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright	

Italian Researcher Abroad – Foreign Operative Unit	
Name and Surname:	Marta Bianciardi
Foreign Institution:	Massachusetts General Hospital / Harvard Medical School, USA
Department/Division/Laboratory:	A.A. Martinos Center for Biomedical Imaging / Department of Radiology
City-State and Country:	Boston, MA, USA
Years of Residence Abroad:	6
Email: martab@nmr.mgh.harvard.edu	Phone: +1 617 724 1723

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Fondazione Santa Lucia	Neuromotor physiology / NeuroImaging / Vestibular rehabilitation unit	Patient recruiting, experiment execution, data analysis
2	Massachusetts General Hospital / Harvard Medical School, USA	A.A. Martinos Center for Biomedical Imaging / Department of Radiology	Developement of anatomical segmentation techniques of brainstem gray matter nuclei; advising on resting state connectivity methodology and physiological noise correction.

Project Title:
Brain Mechanisms of Chronic Subjective Dizziness

Project Code: PE-2013-02355372

Principal Investigator: Indovina Iole

Research Type: Biomedical/Biomedica

Applicant Institution: Fondazione Santa Lucia

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1				
2	Staab Jeffrey	MD, Associate Professor of Psychiatry Mayo Clinic Department of Psychiatry and Psychology 200 1st St SW Rochester, MN	Design of experiments, discussion of results	23/07/1961
3	Passamonti Luca	MD Neurologist at the Institute of Neurological Science National Research Council (CNR) Catanzaro	Supervision of patient recruitment and assessment, protocol definition, analyses supervision, discussion of results	25/12/1975
4	Lacquaniti Francesco	MD, Full Professor of Physiology, University of Rome Tor Vergata and Fondazione Santa Lucia	Design of experiments, discussion of results	24/12/1952

Background and Significance

Chronic Subjective Dizziness (CSD) is a clinical syndrome of persistent (>3 months) non-vertiginous dizziness, unsteadiness, or both that are exacerbated by upright posture, active or passive movement, or exposure to complex visual stimuli. Symptoms may last for years, causing substantial disability. CSD usually follows acute vestibular problems (e.g., peripheral vestibular disorders, migraine, concussion). It affects 15-25% of patients in dedicated dizziness units, but is often overlooked because it has been defined variously over 25 years and its pathophysiologic mechanisms are unknown. An international consensus definition has been proposed for ICD-11, and new research is beginning to elucidate potential mechanisms, offering the promise of better patient outcomes.

Two anxiety-mediated pathological processes have been hypothesized to underlie the balance problems experienced by CSD patients: 1) altered vestibular processing and 2) hypersensitivity to visual motion cues. These processes may involve brain areas mediating instinctive fear reactions (amygdala, hippocampus, orbitofrontal cortex), vestibular function (vestibular insula, brainstem and cerebellum), and space-motion exteroception (occipital and parietal visual cortices), although experimental evidence to support this hypothesis is lacking. This project will examine neuroanatomy, brain function and connectivity in these regions using advanced neuroimaging methods and visual-vestibular stimuli relevant to CSD.

Specific aims

Aim 1: First, we will use functional Magnetic Resonance Imaging (fMRI) to measure brain activity and connectivity in vestibular and anxiety systems during sound evoked vestibular stimulation in normal individuals and patients with CSD. This stimulus is well tolerated and does not induce vertigo, making it unlikely to trigger movements that cause serious MRI artifacts. Our team was one of the first to identify regions in the vestibular cortex associated with gravity perception and balance control. We thus aim at extending this earlier work to brain regions that may mediate CSD. Based on theories of CSD and our pilot data, we will focus the analyses on specific regions of interest (ROIs) such as vestibular nuclei in the brainstem (recently segmented by our Italian collaborator abroad) and cerebellum, insula, amygdala, hippocampus and orbitofrontal cortex.

Aim 2: Second, we will use fMRI to measure brain activity and connectivity in vestibular, visual-motion and anxiety systems during viewing of a virtual reality depiction of a rollercoaster ride in healthy controls and patients with CSD. This will simulate the type of visual flow that often exacerbates CSD symptoms. It may trigger self-motion illusions and anxiety reactions. Based on theories of CSD and our pilot data with this paradigm, we will add visual cortices in our ROIs from Aim 1.

Aim 3: Third, we will use anatomical imaging, resting state and Diffusion Tensor Imaging (DTI) to investigate structural and functional anomalies in vestibular-anxiety system ROIs independent of tasks.

Hypothesis: We hypothesize that patients with CSD compared to healthy controls will show abnormal function of vestibular, visual-motion and anxiety brain systems in response to vestibular and visual-motion stimuli. We also predict that functional alterations in the ROIs may be associated with anatomical abnormalities (e.g., reduced grey-matter volume and altered white-matter patterns). Specifically, we hypothesize that the pattern of anomalies in CSD (e.g., alterations in vestibular, visual-motion and anxiety neural systems) can be interpreted on the basis of anxiety-mediated hypersensitivity to visual motion cues and/or altered vestibular processing. We expect important insights into brain mechanisms underlying CSD to motivate clinical studies to enhance early detection and treatment.

Preliminary data: We acquired 3T structural and functional MRI data during sound evoked vestibular stimulation and rollercoaster simulation in 10 healthy controls and 10 CSD patients. This pilot study showed that insula, hippocampus and fastigial nuclei were deactivated during sound evoked vestibular stimulation and that the insula was deactivated during rollercoaster simulation in CSD patients compared to controls. Voxel based morphometric analysis found gray matter volume reduction in the hippocampus in CSD patients versus controls. These preliminary findings show the viability of our experimental approach and give support to our hypotheses.

Materials and Methods

Subjects: We will recruit 30 patients with CSD and 30 healthy age-matched controls. Participants will undergo systematic neuro-otologic and psychiatric examinations. Those with past or present neuro-otologic, psychiatric or chronic medical illnesses other than CSD will be excluded. CSD will be diagnosed using the recently agreed international definition of the disorder (ICD-11).

Methods: We will obtain 3T structural DTI and functional brain images at rest and in response to the stimuli described in Aims 1 and 2. We will record eye movements (to assess vestibular-visual responses), dizziness handicap, state anxiety and mood as covariates.

Data analysis: Images will be analyzed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm). Study hypotheses will be tested by examining between group differences in ROIs.
A probabilistic in vivo atlas of brainstem nuclei will be provided by our Italian collaborator abroad.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Brain Mechanisms of Chronic Subjective Dizziness
Project Code: PE-2013-02355372	Principal Investigator: Indovina Iole
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Santa Lucia

Project Type: PE- ITALIAN RESEARCHER ABROAD

Impact and Translational Implications

Patients with CSD present a tremendous challenge to physicians in many specialties. Uncertainties about pathogenesis prevent improvement in diagnostic clarity and clinical outcomes. Enigmatic CSD symptoms lead to repeated medical tests at high cost to the NHS. Recent research has produced testable brain/behavioral models of CSD. Our project will directly test some of these models using brain imaging. Results will inform clinical studies to improve diagnosis and treatment.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Impact of carotid endarterectomy and stenting on hemodynamics, fluid-structure interaction, autonomic modulation, and cognitive brain function.
Project Code: PE-2013-02355484	Principal Investigator: Marrocco Trischitta Massimiliano
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Ospedale San Raffaele - Milano
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Metabolic and cardiovascular diseases	
Project Classification IRG:	Surgical Sciences, Biomedical Imaging, and Bioengineering
Project Classification SS:	Bioengineering, Technology, and Surgical Sciences - BTSS
Project Keyword 1:	Fluid mechanics studies of circulation, microcirculation, and transport systems. Biomechanics, computational fluid dynamics, hemodynamics, mathematical modeling, simulation, ventricular remodeling, tissue and organ mechanics and the mechanics of injury.
Project Keyword 2:	baroreflex sensitivity and heart rate and systolic blood pressure variability after carotid revascularization
Project Keyword 3:	cognitive function after carotid revascularization
Project Request: Animals: [ ]	Humans: [X] Clinical trial: [X]
The project has already been presented: [ ]	Project code reference:
[ ] I declare that the object/s of this application is/are under patent copyright	

Italian Researcher Abroad – Foreign Operative Unit	
Name and Surname:	Alessandro Veneziani
Foreign Institution:	Emory University
Department/Division/Laboratory:	Dept. of Mathematics and Computer Science
City-State and Country:	Atlanta, GA. U.S.A.
Years of Residence Abroad:	4
Email: ale@mathcs.emory.edu	Phone: +14047277925

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Ospedale San Raffaele - Milano	Vascular Surgery	patients recruitment and randomization, surgical and endovascular procedures, follow-up, assessment of cardiovascular autonomic control
2	Emory University	Mathematics and Computer Science	numerical modeling of circulation combining medical image analysis and clinical data
3	Università di Pavia	Civil Engineering and Architecture	computational structural analysis and mechanical modelling based on medical images

Project Title:
Impact of carotid endarterectomy and stenting on hemodynamics, fluid-structure interaction, autonomic modulation, and cognitive brain function.

Project Code: PE-2013-02355484

Principal Investigator: Marrocco Trischitta Massimiliano

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Ospedale San Raffaele - Milano

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	CONTI MICHELE	Università di Pavia	Computational structural analysis and mechanical modelling of the carotid wall after treatment (endarterectomy vs. stenting)	08/04/1982
2	Veneziani Alessandro	Emory University	numerical modeling of circulation combining medical image analysis and clinical data	25/05/1969
3	Rimoldi Ornella	Istituto di Bioimmagini e Fisiologia Molecolare, Consiglio Nazionale delle Ricerche IBFM - CNR	Elaboration HR and SBP variability. Image quality assurance and co-ordination of imaging post-processing	26/03/1954
4	Leocani Letizia	Ospedale San Raffaele - Milano	Evaluation and analysis of neurophysiological assessment of cognitive performance	14/08/1967

Background and Significance

The non-inferiority of carotid artery stenting (CAS) compared with carotid endarterectomy (CEA) for the prevention of stroke in patients with internal carotid stenosis (ICS) is still debated (Cutlip, 2012). Short/mid-term endpoints other than perioperative composite risk of death, stroke, or myocardial infarction should be addressed, in view of a longer life expectancy. Impaired cardiovascular autonomic control following ischemic stroke is a prognostic indicator for adverse vascular outcome (Robinson, 2003) and cognitive decline (Bohm, 2012). CEA and CAS may perturb carotid baroreceptors function as a consequence either of surgical trauma or mechanical stimuli related to compliance mismatch between the stented segment and the native artery downstream (Vernhet, 2003, De Santis, Conti 2013, Conti 2011). Geometric features of arteries are key factors in determining rheological parameters, including calculated streamlines and wall shear stress (Cardiovascular Mathematics, 2009, Veneziani A. ed.), and might be useful tools for large scale imaging studies assessing the risk of recurrent atherosclerosis, thrombotic events, (Davies,1997; Hathcock, 2006) and restenosis rates. A major sequela of ICS is cognitive impairment and decline (Johnston, 2004). Yet, in this respect the effects of the different techniques (CAS and CEA) are still unclear and controversial, also due to the low specificity and the expected biases of current psychometric tests (Irvine 1998).

Specific aims

Aim 1: To compare the impact of CEA and CAS on long-term post-operative baroreceptor function and on cognitive brain function, and analyze their influence on clinical outcome. The specific goal is to assess the potential correlation between post-operative autonomic and cognitive function.

Aim 2: To assess the solicitation on the carotid wall due to CAS as compared to CEA through structural analysis and mechanical modeling. The specific goal is to assess the potential correlation between stenting, wall damage, baroceptor impairment, and late neurological sequelae.

Aim 3: To assess the post-operative carotid hemodynamics combining medical image analysis, clinical data, and computer simulations. The specific goal aims at correlating both local (e.g., wall stress stress) and global phenomena (controlateral flow, arterial stiffening) with baroreflex function and post-operative neurological

outcomes.

Hypothesis: Late clinical outcome and prognosis after CAS may be inferior to that after CEA in terms of autonomic modulation, hemodynamic remodeling, and cognitive function.

Preliminary data: We have previously demonstrated that CEA, even when performed on both sides, and regardless the surgical technique, preserves the integrity of baroreflexes and chemoreflexes (Marrocco-Trischitta 2013). Conversely, conflicting results have been reported on baroreceptor sensitivity after unilateral CAS (Huang, 2010; Acampa, 2011), and the effect of bilateral CAS on autonomic control remains unexplored. Preliminary studies from our partners have also shown the value of computer-based simulations for both characterization of arterial flow (Veneziani 2009) and post-stenting cardiovascular mechanics (Conti 2013, 2011). The project aims at combining such recognized expertise to create a multidisciplinary framework allowing a quantitative comparison between the post-operative effects of CEA and CAS, including the baroreflex function.

P300 auditory evoked potentials (AEP) is a highly sensitive and reproducible modality to assess cognitive function (Leocani, 2010). CEA was reported to improve previously impaired cognitive brain function, evaluated by means of P300 AEP measurements, and this beneficial effect appears to be sustained up to 5 years after treatment (Czerny, 2010). No data are available on P300 AEP measurements in patients submitted to CAS, and notably the latter causes significant more cerebral microembolism that were shown to contribute to the development of neurophsycological and cognitive deficits (Pugsley, 1994; Kilo, 2001).

Materials and Methods

CLINICAL ASSESSMENT:
- Population: Patients with >=70% symptomatic or >=80% asymptomatic ICS. Exclusion criteria: age>75 years, previous disabling stroke, contralateral occlusion or >70% stenosis.
- Experimental Design: one center, prospective, two arms, randomized, unblinded. A sample size of 30 patients per arm was calculated based on an ad-hoc power analysis. Follow-up at 12 months by imaging (see below).
- Autonomic modulation: measured by spectral analysis of heart rate and systolic arterial pressure variability
- Cognitive function: assessed by means of P300 AEP measurement, before and after treatment.

IMAGE ACQUISITION: CTA; Phase Contrast MRI.
IMAGE ANALYSIS: in-house developed code using visulization libraries (VTK,VMTK).
STRUCTURAL ANALYSIS: structural finite element analysis (FEA) base on patient-specific geometrical models and accurate constitutive model of the artery.
HEMODYNAMICS ANALYSIS: use of multiscale CFD solver co-developed by A.Veneziani (www.lifev.org).

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Impact of carotid endarterectomy and stenting on hemodynamics, fluid-structure interaction, autonomic modulation, and cognitive brain function.
Project Code: PE-2013-02355484	Principal Investigator: Marrocco Trischitta Massimiliano
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Ospedale San Raffaele - Milano

Project Type: PE- ITALIAN RESEARCHER ABROAD

Impact and Translational Implications

CEA is associated with lower complication rates, and the randomized trial outcomes for CEA can be achieved in community-wide practice (Bunch, 2004). CEA has a better cost effectiveness ratio whereas CAS is comparable to CEA only when performed in high-volume centers and by skilled operators, and at a higher cost. Our results may raise a word of caution against the escalating use of CAS, and restrict its use to selected patients.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: High-end and Low-End Virtual Reality Systems for the Rehabilitation of Friality in the Elderly
Project Code: PE-2013-02355948	Principal Investigator: Riva Giuseppe
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Istituto Auxologico Italiano

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases
Project Classification IRG: Emerging Technologies and Training in Neurosciences
Project Classification SS: Brain Disorders and Related Neurosciences Fellowship - F01
Project Keyword 1: Therapeutic approaches for behavioral, cognitive and emotional disorders.
Project Keyword 2: virtual reality
Project Keyword 3: motor and cognitive rehabilitation
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Gianluca De Leo
Foreign Institution: Old Dominion University
Department/Division/Laboratory: Virginia Modeling Analysis and Simulation Center
City-State and Country: Norfolk, Virginia USA
Years of Residence Abroad: 13
Email: gdeleo@odu.edu Phone: +1 757 683 6733

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Auxologico Italiano	Applied Technology for Neuro-Psychology Lab (Unit 1) and Department of Geriatrics and Cardiovascular Medicine (Unit 2)	Coordination of the whole project and responsibility of the design, development and testing of the VR platforms for reducing cognitive and physical decline in the elderly.
2	Old Dominion University	Virginia Modeling Analysis and Simulation Center	Assisting with the implementation of a user center design approach that will lead the development of VR Systems. Providing technical expertise during the development of the Virtual Reality Systems.
3	Istituto Superiore di Sanità	Department of Technology and Health	Evaluation of adherence in VR-based intervention with definition of prevalence and predictors.

Project Title:
High-end and Low-End Virtual Reality Systems for the Rehabilitation of Friality in the Elderly

Project Code: PE-2013-02355948

Principal Investigator: Riva Giuseppe

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Istituto Auxologico Italiano

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	grigioni mauro	Istituto Superiore di Sanità	Coordination of the evaluation of adherence in VR-based intervention (OU3).	22/04/1958
2	Stramba-Badiale Marco Giovanni	Istituto Auxologico Italiano - Department of Geriatrics and Cardiovascular Medicine	Design, development and testing of the VR platforms for reducing cognitive and physical decline in the elderly (OU1).	12/10/1958
3	Gaggioli Andrea	Istituto Auxologico Italiano - Applied Technology for Neuro-Psychology Lab.	Coordination of the ergonomic analysis of the VR platforms with the frail elderly (OU1).	12/03/1974

Background and Significance

A recent consensus definition defined frailty as a "multidimensional syndrome characterized by decreased reserve and diminished resistance to stressors". In this perspective, frailty may be described as a dynamic process of accelerated ageing, which, in its early phase, is characterised by the absence of disability.
Unfortunately, to date, no healthcare programs or pharmacological treatments are available for frail older people. Nevertheless, in the absence of targeted interventions, the progression of frailty is marked by increased morbidity, disability and poor quality of life. Detecting and contrasting frailty are therefore key goals.
Recently, the "Positive Technology" approach, i.e., the scientific and applied approach to the use of technology for improving the quality of our personal experience through its structuring, augmentation, and/or replacement suggested the possible use of virtual reality (VR) for supporting active aging. Starting from this rationale, the project wants to evaluate the use of VR for reducing cognitive and physical decline in the elderly. Moreover, to facilitate continuity of care, the project aims at defining assessment and treatment protocols using both high-end VR systems in the health care center (Cave Automatic Virtual Environment - CAVE), and low-end VR systems at home (tablets and biosensors). Finally the project aims at evaluating the adherence (prevalence and predictors) of the developed protocols to facilitate their exploitation.

Specific aims

Aim 1: To design, develop and testing the potential of virtual reality (VR) as positive technology for reducing the cognitive decline in the elderly. The theory of flow, developed by Mihaly Csikszentmihalyi, provides a useful framework for addressing this challenge: older adults have the capacity to experience flow when cognitive capacity and intellectual demands are synchronized. Starting from this theoretical premise, Riva and colleagues suggested the possibility of using VR for a new type of applications in positive mental health for the ageing based on a strategy defined as "transformation of flow". The expected effect is a functional reorganization of the brain produced by the broadening of the thought-action repertoire associated with improved self-esteem and self-efficacy.

Aim 2: To design, develop and testing the potential of virtual reality (VR) as positive technology for reducing the physical decline in the elderly. The incidence of falls among frail elderly persons is increasing: non-lethal falls reduce the quality of life of the elderly, who tend to incur fractures or other severe injuries in falls. A VR program aimed at fall prevention among the frail elderly population is therefore important. A primary strength that such a VR program can offer is the creation of simulated realistic home-like environments (homes, supermarkets, etc). in which performance can be tested and trained in a systematic fashion. Moreover, the final environments will allow for the hierarchical delivery of stimulus challenges across a range of difficulty levels. Finally, the use of advanced motion tracking tools, allows the processing of motion parameters in real-time and are directly available for training using

immediate feedback.

Aim 3: To evaluate the acceptability/usability of the VR tools and the adherence to the proposed protocols of the frail elderly. Adherence is a key factor in the evaluation of healthcare initiatives. There are many factors affecting patient adherence, such as age, socio-cultural characteristics, personal experiences, complexity of the protocol, and so on. Hence, it is critical to determine the predictors of adherence in VR-based intervention, in order to enhance acceptability, given the specific patient. Moreover, it will be investigated whether certain modalities are faced with a higher acceptance.

Hypothesis: The use of virtual-reality technology with frail older people may reduce the decline of a wide range of physical, psychological, and cognitive factors. More, the use of both high-end VR systems in the health care center (eg. Cave Automatic Virtual Environment - CAVE), and low-end systems at home will improve the efficacy of the protocols and their adherence.

Preliminary data: The successful "VRehab -Virtual Reality in the Assessment and TeleRehabilitation of Parkinson's Disease and Post-Stroke Disabilities" (RF-2009-1472190) research project supports the use of VR as a tool for the assessment and rehabilitation of the elderly.

Materials and Methods

Five work packages will be included in the project.

WP1 - Human Factors and User Requirements (Coordinator: Andrea Gaggioli, OU1-AUXO): WP1 will be responsible for the human factors and user requirements.

WP2 - System Architecture (Coordinator: Gianluca De Leo, OU2-ODU): WP2 will define the system architecture of both high-end and low-end VR systems.

WP3 - Virtual Reality for Reducing the Cognitive Decline in the Elderly (Coordinator : Giuseppe Riva, OU1-AUXO): WP3 will design, develop and testing the protocols for reducing cognitive decline.

WP4 - Virtual Reality for Reducing the Physical Decline in the Elderly (Coordinator : Marco Stramba Badiale, OU1-AUXO): WP4 will design, develop and testing the protocols for reducing physical decline.

WP5 - Acceptability and Adherence of Virtual Reality with the Frail Elderly (Coordinator: Mauro Grigioni, OU3-ISS): WP5 will evaluate the acceptability/usability of the VR tools and the adherence to the proposed protocols of the frail elderly.

Impact and Translational Implications

On the basis of the results of this project the National Health Service might consider the possibility of introducing a prevention/screening program specifically targeted to the frail elderly to reduce the costs related to increased morbidity, disability and poor quality of life. The development of both high-end and low-end virtual reality solutions and protocols will improve the frail elderly compliance and lower costs.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Evaluation of the diagnostic accuracy of three memory tests for early Alzheimer's disease
Project Code: PE-2013-02356465	Principal Investigator: Clerici Francesca Maria Alessandra
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Regione Lombardia - Direzione Generale Sanità
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases
Project Classification IRG: Brain Disorders and Clinical Neuroscience
Project Classification SS: Clinical Neuroscience and Neurodegeneration - CNN
Project Keyword 1: Alzheimer¿s disease and other dementias.
Project Keyword 2: mild cognitive impairment
Project Keyword 3: memory testing
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Sergio Della Sala
Foreign Institution: University of Edinburgh
Department/Division/Laboratory: Human Cognitive Neuroscience Unit, Psychology Department School of Philosophy, Psychology and Language Sciences
City-State and Country: Edinburgh, UK
Years of Residence Abroad: 21
Email: sergio@ed.ac.uk Phone: 0044(0)1316513242

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Regione Lombardia - Direzione Generale Sanità	Ospedale L. Sacco, Division of Neurology, U.O. Cognitive Disfunction	project coordination, test development, patient recruitment, assessment and follow-up
2	University of Edinburgh	Human Cognitive Neuroscience Unit, Psychology Department School of Philosophy, Psychology and Language Sciences	test development and data analysis
3	IRCCS Ospedale San Raffaele	Division of Neuroscience, Cognitive Neuroscience	test development, patient recruitment, assessment and follow-up

Project Title:
Evaluation of the diagnostic accuracy of three memory tests for early Alzheimer's disease

Project Code: PE-2013-02356465

Principal Investigator: Clerici Francesca Maria Alessandra

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Regione Lombardia - Direzione Generale Sanità

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Cappa Stefano	IRCCS Ospedale San Raffaele	test development and data analysis	01/07/1953
2	Mariani Claudio	Ospedale L. Sacco, Division of Neurology, Director	test development, patient recruitment, assessment and follow-up	12/10/1947

Background and Significance

The present focus of Alzheimer disease (AD) research on early diagnosis has resulted in the definition of new research diagnostic criteria, which emphasize the potential role of biomarkers, in particular cerebrospinal fluid analysis and structural and functional neuroimaging. While a considerable amount of effort is now dedicated to crucial issues about the use of such biomarkers, studies on the cognitive markers of the disease are relatively scant. This is surprising, considering that the only clinical criterion supporting the application of diagnostic biomarkers to individual subjects concerning about cognitive decline is the presence of objective cognitive dysfunction, as assessed by neuropsychological tests. Of paramount importance is the assessment of memory, as the impairment in episodic memory is most commonly seen in subjects with mild cognitive impairment (MCI) who subsequently progress to a diagnosis of AD dementia. Notwithstanding the obvious relevance of this point for any validation of the proposed biomarkers, it is striking that no "gold standard" is available for memory testing of AD. There is clearly the need for tests that are specific for the memory dysfunction of prodromal and early AD, and that are predictive of progression to dementia.

Specific aims

Aim 1: to evaluate the sensitivity and the specificity of the Free and Cued Selective Reminding Test (FCSRT), the Short Term Memory Binding Test (STMBT) and the Dual Task (DT) for sporadic AD dementia

Aim 2: to assess the predictive value of the FCSRT, the STMBT and the DT for progression to AD dementia in subjects presenting with MCI.

Aim 3: to identify the "gold standard" for memory testing of AD

Hypothesis: We hypothesize that the STMBT and the FCSRT will be highly sensitive to prodromal AD.
The DT is supposed to be less sensitive to prodromal AD, but highly specific to AD dementia.
The three tests appear to be very promising candidates to become the "gold standard" for memory testing in AD.

Preliminary data: We have identified from the literature and from our own preliminary work three tests, which appear to be promising candidates to become "gold standards" for the cognitive assessment of AD.
Two tests (STMBT and DT) were developed by the foreign partner.
The first is the STMBT. The impairment of the mechanisms responsible for holding integrated objects in verbal short-term memory was shown to be selectively impaired in mild and moderate AD, in comparison to healthy ageing [7] and chronic depression in the elderly [8]. A subsequent study found defective performance in the task by asymptomatic carriers of a presenilin-1 mutation [9]. Preliminary data from our groups [10]comparing the STMBT performance of 67 subjects with MCI, 27 patients with AD dementia and 46 healthy controls show that MCI subjects performed similarly to AD patients but significantly worse than controls in the STMBT. This support the hypothesis that STMB deficits might be an early marker of sporadic AD.
The second is the DT [11-13]. Performance on the DT has been shown to effectively discriminate AD

from healthy aging and depression [14]. Task performance appears to be preserved in subjects with a diagnosis of MCI, suggesting that the test is suitable to map disease progression, rather than for early diagnosis [15].
The third test is the FCSRT [16]. This traditional memory test has been proposed to have high predictive value for the progression of isolated disorders of memory towards AD. In particular, Dubois and colleagues [17] have claimed that episodic memory loss of the hippocampal type (characterised by a free recall deficit on testing not normalised with cueing) may be the defining feature of prodromal AD. This hypothesis is supported by a study, which indicated the predictive role of defective performance on the FCSRT on progression to dementia in subjects fulfilling the criteria for MCI [18]. The proponent and the Italian partner have validated the Italian version of the FCSRT [19]. Preliminary longitudinal data from our groups [20] on 150 MCI consecutive subjects followed-up for about two years, show that the diagnostic performance of the FCSRT in identifying those subjects who progress to dementia exceeded that of other conventional psychometric tests.

Materials and Methods

a. Cross sectional study: to assess the specificity of the three tests (STMBT, DT and FCSRT) we will recruit 20 patients with mild AD and 20 patients with moderate AD compared to 40 normal controls and to groups with a diagnosis of mild dementia due to Frontotemporal Dementia, behavioural variant (N=20) and Lewy Body Dementia (N= 20).
b. Prospective, longitudinal study: to assess the sensitivity of the three tests (STMBT, DT and FCSRT) to prodromal AD we will recruit 150 subjects with MCI. The participants will be consecutively enrolled at the two following memory clinics of Milan: the Luigi Sacco Hospital and the San Raffaele Scientific Institute. Subjects with MCI and dementia will undergo the same background neurological, imaging and neuropsychological assessment, as well as the three experimental measures (STMBT, DT and FCSRT). The MCI participants will be followed at six months intervals to assess the progression to dementia. We plan a 5 year follow-up through subsequent funding.

Impact and Translational Implications

The proposed widespread use of biomarkers potentially represents a huge cost for the National Health System (NHS). We are convinced that a careful selection of subjects at high-risk for AD on the basis of inexpensive and reliable behavioural testing would represent a major practical advancement also in order to identify the best candidates to biomarkers. The impact on the NHS could be considerable, given the prevalence of cognitive complaints in the growing elderly population.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Microfluidic organotypic model of monocyte transendothelial migration to the joint for the screening of promising therapeutic strategies in obese osteoarthritic patients
Project Code: PE-2013-02356613	Principal Investigator: Lopa Silvia
Research Type: Biomedical/Biomedica	Applicant Institution: Istituto Ortopedico Galeazzi
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Innovative biotechnologies
Project Classification IRG: Musculoskeletal, Oral and Skin Sciences
Project Classification SS: Arthritis, Connective Tissue and Skin - ACTS
Project Keyword 1: Arthritis and Connective Tissue: Inheritable, inflammatory and degenerative diseases of joints and connective tissues.
Project Keyword 2: 3D microfluidic organotypic model
Project Keyword 3: Inflammation-based target screening in osteoarthritis
Project Request: Animals: [X] Humans: [X] Clinical trial: [ ]
The project has already been presented: [ ] Project code reference:
[ ] I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Ivan Martin
Foreign Institution: University Hospital Basel
Department/Division/Laboratory: Department of Biomedicine and Department of Surgery
City-State and Country: Basel, Switzerland
Years of Residence Abroad: 15
Email: IMartin@uhbs.ch Phone: +41612652384

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Ortopedico Galeazzi	Cell and Tissue Engineering Laboratory	Project coordination, patient recruitment, cell and synovial fluid collection, establishment of in vitro organotypic coculture, in vivo study
2	University Hospital Basel	Department of Biomedicine and Department of Surgery	Expertise and supervision in the establishment of in vitro organotypic coculture, researcher exchange for formation and exchange of knowledge
3	Politecnico di Milano	Dipartimento di Elettronica, Informazione e Bioingegneria	Microfluidic chip design, microfabrication, and validation

Project Title:
Microfluidic organotypic model of monocyte transendothelial migration to the joint for the screening of promising therapeutic strategies in obese osteoarthritic patients

Project Code: PE-2013-02356613

Principal Investigator: Lopa Silvia

Research Type: Biomedical/Biomedica

Applicant Institution: Istituto Ortopedico Galeazzi

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Redaelli Alberto	Politecnico di Milano	Associate Professor, microfluidic chip design, microfabrication, and validation	06/02/1966
2	Bongio Matilde	Istituto Ortopedico Galeazzi	PostDoc Research Fellow, cell isolation, establishment of organotypic coculture, in vivo study	05/06/1983
3	Sansone Valerio	Istituto Ortopedico Galeazzi	Orthopaedic Surgeon, patient recruitment, biopsy harvesting	16/10/1958

Background and Significance

Osteoarthritis (OA) is the most common type of arthritis and, given the rising ageing and obesity, is the fastest growing cause of disability worldwide. The pharmacological management of OA is based on symptomatic drugs that do not counteract OA progression. Since increasing evidence indicates that synovial inflammation and macrophage accumulation in the synovium are involved in OA, anti-cytokine drugs have been tested as disease modifying OA drugs (DMOAD), but so far without relevant outcomes. Monocyte (Mo) from obese individuals have been recently shown to display increased expression of chemokine receptors and superior migration ability, which may significantly correlate with the high risk of OA onset and severe progression in obese patients. Hence, targeting chemokine receptors involved in monocyte (Mo) recruitment (i.e. CCR1, CCR2, CCR5, CX3CR1), proposed as therapy for other inflammatory diseases, is likely to be a relevant therapeutic option for OA in obese patients. The development of a 3D microfluidic organotypic model of the joint will allow to investigate Mo migration in a controlled system without neglecting the multiple interactions between joint-resident cells that are disregarded in classical transendothelial migration assays. Furthermore, the possibility to generate models starting from patient-derived cells will represent a key step in DMOAD research area, allowing the reliable screening of therapeutic strategies addressed to a specific class of patients.

Specific aims

Aim 1: To develop an innovative 3D microfluidic organotypic model of the OA joint through the coculture of patient-derived synovial fluid and primary cells, including monocytes, endothelial cells, synovial fibroblasts and articular chondrocytes, allowing the real time monitoring of monocyte transendothelial migration to the inflamed joint.

Aim 2: To evaluate the effect of agents interfering with specific chemokine signaling axes in the organotypic joint model using cells derived from obese OA patients in order to select the most effective therapeutic strategy to reduce monocyte infiltration to the synovial compartment and negative downstream effects on articular chondrocytes.

Aim 3: To verify the level of efficacy of the selected therapeutic strategy in a model of osteoarthritic obese mice on the basis of the ability to reduce cartilage degeneration and OA progression, as well as monocyte infiltration to the joint.

Hypothesis: Our hypothesis is that the screening of agents interfering with chemokine signaling through an organotypic microfluidic model of OA joint specific for obese patients will allow the identification of a promising therapeutic strategy to reduce the severe progression of cartilage and joint degeneration characterizing obese OA patients.

Preliminary data: Prof. Ivan Martin's group at University Hospital Basel (UHBS) has pioneered the field of musculoskeletal tissue engineering, demonstrating that the in vitro recapitulation of developmental

processes can lead to the generation of functional tissues and investigating the influence of environmental factors on cell phenotype and tissue remodeling. UHBS demonstrated that inflammatory factors regulate mesenchymal stromal cells (MSC) differentiation and cartilaginous tissue remodeling. Additionally, they demonstrated a role for tissue repair macrophages in enhancing the cartilage forming capacity of MSC. IOG group characterized different primary cell types resident in the joint, focusing on cells derived from OA patients. They investigated the cross-talk between articular chondrocytes and MSC in vitro and in vivo performing direct contact cocultures or using hydrogels with a spatially-controlled architecture, in collaboration with UHBS and Politecnico di Milano (POLIMI). IOG also evaluated the response of human monocytes to OA synovial fluid demonstrating that stimulation with OA synovial fluid affects the expression of anti-inflammatory mediators, as IL10 and IL1Ra. POLIMI has designed and microfabricated microfluidic chips for different cell culture applications. In particular, they developed microfluidic platforms for the culture of 2D cell monolayers or 3D microaggregates under continuous perfusion of spatiotemporally controlled chemical patterns, defining high-throughput models for soluble factors screening. Moreover, POLIMI demonstrated that it is possible to generate 3D cell-laden gel micropatterns within microfluidic chips through the use of gelatin-based photopolymerizable hydrogels for the culture of MSC and endothelial cells.

Materials and Methods

The microfluidic chip will be designed, microfabricated, and validated. To establish the organotypic coculture, we will isolate outgrowth endothelial cells and monocytes from peripheral blood of obese OA patients. Synovial fluid, synovial fibroblasts and articular chondrocytes will be isolated from the same patients. Known agents interfering with specific chemokine signaling axes (i.e. CCR1, CCR2, CCR5, CX3CR1) will be tested in the organotypic model. Transendothelial migration of monocytes to the synovial compartment and their polarization phenotype will be assessed by immunofluorescence and confocal microscopy. Downstream effects of monocyte infiltration on the expression of matrix metallo-proteases and matrix proteins in articular chondrocytes will be also evaluated. The therapeutic efficacy of the selected strategy will be verified in diet-induced obese mice spontaneously developing OA, evaluating the effect on cartilage degeneration and monocyte recruitment to the joint.

Impact and Translational Implications

The project will contribute to the development of DMOAD addressed to obese patients through the selection of a therapeutic strategy able to reduce the severe OA progression characterizing obese patients, with relevant benefits for patients and National Health Systems. Furthermore, the 3D organotypic model will provide a unique tool for the comprehension of the mechanisms driving OA progression, representing a system that may be easily translated to the modeling of other articular pathologies.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Circulating microRNAs as biomarkers of chemotherapy-related cardiotoxicity and myocardial inflammation
Project Code: PE-2013-02356818	Principal Investigator: CONDORELLI GIANLUIGI
Research Type: Biomedical/Biomedica	Applicant Institution: Istituto Clinico Humanitas
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Metabolic and cardiovascular diseases	
Project Classification IRG:	Cardiovascular and Respiratory Sciences
Project Classification SS:	Cardiac Contractility, Hypertrophy, and Failure - CCHF
Project Keyword 1:	The basic molecular and cellular mechanisms underlying cardiac hypertrophy and failure: myocyte growth, proliferation, metabolism and apoptosis; receptor signaling; transcriptional pathways; inflammatory/ cytokine-mediated processes.
Project Keyword 2:	Chemotherapy; myocardial biomarkers of inflammation; microRNAs
Project Keyword 3:	
Project Request: Animals: ☐	Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐	Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright	

Italian Researcher Abroad – Foreign Operative Unit	
Name and Surname:	Carlo Maria Croce
Foreign Institution:	Ohio State Uinversity
Department/Division/Laboratory:	Comprehensive Cancer Center
City-State and Country:	Columbus, OH, USA
Years of Residence Abroad:	44
Email: Carlo.Croce@osumc.edu	Phone: 001-614-292-8108

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Clinico Humanitas	Research	PI
2	Ohio State Uinversity	Comprehensive Cancer Center	Co-PI

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1				

Project Title:
Circulating microRNAs as biomarkers of chemotherapy-related cardiotoxicity and myocardial inflammation

Project Code: PE-2013-02356818

Principal Investigator: CONDORELLI GIANLUIGI

Research Type: Biomedical/Biomedica

Applicant Institution: Istituto Clinico Humanitas

Project Type: PE- ITALIAN RESEARCHER ABROAD

Background and Significance

Off-target effects are a principal drawback of cancer therapy. Drugs like doxorubicin can accumulate in tissues and induce strong oxidative and genotoxic stresses. In the heart, these stresses lead to inflammation, cell death and impairment of cardiac function. Cardiac toxicity can be assessed by non-invasive echocardiographic analysis determining myocardial stress and strain. In addition, circulating biomarkers for evaluating myocardial necrosis, such as troponin I, or deficits of left ventricular pump function, such as N-terminal pro-brain natriuretic peptide, have been proposed for assessing cardiotoxicity. However, there are no satisfactory biomarkers for predicting adverse effects on myocardial homeostasis and function before substantial damage has occurred.
microRNAs (miRNAs) are approximately 22-nuleotide-long non-coding RNAs that play important roles in gene regulation by inhibiting translation. Their levels have been found to be altered in pathological settings. For example, the cardiac-specific miRNAs miR-1 and miR-133 become down-regulated during stress. Also, miRNAs are highly stable in biological fluids, and cardiac miRNAs can be detected in the blood as a result cardiomyocyte necrosis due to myocardial infarction. However, we have found that the circulating miRNA profile changes during myocardial stress even in the absence of necrosis. Thus, analysis of circulating miRNAs be a way of gauging the functional status of the heart early on in disease.

Specific aims

Aim 1: Determine whether the measurement of specific microRNAs, either freely circulating or found within extracellular vesicles, could be used as an early biomarker of chemotherapy-related myocardial inflammation and stress.

Aim 2: -

Aim 3: -

Hypothesis: microRNAs are active, circulating biomarkers of cardiac inflammation and stress, such as that caused by chemotherapeutic agents, even in the absence of necrosis, which causes passive release of these small RNAs.

Preliminary data:

miR-1 IS RELEASED BY CARDIOMYOCYTES WITHIN EXOSOMES
The intracellular levels of miR-1 and miR-133 - two muscle-specific miRNAs transcribed from the same biscistronic locus - are decreased in cultured cardiomyocytes upon the induction of hypertrophy with the alpha-adrenergic agonist phenylephrine. We found that this reduction was accompanied by an increase in their primary transcripts. To explain this, we measured extracellular miRNAs and found that there was an increased amount expelled within the exosomal fraction present in the culture medium. This was reduced by co-exposure to ammonium chloride, an inhibitor of the multivesicular body pathway to which exosomes belong.

EXOSOME SHEDDING BY CARDIOMYOCYTES IS INCREASED IN PATIENTS WITH CARDIAC HYPERTROPHY
We analyzed n=7 patients with aortic stenosis (a pathology that engenders cardiac hypertrophy and failure) and found that they all had a significantly increased level of exosomes in their plasma. After substitution of the diseased valve with a minimally invasive technique - trancather aortic valve implantation (TAVI) - the level of exosomes decreased immediately to normal. Interestingly, we found that there was a linear correlation between heart wall stress and exosome number.

miR-1 IS AN EXOSOME CARGO THAT IS REDUCED WHEN THE HEART IS DE-STRESSED

Quantitaive real-time PCR analysis for total miR-1 in blood revealed that TAVI reduced the circulating level of this miRNA, while increasing it in the myocardium, suggesting that cardiac stress also in a clinical setting is accompanied by reduced intracellular levels of miR-1 on account of it being released into the circulation.

ANTHRACYCLINS AND EXOSOMES
We have started to study two cancer patients undergoing a treatment regimen consisting in six cycles of Adriamycin. We have found that extracellular vesicles are progressively released into the circulation as the treatment regimen proceeds. This was accompanied by an increase in circulating miR-1 and miR-133 levels. These data are indicative of microRNA-containing extracellular vesicles being an exquisite measure of on-going drug-induced cardiac stress.

Materials and Methods

We will recruit n=60 patients newly diagnosed with either non-Hodgkin or Hodgkin's lymphoma at Humanitas Hospital, using appropriate exclusion/inclusion criteria. Patients will be assessed cardiologically, at the start and end of their respective multi-drug treatment regimen (respectively, Rituximab, Cyclophosphamide, Adriamycin, Vincristine, Prednisone - R-CHOP - and Adriamycin, Bleomycin, Vinblastine, Dacarbazine - ABVD), with noninvasive exams (physical examination, blood pressure, echocardiogram, electrocardiogram) and blood biomarker analyses. For the latter, peripheral blood will be collected for assessment of the miRNA profile with quantitative real-time PCR and for troponins and brain natriuretic peptide levels with standard kits. In the follow-up schedule, samples will be collected during control visits at 1, 3, 6 and 12 months.
Clinical data will be analyzed with SYSTAT version 12.0, and expression data with Stata version 11/SE and GenEx, software.

Impact and Translational Implications

We expect to uncover a signature of circulating miRNAs that is altered upon chemotherapy. Some of the alterations seen in this signature will be due to release of cardiac-specific miRNAs as a result of cardiotoxicity independently from necrosis. Analysis of these miRNAs could enable clinicians to better identify patients at risk of chemotherapeutic-related cardiotoxicity, allowing alterations in the regimen before damage is done.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Etiology and prevention of type 1 diabetes
Project Code: PE-2013-02357094	Principal Investigator: Antonio Toniolo
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Regione Lombardia - Direzione Generale Sanità

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Metabolic and cardiovascular diseases	
Project Classification IRG:	Endocrinology, Metabolism, Nutrition and Reproductive Sciences
Project Classification SS:	Clinical and Integrative Diabetes and Obesity - CIDO
Project Keyword 1:	Genomic approaches that are designed to address questions regarding physiology or pathogenic mechanisms of diabetes and/or obesity.
Project Keyword 2:	Virus
Project Keyword 3:	Prevention
Project Request: Animals: ☐	Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐	Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright	

Italian Researcher Abroad – Foreign Operative Unit	
Name and Surname:	Alberto Pugliese, MD, PhD
Foreign Institution:	University of Miami Miller School of Medicine - 1450 NW 10th Avenue, Miami, FL 33136 USA
Department/Division/Laboratory:	Diabetes Research Institute - Network of Pancreatic Organ Donors (nPOD)
City-State and Country:	Miami, Florida, USA
Years of Residence Abroad:	15
Email: apuglies@med.miami.edu	Phone: +1-305-243.5348

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Regione Lombardia - Direzione Generale Sanità	Ospedale di Circolo e Fondazione Macchi, Varese - Microbiologia Clinica, Endocrinologia e Diabetologia, Endocrinologia Pediatrica	Determination of viral and immunologic markers in T1D specimens. Characterization of Italian T1D patients. Diagnosis, follow up, clinical database of cases, collection of biological samples. Analysis of Clinical/Laboratory data.
2	University of Miami Miller School of Medicine - 1450 NW 10th Avenue, Miami, FL 33136 USA	Diabetes Research Institute - Network of Pancreatic Organ Donors (nPOD)	American samples of diabetic organ donors (pancreas, lymphoid tissues) for search of biomarkers. Donors database with clinico-pathological data and laboratory results. Data analysis.

Project Title:
Etiology and prevention of type 1 diabetes

Project Code: PE-2013-02357094

Principal Investigator: Antonio Toniolo

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Regione Lombardia - Direzione Generale Sanità

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	SALVATONI ALESSANDRO		Selection of Italian pediatric patients with type 1 diabetes. Diagnosis and follow up. Clinical database, collection of biological samples, prescription of metabolic and genetic tests. Analysis of Clinical data and Laboratory results.	25/10/1951
2	Bartalena Luigi	Ospedale di Circolo e Fondazione Macchi, Varese - Unità Operativa di: Endocrinologia e Diabetologia	Selection of Italian adult patients with type 1 diabetes. Diagnosis and follow up. Clinical database, collection of biological samples, prescription of metabolic and genetic tests. Analysis of Clinical data and Laboratory results.	21/11/1950

Background and Significance

The National Diabetes Program has been implemented in Lombardy with two major aims: 1) improving the use of allocated health resources, and 2) improving prevention. Validated biomarkers capable of assessing the risk of T1D, its progression rate and complications are required to prevent the disease and to conduct clinical trials. Recent studies show that virus infections play a critical role in the etiology of T1D. Thus, virus detection is now included among alternative biomarkers (gene expression patterns, innate immunity, beta cell-specific T cells) that may be expressed earlier, be more predictive and/or more cost effective than classical markers. Likely, a combination of biomarkers will be required for predicting T1D and staging its progression.
The Virology and Diabetology group at the Ospedale di Circolo in Varese has been working with the Diabetes Research Institute in Miami to address the role of viral infections in T1D. Research was based on examination of specimens from pediatric and adult patients as well as from tissues of organ donors with diabetes. The major questions are:
1. Are enteroviruses associated with T1D? Is there an acute or chronic viral infection?
2. Can a relevant virus be identified? Are there differences between Italy and the US?
3. Which cells are infected (pancreas, islet cells, leukocytes from spleen, lymph node, blood)?
4. What functional consequences are brought on beta cells?
5. Is virus in blood a marker of pancreatic islet cells infection?

Specific aims

Aim 1: Are enteroviruses associated with T1D? Is there an acute or chronic viral infection? The question will be addressed using highly sensitive methods for measuring virus prevalence in T1D cases vs. non-diabetic controls. With the same methods, virus presence will be assessed in autoantibody-positive non-diabetics, T1D cases at the clinical onset, long-standing cases.

Aim 2: Can a relevant virus be identified? Are there differences between Italy and the US in terms of virus species/types that are associated with T1D? This point will be addressed by identifying the involved enteroviruses through immunologic and genomic approaches. It will be critical to assess if one or more specific enterovirus type(s) is/are associated with T1D both in two different populations.

Aim 3: Is virus in blood a marker of pancreatic islet cells infection? Blood is a convenient specimen for detecting

biomarkers. Using nPOD specimens it will be possible to assess whether the virus is present at the same time in peripheral blood, pancreatic islets, lymphoid tissues.

Hypothesis: An increasing body of evidence implicates enterovirus (EV) infection of pancreatic beta-cells in T1D pathogenesis. It has also become clear that, in T1D, such infections do not follow the typical course of acute infection with extensive cell lysis. Rather, infection appears to be atypical in that it does not cause acute damage to islet cells, but viral antigen and genome can be detected for prolonged times specifically in beta cells. In T1D, infections appear to exist in a persistent form, such that viral replication occurs very slowly and leads to subtle changes in islet cell physiology, which ultimately culminate in the development of autoimmunity rather than cell lysis. Thus, we assume that defective enterovirus type(s) have tropism for beta cells, that these cells are slowly damaged, that autoimmune response is induced to intracellular antigens and that virus- and immune-mediated damage causes progressive beta cell impairment and insulin deficiency.

Preliminary data: One strength of the present consortium is that it has access to pediatric and adult patient cohorts from a single geographic area (Varese, Italy) and to a unique biobank of tissues from organ donors with T1D (nPOD; Miami, Florida). Preliminary work showed that EVs of different species were detected in pancreas and lymphoid tissues of 4/8 nPOD cases (vs. 1/6 non-diabetic controls). Extensive work on over 130 Italian patients at the clinical onset and with long-standing disease showed that different EV species are present in 80% of cases at onset (vs. 4% in non-diabetics) and in a lower percentage in long-standing T1D cases. Notably, at onset, EV infections are spreading among family members without causing clinical symptoms. We showed that EVs are highly prevalent in blood at the clinical onset, but that can also be found up to 33 years of disease. In some cases, sequencing methods (that need to be improved) have identified CAV1, Echo30, CBV1 as viral agents associated to T1D.

Materials and Methods

Detection of virus markers: prior to RT-PCR, samples of T1D patients or pancreas donors are cultured on EV-susceptible cell lines in order to increase sensitivity of the assay. RNA is extracted from 0.6 ml of cell culture supernatant. Alignment-based primers for detecting virtually all EV types have been designed (5'UTR, 5'UTR-VP2, 3D RNA polymerase, 2C helicase regions). Specificity and sensitivity of the combined culture-PCR assays is superior to that of published tests. Performance: 18/19 CSF samples of post-polio cases positive for polioviruses up to 80 yrs after acute polio; 89/103 T1D cases EV-positive at the clinical onset. T1D cases were EV-positive from 1 month to 33 yrs after clinical onset. Direct RNA sequencing identified PV1, CAV1, CBV3, Echo30. Diabetes-related autoantibodies are determined by liquid-phase immunoassays. Measurement of peripheral blood T cells specific for pancreatic autoantigens is performed using in vitro proliferation assays and/or tetramer staining.

Ministero della Salute
Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti

BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero

Project Title:
Etiology and prevention of type 1 diabetes

Project Code: PE-2013-02357094

Principal Investigator: Antonio Toniolo

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Regione Lombardia - Direzione Generale Sanità

Project Type: PE- ITALIAN RESEARCHER ABROAD

Impact and Translational Implications

While both type 1 and type 2 diabetes result in hyperglycemia, the pathophysiology and etiology of the diseases are distinct and require different preventive measures. T1D incidence is increasing in Italy and the accumulating patients represent up to 0,7% of population. Preventive measures for T1D have been proposed, including viral vaccines. Preventive measures have to target at-risk subjects identified through viral, genetic, immunologic biomarkers now being validated for screening purposes.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Circulating cell-derived microvesicles in coronary artery disease: molecular signature, functional properties, and predictive value in coronary artery bypass graft patency
Project Code: PE-2013-02357476	Principal Investigator: Camera Marina
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Centro Cardiologico S.P.A. Fondazione Monzino
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Metabolic and cardiovascular diseases
Project Classification IRG: Vascular and Hematology
Project Classification SS: Atherosclerosis and Inflammation of the Cardiovascular System - AICS
Project Keyword 1: Atherosclerosis and Inflammation of the Cardiovascular System - AICS
Project Keyword 2: cell-derived microvesicles
Project Keyword 3: coronary artery bypass graft
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Mauro Perretti
Foreign Institution: Queen Mary & Westfield College
Department/Division/Laboratory: William Harvey Research Institute/Barts and The London School of Medicine/Centre for Biochemical Pharmacology
City-State and Country: London, United Kingdom
Years of Residence Abroad: 20
Email: m.perretti@qmul.ac.uk Phone: 00442078828782

BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero

Project Title:
Circulating cell-derived microvesicles in coronary artery disease: molecular signature, functional properties, and predictive value in coronary artery bypass graft patency

Project Code: PE-2013-02357476

Principal Investigator: Camera Marina

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Centro Cardiologico S.P.A. Fondazione Monzino

Project Type: PE- ITALIAN RESEARCHER ABROAD

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Centro Cardiologico S.P.A. Fondazione Monzino	Unit of Cellular and Molecular Biology	Evaluation of the molecular signature of circulating microvesicles in patients undergoing elective surgical myocardial revascularization.
2	Queen Mary & Westfield College	College William Harvey Research Institute/Barts and The London School of Medicine/Centre for Biochemical Pharmacology	Evaluation of the molecular mechanisms responsible for microvesicle heterogeneity according to the cell of origin and the stimulus responsible for their generation.
3	Università del Piemonte Orientale	Department of Health Sciences, School of Medicine, Laboratory of Immunopharmacology	Evaluation of the different effects evoked by different microvesicles on target cells relevant in CVD.

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Brunelleschi Sandra	Università del Piemonte Orientale	Coordinator Operative Unit 3.	25/06/1954
2	Perretti Mauro	Queen Mary & Westfield College	Coordinator Operative Unit 2.	11/05/1960
3	Brioschi Maura	Centro Cardiologico Monzino IRCCS	She will be in charge of the proteomic analysis of microvesicles.	18/12/1978

Background and Significance

Cell-derived microvesicles (MVs) are emerging as novel players in cardiovascular disease (CVD). They are involved in intercellular communication being vectors of biological messages and can participate in the pathophysiology and development of disease. Circulating MVs have been considered as biomarkers of vascular injury and inflammation in several CVD including atherothrombosis and myocardial infarction where elevated levels of MVs have been correlated with the severity of disease. MVs are heterogenous in nature, varying in size and content (proteins, lipids and mRNA), and are now recognized to play different roles on target cells depending on the cell of origin and the stimulus responsible for their generation. Graft patency and completeness of revascularization are major determinants of long-term outcome following coronary artery bypass grafting (CABG). Occlusion rates can be as high as 28% (per graft) or 45% (per patient) 12-18 months thereafter. Endothelial damage, inflammation and intimal hyperplasia are among the main candidates responsible for the early, and possibly late, graft failure. The issue of identifying predictors of graft patency after CABG has been addressed by several studies, which mainly focused on the presence of conventional risk factors, genetic markers, features of coronary targets, or technical aspects. Fewer studies focused on biological markers, such as perioperative inflammatory factors, but none has assessed the potential involvement of MVs.

Specific aims

Aim 1: Elucidate whether a specific "signature" of circulating MVs is associated with postoperative bypass graft

occlusion, in patients undergoing elective surgical myocardial revascularization (WP1).

Aim 2: Evaluate the effects and the global proteomic changes exerted by different cell-derived MVs prepared from healthy subjects and CAD patients (also looking for gender differences) on target cells relevant in atherosclerosis (monocytes, endothelial cells, EC, smooth muscle cells, SMC) (WP2).

Aim 3: Assess the contribution of Tissue Factor (TF)+ MVs, compared to that of TF+ platelets and monocytes, to the increased procoagulant phenotype associated with CVD (WP3).

Hypothesis: WP1: The patient's activation status before surgery might influence the long-term outcome following CABG. The MP signature (cell of origin, prothrombotic potential, proteomic profile and effects on target cells) is likely to reflect the activation state of endothelial and circulating cells.

WP2: MVs of different cell origin and generated by different stimuli may exert different effects (i.e. pro- or anti-inflammatory, pro- or anti-atherosclerotic, pro- or anti-thrombotic) on target cells relevant in CVD such as monocytes, EC, SMC.

WP3: MVs may exert a prominent role in the thrombotic complications of CVD by providing phosphatidylserine (PS) and TF on their membrane surface.

Preliminary data: WP1: OU1 will take advantage from an existing biobank of plasma samples prepared from a cohort of 330 consecutive patients enrolled for elective surgical myocardial revascularization at Centro Cardiologico Monzino, Milan (Fig.1). Patients underwent a 64-rows CT scan evaluation of graft patency after 18-24 months. Plasma samples (-80°C) were collected the day before CABG and at 18-month follow-up. Patent and occluded grafts were observed in 75% and in 25% of patients, respectively. No significant differences in clinical characteristics and in pharmacological treatment were observed among patients with patent and occluded grafts (Fig.2). By contrast flow cytometry analysis of MVs showed a significantly higher number of total and of Annexin V-, CD41-, CD62P-, TF-positive MVs in patients with occluded graft compared to those with patent graft (n=8 for each group; Fig.3-4).

WP2: UO1 verified the feasibility of MS analysis to characterize MV proteome on EC-derived MVs (Fig.5). OU3 set up conditions to assess oxyradical production, proinflammatory cytokine release, NF-kB activation and PPARg protein expression on human monocyte/macrophages stimulated with MVs of different cell origin (monocyte, M2 macrophages, neutrophils; Fig.6). OU2 set up conditions to study the heterogeneity of polymorphonuclear leukocyte-derived MVs according to the stimulus used to generate them (Fig.7). Functionality of MVs in vascular calcification was assessed in the BioHybrid system employing SMC (Fig.8).

WP3: OU1 verified the feasibility to compare the thrombin generation capacity of monocytes, platelets and MVs isolated from the same donor (Fig.9).

Project Title:
Circulating cell-derived microvesicles in coronary artery disease: molecular signature, functional properties, and predictive value in coronary artery bypass graft patency

Project Code: PE-2013-02357476

Principal Investigator: Camera Marina

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Centro Cardiologico S.P.A. Fondazione Monzino

Project Type: PE- ITALIAN RESEARCHER ABROAD

Materials and Methods

MVs will be characterized by flow cytometry (FACSAriaIIu) on plasma samples collected before CABG and one year after. Antibodies will be used to establish the cell origin (CD41, CD31, CD45, glycophorin etc) and the activation state (AnnexinV, CD62p, PAC-1, TF etc).
MV pro-thrombotic potential will be measured using the Calibrated Automated Thrombogram (Thrombinoscope).
Proteomic profiling of MVs and MV-treated cells will be analysed on a hybrid quadrupole orthogonal acceleration time-of-flight Q-Tof mass spectrometer with an ESI source, Synapt-MS, connected to a Nano-Acquity UPLC using a label free quantitative LCMSE method.
MV functional properties (calcification, proliferation, migration, apoptosis, autophagy) on target cells will be assessed by the BioHybrid system (a fast surrogate readout for in vivo bio-activity of MVs) as well as by evaluation of superoxide anion production, cytokine release, NFkB, PPARg protein and gene expression, NO release on human monocytes and HUVEC.

Impact and Translational Implications

This multi-faced approach will extend the knowledge in the field of cell-derived MVs a) revealing whether a pre-existing circulating MV signature may be associated with or predict long-term CABG outcome. If this were the case, one could think of a pharmacological intervention aimed at reducing the activation status of the patient before surgery; b) unveiling the relative contribution of the different cell-derived MVs on key cellular processes, involved in the onset and progression of CVD.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: The oncogenic potential of the AID/APOBECs: involvement in tissue transformation and oncogenesis - new tools to better model cancer
Project Code: PE-2013-02357669	Principal Investigator: Conticello Silvestro
Research Type: Biomedical/Biomedica	Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Oncology
Project Classification IRG: Oncology 1 - Basic Translational
Project Classification SS: Cancer Etiology - CE
Project Keyword 1: DNA adducts, DNA damage and repair mechanisms, metabolism of endogenous and exogenous compounds that modulate early events in carcinogenesis
Project Keyword 2: DNA/RNA editing
Project Keyword 3: Oncogenes
Project Request: Animals: [X] Humans: [X] Clinical trial: [ ]
The project has already been presented: [ ] Project code reference:
[ ] I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Pier Paolo Pandolfi
Foreign Institution: Beth Israel Deaconess Cancer Center
Department/Division/Laboratory: Division of Genetics, Department of Medicine
City-State and Country: Boston, MA, USA
Years of Residence Abroad: 18
Email: ppandolf@bidmc.harvard.edu Phone: +1-617-735-2121

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Toscana	Core Research Laboratory - Istituto Toscano Tumori, Azienda Ospedaliero Universitaria Careggi, Firenze	Coordinator. Aims 1, 2, 3
2	Beth Israel Deaconess Cancer Center	Division of Genetics, Department of Medicine	Aim 3: Analysis of the murine models
3	IFOM - Istituto FIRC di Oncologia Molecolare	DNA Editing in Immunity and Epigenetics	Part of Aims 2 and 3

Project Title:
The oncogenic potential of the AID/APOBECs: involvement in tissue transformation and oncogenesis - new tools to better model cancer

Project Code: PE-2013-02357669

Principal Investigator: Conticello Silvestro

Research Type: Biomedical/Biomedica

Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Petersen-Mahrt Svend	IFOM - Istituto FIRC di Oncologia Molecolare	Part of Aims 2 and 3	30/08/1967

Background and Significance

The AID/APOBECs are cytosine deaminases targeting nucleic acids to induce DNA damage. Each of these enzymes plays a central role in its physiological context, however their ability to mutate comes at a steep price: AID, the trigger of the antibody diversification processes, is essential in the onset of mature B-cells tumors, while the mutational signature of the AID/APOBECs is present in the genomes of many tumors.
The overall aim of the proposal is to investigate the specific mechanisms that affect AID/APOBECs activity in cancer development, while at the same time develop them into novel tools to model cancer. We will focus on APOBEC1, an RNA editing enzyme which we have recently demonstrated to act as a DNA mutator in human cells, and whose aberrant expression could be linked to the onset of genetic alterations in esophageal adenocarcinomas. Indeed, transgenic mice overexpressing APOBEC1 in the liver develop hepatocellular carcinomas, while APOBEC1 deficiency in a cancer-prone mouse model reduces the onset of colon tumors.
Modelling specific human cancer mutations in animal models has provided few breakthroughs. On the other hand, intracellular expression of AID/APOBECs has been used to evolve endogenous proteins, and their mutation profile can be detected in human tumour suppressor loci. Hence, in vivo targeting DNA deaminases to oncogenic loci will select the required DNA lesions for transformation and thus provide a novel approach in modelling neoplasia.

Specific aims

Aim 1: Dissect the oncogenic potential of APOBEC1 - We will assess the rise of tumorigenic features in tissues/cells expressing APOBEC1, either wild type or mutant. Beyond its mutagenic activity, APOBEC1 has been shown to alter the cellular state by targeting RNA molecules. We will thus explore this interplay between DNA and RNA targeting. We will identify the cellular processes altered by APOBEC1 expression and whether specific genomic regions are susceptible to its action.

Aim 2: Identify factors that are associated, positively or negatively, with APOBEC1 expression during the early phases of cancer development - Due to the characteristic expression pattern of APOBEC1, we will compare tissues in which APOBEC1 is physiologically expressed (e.g. small intestine) with those in which it is not (e.g. esophagus). We will compare upstream (e.g. DNA targeting) and downstream (e.g. DNA repair) pathways in these cells, and whether physiological interactors of APOBEC1 provide protection against its mutagenic activity.

Aim 3: Exploit the mutagenic activity of the AID/APOBECs to model cancer onset/progression - We will target oncogene and tumour suppressor loci for continuous mutagenesis using chimeras of AID/APOBECs and Transcription-Activator-Like-Effectors (TALEs). The targeted DNA lesion generation will be characterised in the context of different cellular states and pathways (e.g. cell cycle state and/or DNA repair pathways).

Hypothesis: The oncogenic potential of these deaminases depends on factors that affect their mutagenic activity, either directly (e.g. ability to target RNA, turnover kinetics) or indirectly (association to target specific loci, pathway choice in DNA repair). Identification of these factors will elucidate the effective role of APOBEC1 in cancer development.
Our tissue and cell specific analysis will shed light on the local requirements for their mutagenic activity, while the continuous site-specific DNA damage model will generate a global approach to oncogenesis.

Project Title:
The oncogenic potential of the AID/APOBECs: involvement in tissue transformation and oncogenesis - new tools to better model cancer

Project Code: PE-2013-02357669

Principal Investigator: Conticello Silvestro

Research Type: Biomedical/Biomedica

Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

Preliminary data:

1) We have selected mutants of APOBEC1 that lack editing activity on RNA while retaining their ability to mutate bacterial DNA. We have tested assays in which overexpression of APOBEC1 leads to acquisition of tumorigenic features (soft-agar, transformation, self-renewal). We have developed a deep-sequencing approach that allows the analysis of the mutation rate at specific loci up to 0.002%.
2) We have selected genes that are differentially expressed between mucosa from small intestine, Barrett's esophagus and esophageal adenocarcinoma. We have developed an assay to measure the efficiency of specific pathways of DNA repair in cellular extracts.
3) We have verified expression and targeting of TALE-AID and TALE-APOBEC1 fusion proteins in cell lines. We verified their potential as DNA mutagens and sequence specific DNA binding factors. We also generated transgenic TALE-AID zebrafish lines.

Materials and Methods

1) We have developed assays in our labs to identify mutants and test for mutator phenotypes. Standard techniques will be used to test the oncogenicity of APOBEC1 and its mutants (substrate-independent growth, self-renewal, tumorigenicity in mice).
2) Knock-in/out of selected genes will be performed in APOBEC1-expressing cells. The mutagenic outcomes will be assessed in these cells through standard assays. The efficiency of DNA repair pathways will be assayed through a built-to-purpose assay developed in the labs.
3) TALE-AID and TALE-APOBEC1 (and catalytic mutants of the deaminase motif) fusion proteins will be expressed in cell lines and targeted to the p53 locus. We will assess the mutagenic potential (deep sequencing) and cellular transformation activity (see above). Analogous TALE-AID fusion proteins will be generated to induce p53 mutations and tumours in transgenic animals.

Impact and Translational Implications

Understanding the processes mediating the oncogenic potential of APOBEC1 could identify risk factors involved in cancer development and provide the rationale to improve current diagnostic protocols for tumors in which APOBEC1 is involved.
AID/APOBEC-targeted murine models of human cancer can lead to the development of a more 'physiological' modelling of cancer that could be used to test and develop novel pharmacological approaches for patient care.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Coagulation/complement activation and cerebral hypoperfusion in relapsing-remitting multiple sclerosis
Project Code: PE-2013-02357745	Principal Investigator: Koudriavtseva Tatiana
Research Type: Biomedical/Biomedica	Applicant Institution: Istituti fisioterapici ospitalieri - Istituto Regina Elena

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases
Project Classification IRG: Brain Disorders and Clinical Neuroscience
Project Classification SS: Clinical Neuroscience and Neurodegeneration - CNN
Project Keyword 1: Neuroimaging, functional, biochemical, and neuropathological studies to assess the onset, progression, treatment, and development of biomarkers for brain disorders.
Project Keyword 2: Multiple Sclerosis
Project Keyword 3: Coagulation
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Maria Matilde Inglese
Foreign Institution: Mount Sinai Hospital
Department/Division/Laboratory: Department of Neurology
City-State and Country: New York, NY, USA
Years of Residence Abroad: 11
Email: matilde.inglese@mssm.edu Phone: 001-212-241-4379

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituti fisioterapici ospitalieri - Istituto Regina Elena	Unit of Neurology, Multiple Sclerosis Regional Centre	Study design and coordination. Patient enrollment and clinical evaluation. Clinical, biochemical and MRI data interpretation. Publication of the study results.
2	Mount Sinai Hospital	Department of Neurology, Unit of Imaging Research	MRI data elaboration and interpretation.
3	Istituti fisioterapici ospitalieri-Istituto Regina Elena	Unit of Clinical Pathology	Laboratory testing and biochemical data interpretation.

Project Title:
Coagulation/complement activation and cerebral hypoperfusion in relapsing-remitting multiple sclerosis

Project Code: PE-2013-02357745

Principal Investigator: Koudriavtseva Tatiana

Research Type: Biomedical/Biomedica

Applicant Institution: Istituti fisioterapici ospitalieri - Istituto Regina Elena

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Conti Laura	Istituti fisioterapici ospitalieri-Istituto Regina Elena	Laboratory testing and biochemical data interpretation.	24/11/1955
2	D'AGOSTO GIOVANNA	Istituti fisioterapici ospitalieri-San Gallicano	Laboratory testing and biochemical data interpretation.	27/02/1966
3	Anelli Vincenzo	Istituti fisioterapici ospitalieri-Istituto Regina Elena	MRI performing, MRI data elaboration and interpretation.	20/06/1958

Background and Significance

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system. It has been demonstrated that not only adaptive immunity but also innate immune system plays a relevant role in MS pathogenesis (Gandhi 2010; Mayo 2012). Innate immunity represents the immediate nonspecific defense against infections acting through its essential arms such as inflammation and coagulation that can regulate each other in a concerted action when activated (Esmon 2011). There are several studies confirming platelet (Horstman 2010;Nurden 2011;Behari 2013) and complement (Horstman 2011;Veerhuis 2011;Ingram 2012) involvement in MS, having an important role in the innate immune response by linking inflammation and coagulation.
Moreover, decreased cerebral blood volume and flow, as well as its prolonged mean transit time, have been demonstrated in all forms of MS, both in white and grey matter, by dynamic susceptibility contrast-enhanced MRI (Adhya 2006, Inglese 2007, Varga 2009). It may be hypothesized that the widespread cerebral hypoperfusion in MS may prevalently be determined by blood flow deceleration in the venous bed due to the inflammatory-thrombotic processes (Koudriavtseva 2014).
The aim of our study is to evaluate the serum/plasma levels of coagulation/complement factors and to assess the presence of brain hemodynamic changes in relapsing MS patients compared to remitting ones and to healthy controls in order to correlate patient coagulation status with MRI perfusion data.

Specific aims

Aim 1: To evaluate the serum/plasma concentrations of complement/coagulation factors [complement C3, C4, C4a and C9, factor II and VIII, D-dimer, antithrombin, protein C and S, von Willebrand factor, soluble thrombomodulin and soluble Endothelial Protein C Receptor, antiphospholipid antibodies, lupus anticoagulant, complete blood count] in 3 groups of subjects: relapsing-remitting (RR)MS pts in relapse (REL), RRMS pts in remission (REM) and age- and sex-matched healthy controls (CTR)

Aim 2: To evaluate absolute cerebral blood flow (CBF), blood volume (CBV) and mean transit time (MTT), by dynamic susceptibility contrast-enhanced 3.0-T MRI in the same 3 groups

Aim 3: To correlate the serum/plasma levels of complement/coagulation factors with both MRI perfusion data and demographic/clinical (age, gender, disability and disease duration) features of MS pts

Hypothesis: The activation of coagulation/complement system with pro-inflammatory cerebral endothelial alteration could determine the deceleration of CBF/MTT and decrease of CBV during the inflammatory-thrombotic processes in the course of MS relapse compared to remission and healthy controls

Project Title:
Coagulation/complement activation and cerebral hypoperfusion in relapsing-remitting multiple sclerosis

Project Code: PE-2013-02357745

Principal Investigator: Koudriavtseva Tatiana

Research Type: Biomedical/Biomedica

Applicant Institution: Istituti fisioterapici ospitalieri - Istituto Regina Elena

Project Type: PE- ITALIAN RESEARCHER ABROAD

Preliminary data:

We identified by proteomics analysis differently expressed serum proteins: ceruloplasmin, clusterin, apolipoprotein E and in particular anti-thrombin (AT) and complement C3 in 18 MS pts compared to 7 CTR. Moreover, AT has been found oxidatively modified in relapse compared to remission reinforcing the importance of the coagulation system in MS (Fiorini, Koudriavtseva et al. PLoS One. 2013).

We also tested sera from 58 REM, 26 REL, 16 pts with secondary progressive (SP)MS and 60 CTR for a large spectrum of antiphospholipid antibodies (aPL) using enzyme immunoassays. The overall rate of positivity for at least one aPL was significantly higher in MS pts compared to CTR (32% vs 7% respectively, $p<0.0001$), and in REL compared to REM and SPMS (53.8, 20.7 and 37.5% respectively, $p=0.002$). In the single aPL analysis, the rate of positivity was significantly higher in MS pts compared to CTR for anti-prothrombin IgM (7% vs 0, $p=0.05$), and in REL compared to REM and SPMS for anti-ß2glycoproteinI IgM (26.9, 1.7, 6.3% respectively, $p<0.0001$), anti-prothrombin IgM (15.4, 3.4, 6.3% respectively, $p=0.05$) and IgG (19.2, 5.2, 0% respectively, $p=0.05$). These data suggest that aPL occurrence in MS could be an expression of inflammatory-thrombotic processes during the relapse (Koudriavtseva et al. Neurol Sci 2014). In the same pts anti-annexinV IgG were associated with high total and low density cholesterol levels supporting the relevance of thrombogenic mechanisms in MS (Mandoj et al, submitted to J Neuroimmunol).

Previously, hypoperfusion and especially decreased CBF has been demonstrated in all forms of MS and even in clinically isolated syndrome, both in normal-appearing white and grey matter and in lesions, by dynamic susceptibility contrast-enhanced MRI (Adhya 2006, Inglese 2007, Varga 2009).

Materials and Methods

Informed and consenting MS pts (1° group: REL; 2° group: REM) and age- and sex-matched CTR (3° group) will be enrolled in the study. Pts' level of disability will be evaluated using the EDSS score and MSFC score.

All pts and CTR will be tested for complement/coagulation and soluble markers of endothelial damage assays, and will undergo dynamic susceptibility contrast-enhanced MRI using a 3.0-T scanner to evaluate CBF, CBV and MTT, lesion number and volume.

Sample size calculation

Overall 90 subjects (30 for each group) will be enrolled in order to compare the level of complement C4a (Ingram 2012). By using the ANOVA test, this sample size will allow to detect effect size values [delta=(miA-miB)/sigma] equal to at least 0.71, with a statistical power of 80%, to a level of significance of 5%.

Statistical Analysis

To measure differences between MS pts and CTR unpaired t-tests will be used; univariate correlations will be calculated using Spearman rank correlation coefficient.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Coagulation/complement activation and cerebral hypoperfusion in relapsing-remitting multiple sclerosis
Project Code: PE-2013-02357745	Principal Investigator: Koudriavtseva Tatiana
Research Type: Biomedical/Biomedica	Applicant Institution: Istituti fisioterapici ospitalieri - Istituto Regina Elena

Project Type: PE- ITALIAN RESEARCHER ABROAD

Impact and Translational Implications

Identifying a link between activation of coagulation/complement system and cerebral hypoperfusion in MS pts could lead to the development of new effective therapeutic strategies for MS pts. Even if the activation of coagulation system, linked to innate immunity, is a mandatory process following different types of tissue damage and it is not the primary cause of the formation of demyelinating plaque, interfering with coagulation system could represent a new therapeutic target in MS.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Chronic Kidney Disease as a Dysmetabolic Determinant of Disability among Older People (CKD-3D)
Project Code: PE-2013-02357826	Principal Investigator: Corsonello Andrea
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Istituto Nazionale di Riposo e Cura per Anziani

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in
Metabolic and cardiovascular diseases

Project Classification IRG: Population Sciences and Epidemiology

Project Classification SS: Kidney, Nutrition, Obesity and Diabetes - KNOD

Project Keyword 1: Development and improvement of research designs and methodologies addressing epidemiologic questions in kidney diseases/conditions, obesity, diabetes, gastro-intestinal conditions, environmental and nutritional influences on health outcomes in human populations in relation to time, place, and personal characteristics.

Project Keyword 2: Disability and geriatric assessment

Project Keyword 3: Older patients

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

The project has already been presented: ☐ Project code reference:

☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit

Name and Surname: Francesco Mattace-Raso

Foreign Institution: Erasmus University Medical Center Rotterdam

Department/Division/Laboratory: Department of Internal Medicine, Division of Geriatrics

City-State and Country: Rotterdam, The Netherlands

Years of Residence Abroad: 15

Email: f.mattaceraso@erasmusmc.nl Phone: +31 10 703 59 79

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Nazionale di Riposo e Cura per Anziani	Research Hospital of Cosenza, Unit of Geriatric Pharmacoepidemiology	Principal investigator
2	Erasmus University Medical Center Rotterdam	Department of Internal Medicine, Division of Geriatrics	Study design, training and data analysis
3	University of Parma	Geriatric and Rehabilitation Dpt, Section of Geriatrics	Study design, data collection and analysis

Project Title:
Chronic Kidney Disease as a Dysmetabolic Determinant of Disability among Older People (CKD-3D)

Project Code: PE-2013-02357826

Principal Investigator: Corsonello Andrea

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Istituto Nazionale di Riposo e Cura per Anziani

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Maggio Marcello	University of Parma	Study design, data collection and analysis	06/01/1969
2	Mattace Raso Francesco	Erasmus University Medical Center Rotterdam, Department of Internal Medicine, Division of Geriatrics	Study design, training and data analysis	16/12/1967

Background and Significance

Chronic kidney disease (CKD) in the older population represents a relevant public health burden, resulting in an increased risk of morbidity and mortality. Current evidence suggests that CKD in older patients is harmful but treatable if patients at risk are correctly identified. However, currently available creatinine-based measures of kidney function are plagued by some degree of inaccuracy (Pedone et al 2006; Corsonello et al 2011). Furthermore, several studies showed the existence of a U-shaped relationship between creatinine-based glomerular filtration rate (eGFR) and mortality in frail and older people(Cox et al 2008; Tonelli et al 2011; Shastri et al 2012; Peters et al 2013), while other filtration markers not affected by muscle loss may better predict negative outcomes (Foster et al 2013). Finally, CKD also affects outcomes relevant to older people, such as frailty, disability, cognitive impairment, depression, malnutrition, sarcopenia, and adverse drug reactions(Duenhas et al 2003; Seliger et al 2004; Shlipak et al 2004; Corsonello et al 2005; Foley et al 2007; Yaffe et al 2010; Pedone et al 2012; Reckert et al 2013; Walker et al 2013; Lattanzio et al 2012). Despite this bulk of epidemiological evidence, a comprehensive study aimed at investigating CKD among older patients taking into account innovative biomarkers, as well as the numerous functional dimensions that CKD is able to impair does not currently exist.

Specific aims

Aim 1: To build an observational database specifically targeting Chronic Kidney Disease (CKD) and its consequences among older patients discharged from acute care hospital.

Aim 2: To investigate the relationship between kidney function and major determinants of disability, including frailty, mobility limitation and dependency, cognitive impairment, depression, malnutrition, sarcopenia, quality of life and adverse drug reactions, among older patients discharged from acute care hospitals.

Aim 3: To investigate whether innovative biomarkers of kidney function, such as cystatin C, beta-trace protein and beta2-microglobulin are able to predict prognosis and to individuate patients carrying disability determinants with better accuracy compared to creatinine-based measures. The interactions between different measures of kidney function and major determinants of disability in predicting prognosis, including overall mortality, cardiovascular (CV) mortality, CV events, and use of health care resources, will be also investigated.

Hypothesis: Chronic kidney disease (CKD) is an important public health problem that is characterized by poor health outcomes, high incidence of disability and very high health care costs. Since the prevalence of CKD is higher in older people, the impact of population aging on the healthcare systems will depend also on how the community responds. Since the determinants, trajectories, and interacting factors of CKD-related disability are not fully elucidated, we hypothesize that an observational prospective study including real-world patients aged 75 years or more, measurement of innovative biomarkers and systematic use of comprehensive geriatric assessment may provide useful data to develop sound interventions in the

community. We hypothesize that such a study may lead to significantly improvement in knowledge about CKD in older patients, clinical practice, and decision making.

Preliminary data: Over the past 10 years we worked on the clinical application of equations for estimating renal function in elderly patients in relation to several outcomes (Corsonello et al 2005; Corsonello et al 2006; Pedone et al 2006; Pedone et al 2008; Corsonello et al 2011; Pedone et al. 2011), including mortality (Corsonello et al 2010; Corsonello et al 2010), adverse drug reactions (Corsonello et al 2011), worsening disability (Pedone et al 2012), and physical performance as objectively measured by the Short Physical Performance Battery (Lattanzio et al 2012). More recently, we reported a U-shaped relationship between eGFR and mortality among community-dwelling nonagenarians using the Berlin Initiative Study 1 (BIS1) equation (Montesanto, et al 2014).

Materials and Methods

We will carry out a prospective observational study of patients aged 70 or more discharged from participating acute care geriatric wards. The outcomes of the study will be: eGFR decline, mortality, hospital admissions and number of in-hospital days, occurrence of adverse drug reactions (ADRs), physical performance, dependency, cognitive impairment, depression, malnutrition, quality of life, services utilization. Assessment will include: Demographics and pre-enrollment data, clinical variables (including diagnoses and medications), laboratory parameters (including innovative kidney function biomarkers), comprehensive geriatric assessment (CGA), healthcare resource consumption, quality of life. The duration of follow-up will be 12 months after discharge.

Impact and Translational Implications

The project will significantly impact current knowledge about CKD in older patients in the context of a real world observational study. The project will allow to compare the ability of traditional and innovative biomarkers in predicting trajectories of disability over time. The systematic use of CGA will be extremely useful to investigate these outcomes. The project findings might therefore support changes in clinical practice and decision making.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: A registry of severe malaria in returning travellers from endemic countries: clinical outcome and adverse events including haematological and pharmacokinetic data. NetwOrk for severe MALaria treatment - NOMAL study
Project Code: PE-2013-02357936	Principal Investigator: Nicastri Emanuele
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Istituto per le Malattie Infettive Lazzaro Spallanzani
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Infectious and immunological diseases
Project Classification IRG: Infectious Diseases and Microbiology
Project Classification SS: Clinical Research and Field Studies of Infectious Diseases - CRFS
Project Keyword 1: Design and execution of investigator-initiated clinical studies for testing agents or strategies for preventing or treating infectious diseases
Project Keyword 2: Registry of imported severe malaria cases: clinical outcome
Project Keyword 3: Registry of imported severe malaria cases: drug monitoring, pitting rate and adverse events
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☒
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Umberto d'Alessandro
Foreign Institution: Medical Research Council Unit, The Gambia
Department/Division/Laboratory: Director
City-State and Country: Banjul, The Gambia Atlantic Boulevard, Fajara P. O. Box 273.
Years of Residence Abroad: 20
Email: udalessandro@mrc.gm Phone: +(220) 4496715

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto per le Malattie Infettive Lazzaro Spallanzani	Clinical and clinical research department	Principal investigator ¿Enrollment of severe malaria cases¿Acquisition of IA&IQ. ¿IA&Q use, clinical outcome, drug monitoring and adverse event registry¿Coordination of the project
2	Medical Research Council Unit, The Gambia	Directorate	Italian researcher abroad. Study design; protocol definitions; Supervision of IQ&IA drug monitoring; Supervision of hematological assay including pitting phenomenon MD & PhD training activities

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: A registry of severe malaria in returning travellers from endemic countries: clinical outcome and adverse events including haematological and pharmacokinetic data. NetwOrk for severe MALaria treatment - NOMAL study
Project Code: PE-2013-02357936	Principal Investigator: Nicastri Emanuele
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Istituto per le Malattie Infettive Lazzaro Spallanzani

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1				

Background and Significance

Around 800 to 1000 cases of imported P. falciparum malaria per year are reported to the TropNet surveillance system, approximately 4% of all imported malaria cases progress to severe malaria, and the overall mortality rate of imported Plasmodium falciparum malaria in Europe is 0.4%. WHO guidelines recommend intravenous artesunate (IA) as first-line therapy for severe malaria, but it is not still approved by the European Medical Agency, EMA and Italian Drug Agency, AIFA. In few excellence centres in Italy, there is an increasing use of IA due to evidence-based clinical benefits, but all over the country, most clinical centres still use intravenous quinine (IQ), an old antimalarial drug with a well- known efficacy and toxicity profiles. Finally, patients with severe malaria admitted in ICU often experience the combined use of both IQ & IA. Recently, first reports of post-artesunate delayed hemolysis (PADH), raise concerns on a drug until now considered to have a good toxicity profile in endemic countries.
In our proposed cohort of patients with severe malaria, efficacy and safety profiles jointly with in-vivo pharmacological and haematological data will be collected. Implementing observational trials reporting pharmacological parameters, pharmacy surveillance, drug resistance and clinical outcome could constitute a major step to choose the best therapy for severe malaria in non-endemic countries.

Specific aims

Aim 1: Severe malaria registry: to develop an Italian cohort of severe malaria cases in returning travellers from malaria endemic countries in 6 national sentinel sites.

Aim 2: Severe malaria efficacy study: to assess epidemiological, diagnostic, therapeutic and drug resistance parameters likely to be related to the clinical outcome of patients.

Aim 3: Severe malaria toxicity study: to assess haematological and pharmacological parameters likely to be related to the occurrence of adverse events: peak and trough determination, area-under-the-curve (AUC) of IA and IQ drug concentration and pitting hematological data.

Hypothesis: After the WHO recommendations of IA as first line therapy in severe malaria, IQ is currently replaced by an increasing use of this regimen in Italy. The occurrence of a PADH in one fifth of treated patients in non-endemic countries raises major concerns on a drug until now considered to have a good toxicity profile in endemic countries. In non-endemic countries, efficacy and toxicity data on severe malaria cases treated with different regimen can only be obtained by national registries (see data from the French artesunate surveillance programme) or in tropical diseases surveillance networks (see data from TropNet or GeoSentinel). In Italy until now no data registry on severe malaria cases exist.

Preliminary data: In 1984-2003, 507 patients (pts) were admitted at Spallanzani institute for malaria; 393 (77%) with P. falciparum infection. Severe malaria was reported in 59 (11.6%) pts: of them, 11, all with severe P. falciparum infection from Africa died. The overall case fatality rate was 2.2%, and the case-fatality rate of patients with severe malaria was 18.6%. Lack of chemoprophylaxis and time to defervescence were independently associated with severe malaria occurrence.
More recently in last 6 years, 67 pts were diagnosed as severe malaria: 10, 14.9% with SNC

involvement, 31, 46.3% with liver involvement; 61, 91% were resident in Italy but 23, 34.3% were immigrants; 49, 73% came from West Africa; 61, 91% did not receive any prophylaxis. 18, 27.7% were treated with IA as first line regimen; 10, 14.9% were treated with both IA&IQ all over the in-hopital stay. No patient died, but an independent association between more severely affected patients and cerebral involvement and country origin (West Africa) was reported. No significant difference in clinical outcome was reported between IQ and IA treated patients. However, in 2011-2 we reported two cases of severe malaria with PADH, the first patient treated with oral arthemeter/lumefantrin only, and the second patient treated with iv and oral artemisinine derivatives. Both patients fully recovered after blood transfusions. In recent literature, a 22-24% of PADH has been reported in TropNet cohort and in the French Registry. Finally, D'Alessandro recently reported an alarming case of a traveler from Angola with severe malaria not responsive to iv and oral artemisinine derivatives

Materials and Methods

NOMAL is a cohort of severe malaria cases enrolled in 6 centres: Spallanzani Institute, Rome; Brescia University; Negrar Hospital, Verona; Turin University; La Sapienza Rome University; Naples II University. 70 severe malaria cases treated with IA, IQ, or both drugs accoording to the single center policy, will be enrolled in 3 years.All patients will sign an informed consent (IC). Inclusion criteria: 1. Patients with severe malaria. Exclusion criteria: 1. Patients who cannot sign IC. IA and IQ will be used according to WHO guideline. Oral therapy will be placed as soon as possible. Daily, patients will be monitored for time to parasite clearance, defervescence, discharge, and adverse events. IQ and IA drug levels will be performed by HPLC. Pitting rate will be quantified by fluorescence microscopy and flow cytometry. In case of clinical failure, drug resistance will be performed. D'Alessandro will provide protocol & study supervision and clinical and therapeutic training activities.

Impact and Translational Implications

IA is not approved nor available in most Italian hospitals. Despite IA has been proven to be safe and effective, the emergence of data on PADH could represent a major concern for clinicians. IQ, current standard of care in Italy, has less rapid parasite clearance, cardiotoxicity and hypoglycemic effect. NOMAL data could help choosing the best antimalarial regimen in order to improve safety and toxicity and to assess clinical outcome of patients with severe malaria in non-endemic countries.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Virtual and Augmented Reality Support for Transcatheter Valve Implantation by using Cardiovascular MRI
Project Code: PE-2013-02357974	Principal Investigator: Positano Vincenzo
Research Type: Biomedical/Biomedica	Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Metabolic and cardiovascular diseases	
Project Classification IRG:	Surgical Sciences, Biomedical Imaging, and Bioengineering
Project Classification SS:	Biomedical Imaging Technology - BMIT
Project Keyword 1:	Development of image-based methods and strategies to characterize tissue or for the support of image-guided surgical or physical interventions that require high performance computing and display of images for interactive man-machine environments that simultaneously, or sequentially, diagnose, plan, treat, update, and follow-up.
Project Keyword 2:	image-guided surgery
Project Keyword 3:	Finite Element Modeling
Project Request: Animals: [X]	Humans: [ ] Clinical trial: [ ]
The project has already been presented: [X]	Project code reference: PE-2011-02346884
[ ] I declare that the object/s of this application is/are under patent copyright	

Italian Researcher Abroad – Foreign Operative Unit	
Name and Surname:	Claudio Capelli
Foreign Institution:	UCL (University College London)
Department/Division/Laboratory:	Institute of Cardiovascular Science
City-State and Country:	London, United Kingdom (UK)
Years of Residence Abroad:	5
Email: c.capelli@ucl.ac.uk	Phone: +44 (0) 75 333 422 36

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Toscana	Fondazione Toscana G Monasterio, Clinical Engineering Department, Radiology Department, Pediatric and GUCH Catheterization	Coordination of the Project; Medical advice, MR image acquisition and animal experiments
2	UCL (University College London)	Institute of Cardiovascular Science	Mechanical modelling of patient specific procedure by Finite Element Analysis
3	University of Pisa	ENDOCAS- Center for Computer Assisted Surgery, Department of translational research and new technology in medicine	Integration of the navigation system with patient specific MR data and Finite Element Analysis models

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Virtual and Augmented Reality Support for Transcatheter Valve Implantation by using Cardiovascular MRI
Project Code: PE-2013-02357974	Principal Investigator: Positano Vincenzo
Research Type: Biomedical/Biomedica	Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Ferrari Vincenzo	University of Pisa	Coordination of ENDOCAS activity	27/06/1976
2	Schievano Silvia	Institute of Cardiovascular Science, UCL (University College London)	Coordination of UCL activity	11/03/1978
3	Spadoni Isabella	Fondazione Toscana G Monasterio, Massa,Head of Pediatric and GUCH Catheterization Laboratory	MD advice and animal model experiments	12/12/1955
4	Chiappino Dante	Fondazione Toscana G Monasterio, Pisa, Director of the Radiology Department	MD advice and coordination of animal experiments	08/12/1954

Background and Significance

Patients with congenital heart defects (CHD) and associated valve diseases may undergo several open heart surgeries over their lifetime, with correlated increasing peri- and post-surgical risk. Transcatheter Valve Implantation (TVI) enables treatment of valvular disease with no need for open heart surgery, improving the overall condition of the heart and facilitating patient recovery. However, application of TVI, especially to patient with congenital heart disease, still poses many challenges. First, the implantation site of these patients is highly variable in size, geometry and dynamics, so an accurate planning of the suitability of the currently available device in respect to patient-specific anatomy is needed. Secondly, during TVI procedure the precision in the placement of the device is critical for the optimal treatment outcome. As the TVI procedure is conducted by X-ray fluoroscopy, a high radiation dose may be needed for precise implantation, with a high associated radiological risk especially in the paediatric population. These drawbacks have still limited the use of TVI to a relative low number of patients, as stated in the current guidelines. To overcome the above cited limitations and to extend the benefit of such minimally invasive approach, this work aims to improve the current patient selection for TVI procedure and reduce the associated procedural comorbidities by integrating Magnetic Resonance Imaging (MRI) with computer based navigation system.

Specific aims

Aim 1: To develop a TVI planning (TVI-PLAN) tool. A software application will be implemented providing planning of TVI procedures as based on realistic simulations of catheter insertion, movement, operation and device implantation. The TVI-PLAN will include the input of MR data set, as it is standardly acquired prior to the intervention in patients scheduled for TVI. Hence, the patient-specific vascular tract will be modelled by mean of a geometrical mesh. Distensibility of cardiovascular structures will be also modelled by combining imaging data with physiological parameters. Finite element analysis will be performed to simulate the implantation of the TVI device. The simulation will allow a prediction of the interventional feasibility including assessment of mechanical stability of each specific implant.

Aim 2: To develop a TVI procedural assisting device (TVI-AD). Purpose of this device is reducing the need of X-rays and to accurately guide the valve implantation as optimized by TVI-PLAN. TVI-AD will integrate the graphic user interface (GUI) of a navigator system to monitor the real time position and orientation of the endovascular tools (guidewires, catheters and stent) in respect to a 3D model of the patient specific implantation site and to show the instruments in the virtual model of the arteries.

TVI-AD can be used in combination with a so called XMR suite, a system which integrates MRI and fluoroscopy in the same laboratory, improving the registration between virtual and real patient's data. MRI data acquired immediately before the intervention could be used to create a virtual model of the arteries by 3D computer reconstruction. The bed can be shifted on a rail from the MR to the treatment area, where an electromagnetic localizer will localize the sensorized endovascular instruments. The system will model anatomy movements, due to breathing and cardiac cycle.

Aim 3: To validate TVI-PLAN and TVI-AD by means of animal models. Validation of the implemented tools will be crucial for ensuring the clinical benefit, and will be carried out by continuous interaction with interventional cardiologists of OU1. Validation phase will allow to evaluate the modelling reliability, to test materials, to calibrate sensors, to quantify the radiation reduction and to assess learning curve.

Hypothesis: The use of the TVI-PLAN and TVI-AD, integrating patient-specific anatomy from MRI, mechanical model of the cardiovascular devices, and sensorized endovascular instruments will improve safety and effectiveness of TVI procedures.

Preliminary data: The OU1 has a large competence in MR image processing, image registration and animal model experiments in a MRI environment. The cath lab of OU1 has considerable experience in interventional treatment of CHD including TVI. The previous work of the Italian Researcher Abroad and the OUA demonstrated effective finite elements modelling of implanted devices, as stents and artificial valves. The OU2 realized several navigation systems.

Materials and Methods

Full imaging assessment will be performed at our centres by MRI facilities at FTGM (two MRI scanners in Pisa, one XMR suite in Massa).
A FEM modelling software Abaqus/Explicit (Simulia) is available at UCL in London and will be used for mechanical modelling of devices.
An Electromagnetic localizer AURORA (Northen Digital), including sensors and graphic workstation, is available at EndoCAS in Pisa and could be used to support the project.
The animal facility located in the CNR research area in Pisa will be exploited to provide the animal models in the validation phase. Farm pigs will be included in this study. TVI in the pulmonary position will be performed with the support of the developed tools by an experienced interventional cardiologist. The pulmonary valve will be preferred because of the access route which is more similar to human case. Moreover, pulmonary valve diseases are more frequent in patients born with congenital heart diseases.

Impact and Translational Implications

According to current guidelines, a limited number of patients can access TVI, due the current limitations of the technique. The aim of this project is to develop navigation tools based on patient-specific images to improve the TVI effectiveness predicting risk factors, allowing safe extension of the methodology to a wider patient population, that requires treatment for cardiac valve disease. The proposed project will also support the technology development around the novel XMR suite in OU1.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Does intensive exercise induce plasticity-related changes in Parkinson's Disease?
Project Code: PE-2013-02357980	Principal Investigator: Russo Margherita
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Centro Neurolesi Bonino Pulejo

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases

Project Classification IRG: Brain Disorders and Clinical Neuroscience

Project Classification SS: Clinical Neuroscience and Neurodegeneration - CNN

Project Keyword 1: Parkinson¿s disease and other movement disorders (Huntington¿s, Dystonias, Ataxias).

Project Keyword 2: Neuronal plasticity

Project Keyword 3: Neuro-rehabilitation

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

The project has already been presented: ☐ Project code reference:

☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit

Name and Surname: Mara Felice Ghilardi

Foreign Institution: Cuny College of New York

Department/Division/Laboratory: Physiology, Pharmacology and Neuroscience

City-State and Country: New York United States

Years of Residence Abroad: 30

Email: lice.mg79@gmail.com Phone: 0012126505814

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Centro Neurolesi Bonino Pulejo	Neuro-Rehabilitation Dept.	coordination, project organization, experimetal and data collection, discussion
2	Cuny College of New York	Mov Disorder Division	project organization, data analysis and discussion
3	AOU Policlinico G. Martino Messina	Department of neuroscience	project organization, experimetal and data collection, discussion

Project Title:
Does intensive exercise induce plasticity-related changes in Parkinson's Disease?

Project Code: PE-2013-02357980

Principal Investigator: Russo Margherita

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Centro Neurolesi Bonino Pulejo

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Quartarone Angelo	AOU Policlinico G. Martino Messina	project organization, experimetal and data collection, discussion	18/10/1965
2	Ghilardi Maria Felice	Cuny College of New York	project organization, data anlysis, discussion	12/09/1957

Background and Significance

In Parkinson's disease (PD), progression of motor and non-motor symptoms is paralleled by relentless neuronal loss. While reducing some of the symptoms, current pharmacological and surgical treatments do not stop the disease progression. Recent studies showed that PD affects long-term potentiation (LTP) mechanisms within motor loop. LTP impairment may underlie some of PD symptoms but it might hamper mechanisms related to neurorepair and neuroprotection. It is plausible that exercise might enhance plasticity in PD, as suggested by experimental animal model showing that it may promotes cell proliferation, neuronal differentiation and neurotrophic factors. In patients with PD, intensive exercise induces long-lasting clinical improvement and decreases L-dopa intake. The study's primary goal is to ascertain whether a multidisciplinary intense training program (MITP) in PD may produce clinical improvements and enhances brain plasticity. We will then ascertain which program component contributes more to clinical and plasticity improvement and the duration of these changes. For these aims we will use indices of brain plasticity derived from behavioral test, imaging, electrophysiology and biochemistry. The proposed studies might change our understanding of PD, disclose novel therapeutical options to approach PD and possibly other conditions of impaired plasticity- and decrease economical, physical and psychological costs.

Specific aims

Aim 1: To determine if MITP in patients with PD produces clinical improvements and enhances brain plasticity

Aim 2: To determine if aerobic exercise may further enhance brain plasticity comparing the effect of MITP vs traditional rehabilitation approach and pharmacological treatment alone

Aim 3: To determine if the changes in cortical plasticity induced by MITP are long lasting

Hypothesis: We hypothesize that a systematic exercise program - MITP, a 8-week (5 days/week) intensive rehabilitation treatment in PD patients Hoehn and Yahr stage 2-2,5, involving cardiovascular fitness, gait and balance training using treadmill and/or Lokomat promotes better clinical improvements in motor domain and quality of life and changes in biochemical, imaging and electrophysiological indices of brain plasticity as compared with conventional rehabilitation treatment. Hence we may speculate that the aerobic component of the MIPT is the most important determinant of clinical improvement and plasticity enhancement. Finally, we would like to ascertain if such changes in clinical and cortical plasticity will persist over the following 12 months.

Preliminary data: In previous studies, it has been demonstrated that a similar rehabilitation protocol in patients in early PD reduced motor disability immediately after treatment. Comparison with a group of patients in the same pharmacological regimen without rehabilitation showed a reduced rate of disease progression one year later (Frazzitta et al., in press). Similar results were obtained in patients in more advanced stage. These data suggest that intensive exercise might slow down the natural progression of PD.

Preliminary evidence also showed that this treatment has an effect on biochemical plasticity indices: BDNF serum levels increased after a cycle of treatment but not after a similar period of time in a group of patients with PD that did not undergo the treatment protocol (Frazzitta et al., 2013). In a pilot collaborative study, we have measured BDNF-induced Trk-B activation in lymphocytes of ten patients before and after a cycle of complete rehabilitation treatment. On one side, lymphocytes signaling can provide an in vivo marker of plasticity in humans, since the levels of activation of TrkB in lymphocytes and in cortical neurons are strongly correlated. On the other, it could provide a marker of immune function that is likely impaired in PD, since neuro-inflammation is considered part of PD pathogenesis. We found that after a treatment cycle, BDNF-TrkB signaling and TrB-NMDAR interaction increased compared to baseline values. In these patients, we also found that retention of visual sequences improved after the treatment compared to baseline.

Materials and Methods

We will test 120 patients with PD stage 2 and 2,5 divided in four groups: 1) 8-week MITP + treadmill; 2) 8-week MITP +Lokomat; 3) 8-week traditional rehabilitation 4) pharmacological treatment alone. Each MITP session will include: cardiovascular warm-up, relaxation, muscle stretching, range of motion, muscles reinforcement; exercises for balance and gait using treadmil and/orLokomat. Effects of treatment will be assessed before, immediately after, and 1, 6 and 12 months later, by measuring: a. clinical assessment: UPDRS II & III scores, 6-min walking, Berg balance, Timed up and go, PD Disability Scale, quality of life; Hamilton Depression Score. b. biochemical changes: serum BDNF levels, activation of lymphocyte TrkB; c. electrophysiological changes: LTP induction with rTMS, EEG at rest and during tasks. d. MRI-related changes: voxel-based morphology; tractography; fMRI. Mixed model ANOVAs and correlative analyses will be performed.

Impact and Translational Implications

PD is a chronic progressive disease, imposing heavier burdens to western countries as the number of PD patients is expected to rise with increase in life expectancy. If successful, the results of this study will improve our understanding of the pathology, open novel therapeutical clinical strategies to approach PD and to slow down its progression, thus decreasing the economical and psychological burdens for the patients, the caregivers and the society at large.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Reduction of breast cancer recurrence in women: lifestyle strategies and microRNA expression
Project Code: PE-2013-02358099	Principal Investigator: Montella Maurizio
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Istituto nazionale tumori Fondazione Giovanni Pascale
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Oncology
Project Classification IRG: Oncology 2 - Translational Clinical
Project Classification SS: Chemo/Dietary Prevention - CDP
Project Keyword 1: Development and validation of biomarkers important in prevention, including markers of cancer risk and progression
Project Keyword 2: breast cancer, diets, vitamin D, blood glucose and markers of insulin resistance, miRNA expression
Project Keyword 3: lifestyle strategies, recurrence, survival
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☒
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Livia Silvia Augustin
Foreign Institution: St. Michael's Hospital
Department/Division/Laboratory: Clinical Nutrition and Risk Factor Modification Centre
City-State and Country: Toronto, Ontario, Canada
Years of Residence Abroad: 4
Email: livia.augustin@utoronto.ca Phone: +1- 416 - 867 - 7475

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto nazionale tumori Fondazione Giovanni Pascale	Epidemiology Unit	study design and statistical analysis, manage data of clinical patients, recruitment of cases and collection of biological samples
2	St. Michael's Hospital	Clinical Nutrition and Risk Factor Modification Centre	processin dietary and lifestyle protocol, monitoring interviews, the development food database.
3	University of Catania	Scienze Biomediche	Micro RNA analysis and end recruitment of cases and collection of biological samples

Project Title:
Reduction of breast cancer recurrence in women: lifestyle strategies and microRNA expression

Project Code: PE-2013-02358099

Principal Investigator: Montella Maurizio

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Istituto nazionale tumori Fondazione Giovanni Pascale

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Libra Massimo	University of Catania	Coordinator	03/12/1970
2	Grimaldi Maria	Istituto nazionale Fondazione Giovanni Pascale	Researcher	10/04/1971

Background and Significance

Breast cancer (BC) is the second leading cause of cancer deaths in women. Obesity, metabolic syndrome and type 2 diabetes are associated with higher risk of BC development and progression. The proposed mechanisms are linked to hyperinsulinemia and hyperglycemia and their interplay with estrogen levels. Excess adipose tissue results in increased plasma levels of insulin and insulin-like growth factor-1 (IGF-1), markers of inflammation and oxidative stress which are all involved in mitogenic activity and breast tumor progression. It has been suggested that vitamin D deficiency is common in BC patients, while its supplementation may have beneficial effects on glycemic control. Dietary interventions have been associated with molecular changes that alter cellular signaling. These include changes in microRNA (miRNA) expression. MiRNAs are small non-coding RNAs that alter gene expression through posttranscriptional silencing or activation. Strategies aimed at lowering large fluctuations in blood glucose and insulin, including low glycemic index (GI) diets, vitamin D supplementation and exercise, may reduce body weight and estrogen levels with consequent reduction of BC progression. Furthermore, the analysis of distinct plasma miRNA expression patterns associated with lifestyle strategies may identify novel molecular markers associated with BC recurrence. Overall, these strategies could result in relevant public health impact and help to reduce healthcare costs.

Specific aims

Aim 1: The principal aim of this lifestyle intervention program is to test the effect of the combination of low GI foods, vitamin D and exercise at two levels of intensity, on secondary prevention: on recurrence of BC in the same or opposite breast, on distant metastasis and on disease-free survival and mortality from BC and all-cause.

Aim 2: To investigate the effect of the proposed lifestyle intervention program on metabolic syndrome factors, markers of insulin resistence, circulating levels of glucose, insulin and lipids; and on hormonal markers including serum estradiol levels, testosterone, sex hormone binding protein; and factors affecting cancer growth including insulin-like growth factors and their binding proteins, inflammatory factors, markers of oxidative stress and antioxidant status, serum vitamin D, and incidence of metabolic syndrome, prediabetes and type 2 diabetes.

Aim 3: To identify, distinct plasma miRNA expression patterns associated with BC recurrence. miRNA expression patterns will be also analyzed according to the lifestyle and dietary intake. Blood-based miRNA expression profiling has several crucial advantages, such as easy accessibility using a minimally invasive method, and the potential of developing a test for population screening.

Hypothesis: The evidence suggests that reduced glycemia, body weight and metabolic syndrome factors improve prognosis in women with BC. Low GI diets and exercise have been shown to improve glucose control, body weight, metabolic syndrome markers, estradiol levels and reduce the incidence of BC. Vitamin D has also been shown to reduce BC recurrence and improve glycemic control and vitamin D levels tend to be below optimal levels in cancer patients. Our hypothesis is that a lifestyle program combining low glycemic index foods + vitamin D + exercise given at higher intensity in the test group compared to the lower intensity

program in the control group will result in significantly lower BC recurrence, distant metastasis, increased disease-free survival, reduced mortality from BC, and improved markers of cancer progression, in the test group compared to the control group.

Preliminary data: Low GI foods are by definition carbohydrate foods with lower glycemic impact than high GI food. Low GI diets have been shown to reduce the risk of developing type 2 diabetes, coronary heart disease and some cancers and in clinical trials to improve glycemic control, body weight and other metabolic syndrome factors. Low GI foods have also shown to beneficially influence the IGF axis in acute studies. Vitamin D affects insulin secretion, insulin resistance and inflammation and circulating levels of vitamin D appear inversely related to type 2 diabetes development, the metabolic syndrome and glycemic control. Vitamin D supplementation has also been shown to improve metabolic syndrome markers. Exercise is known to reduce fat mass and improve the metabolic profile including improving insulin resistance and glycemia and it is related to improved BC outcomes.

Materials and Methods

Breast cancer surgically removed will be randomized to either the test or the control arm. Both arms will include the program but at two intensity levels: 1) Low GI diet + vitamin D + exercise, intensive treatment (test) and 2) low GI diet + vitamin D + exercise, lower intensity (control). The healthy diet will emphasize low GI foods high vegetable, fruit, low red meat. The exercise component will include brisk walking. The test group will receive more intense advice. Counseling will be given during clinic visits at time zero (baseline) and every 6 months thereafter. Blood samples and anthropometric measures will also be collected at each visit. A 3-day food record prior to each clinic visit and between visits. The analysis of miRNA will be performed in plasma samples from patients before and after the specified dietary and life style strategies according to the miScript miRNA PCR Array. Disease-free and overall survival will estimated by using the Cox regression model.

Impact and Translational Implications

It is expected that both arms of the study will benefit from the lifestyle intervention program but a significantly lower breast cancer recurrence rate would be expected in the intensive treatment group (test). Should there be a significant lower recurrence rate in one or both groups this study could help to form part of the recommendations in national dietary guidelines for the prevention of secondary breast cancer.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Clinical, structural and functional markers for recovery of consciousness
Project Code: PE-2013-02358145	Principal Investigator: Estraneo Anna
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Fondazione Salvatore Maugeri
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases
Project Classification IRG: Brain Disorders and Clinical Neuroscience
Project Classification SS: Clinical Neuroscience and Neurodegeneration - CNN
Project Keyword 1: Neuroimaging, functional, biochemical, and neuropathological studies to assess the onset, progression, treatment, and development of biomarkers for brain disorders.
Project Keyword 2: disorders of consciousness
Project Keyword 3: brain plasticity
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Andrea Soddu
Foreign Institution: The University of Western Ontario
Department/Division/Laboratory: Brain and Mind Institute, Physics and Astronomy Department
City-State and Country: London ON, Canada
Years of Residence Abroad: 10
Email: asoddu@uwo.ca Phone: (519) 661-2111 x 82669

BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero

Project Title:
Clinical, structural and functional markers for recovery of consciousness

Project Code: PE-2013-02358145

Principal Investigator: Estraneo Anna

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Fondazione Salvatore Maugeri

Project Type: PE- ITALIAN RESEARCHER ABROAD

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Fondazione Salvatore Maugeri	Neurorehabilitation Unit and Laboratory for Disorders of Consciousness	Principal Investigator Project coördinator Enrollment of DOC patients Clinical evaluation and quantitative assessment of visual behavior in DOC patients Data analysis
2	The University of Western Ontario	Brain and Mind Institute, Physics and Astronomy Department	Principal investigator Neuroimaging analysis and modeling Enrollment and neuroimaging acquisition in healthy subjects Data analysis
3	SDN-IRCCS	Neuroradiology Department	Coordinator UO 3 Neuroimaging acquisition in DOC patients and healthy subjects. Data analysis

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Cavaliere Carlo	SDN-IRCCS	Coordinator UO 3 Neuroimaging acquisition of DOC patients and healthy subjects. Data analysis	31/08/1983
2	Stancanelli Annamaria	Fondazione Salvatore Maugeri	Quantitative assessment of visual behavior in DOC patients	05/01/1964

Background and Significance

The improvement of intensive care techniques and the failure of treatments to restore brain functions after a severe brain injury increased the number of patients with Disorders of Consciousness, i.e. patients in vegetative state (VS) or in minimally conscious state (MCS), with strong ethical and social implications, and impact on health care policies. VS and MCS can be distinguished by detecting volitional versus reflexive responses to stimuli. Standardized clinical scales and quantitative assessment of visual tracking abilities can contribute to identify early signs of responsiveness and to monitor their evolution. Nonetheless, this remains a very difficult task because of marked fluctuations of patients' conditions and frequent presence of severe visuo-perceptual, motor or language disabilities.
Neuroimaging methods, such as resting-state fMR, DTI, or 18F FDG-PET, can help to recognize neural activity specifically associated with awareness in such patients, independently from their abilities to produce purposeful behaviors, thus possibly identifying a higher number of responsive patients and redefining classical diagnostic criteria. This is particularly relevant since late recovery of responsiveness and consciousness in VS can no longer be regarded as exceptional. Moreover, to identify patterns of structurally and functionally connected brain regions might help to characterize subsamples of VS and MCS patients with different long-term outcome, to optimize care management.

Specific aims

Aim 1: To characterize structural alterations and residual functional activity (and their relationships) associated to levels of consciousness in patients with Disorders of Consciousness (DoC) of different aetiology, to clarify the pathophysiological mechanisms of DoC.

Aim 2: To identify clinical and biological prognostic markers of long-term outcome in DoC patients.

Aim 3: To investigate clinical, structural and functional changes associated to clinical outcome in order to comprehend the physiological mechanism underlying recovery of consciousness.

Hypothesis: Combining structural and functional neuroimaging data (using a neuronal model framework) with clinical quantitative assessment of awareness signs might improve our understanding of collective behavior of neuronal populations at different levels of consciousness. Moreover, structural-functional patterns can be useful to recognize sub-clinical signs of recovery and to identify prognostic biomarkers. Integrated quantitative clinical and neuroimaging data recorded over a long time post-onset can contribute to comprehend evolution of DoC and provide insight to develop new treatments and optimize use of National Health care resources.

Preliminary data: Late recovery of responsiveness and consciousness cannot be considered as exceptional in traumatic or non-traumatic VS (Estraneo et al., 2010); this strongly supports the need for long-term monitoring to comprehend the natural history of prolonged DoC (Estraneo et al., 2013, 2014).
Within this perspective, the participating units gathered several data on the importance of multimodal assessment of DoC patients. Non-reflexive visual behaviour is the most frequent clinically-detected intentional response in MCS (Estraneo et al., submitted). A quantitative computerized assessment of visual tracking abilities (Trojano,..., Estraneo, 2012, 2013) might provide additional elements for diagnosis of DoC and identify early signs of responsiveness as possible prognostic markers.
In addition, brain fMR (Soddu et al. 2009, 2011, 2012, 2013; Estraneo et al SINC 2011; Estraneo, ...Soddu et al., in preparation) and 18F FDG-PET at rest (Stender, ...Soddu et al. 2014) could complement bedside examination, also to predict long-term recovery in VS. Such techniques might also investigate brain structural and functional reorganization associated to behavioral amelioration, and identify the main indices for plasticity (Demertzi, ...Soddu et al. 2011, Estraneo, ...Soddu et al., in preparation). DTI can reveal structural damage in tissue that appears normal in conventional MR (Molino, Cavaliere et al. 2014), and also assess neuropathology of VS and MCS in vivo (Fernandez-Espejo et al. 2011).
On the basis of such preliminary data, our project aims to provide further insights on the neural correlates of consciousness not only at a clinical level (i.e., medical management and rehabilitation) but also from a scientific-theoretical perspective (i.e., brain plasticity).

Project Title:
Clinical, structural and functional markers for recovery of consciousness

Project Code: PE-2013-02358145

Principal Investigator: Estraneo Anna

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Fondazione Salvatore Maugeri

Project Type: PE- ITALIAN RESEARCHER ABROAD

Materials and Methods

This study will enroll 40 patients with prolonged DoC (VS or MCS; time from brain injury longer than one month) of different aetiology and follow-up them until 2 years post-onset. Complete assessments will be performed at study entry and at 6, 12 and 24 months after onset and every time the clinical conditions will change. Assessment procedures will include:
Clinical assessment: patients level of consciousness, responsivity and functional disability will be assessed by standardized scales;
Quantitative assessment of visual behavior: number and duration of fixations during visual tracking of moving stimuli will be recorded by a computerized eye tracking system;
Neuroimaging Study: all patients will undergo neuroimaging evaluation at rest by means of a simultaneous 3 Tesla MR/PET scanner (Biograph mMR, Siemens Healthcare) to gather structural and functional data by means of advanced analytical techniques (such as sparce principal component analyses and dictionary learning approach).

Impact and Translational Implications

Our project will provide clinical insights, improve diagnostic procedures, and define prognostic markers for disorders of consciousness. The results will contribute to optimize use of resources of the National Health care system and might help clinicians identifying patients eligible for specific treatments. Our project will also address mechanisms of brain plasticity, to identify biomarkers for establishing an evidence-based prognosis and conveying correct information to patients families.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: PREDICTING THE FUTURE HEALTH CARE NEEDS OF CANCER SURVIVORS IN AGEING POPULATIONS (FORECARE)
Project Code: PE-2013-02358393	Principal Investigator: De Angelis Roberta
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Istituto Superiore di Sanita'
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Methodological, epidemiological, socio-economic, organizational, managerial emerging public health issues related to the above reported areas
Project Classification IRG: Population Sciences and Epidemiology
Project Classification SS: Epidemiology of Cancer - EPIC
Project Keyword 1: Development and improvement of research designs and methodologies addressing epidemiologic questions in cancer
Project Keyword 2: prevalence, morbidity, forecasting
Project Keyword 3: population-based cancer registries
Project Request: Animals: ☐ Humans: ☐ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: ANGELA MARIOTTO
Foreign Institution: National Cancer Institute (NCI)
Department/Division/Laboratory: Surveillance Research Program/Data Modeling Branch
City-State and Country: Bethesda-Maryland, United States of America
Years of Residence Abroad: 14
Email: mariotta@mail.nih.gov Phone: +1 (240) 276-6698

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Superiore di Sanita'	Centro Nazionale di Epidemiologia Sorveglianza e Promozione della Salute (CNESPS)/Reparto di Epidemiologia dei Tumori	Principal Investigator Unit - Coordination of all project's activities - Statistical methods, software design and development, applications, dissemination
2	National Cancer Institute (NCI)	Surveillance Research Program/Data Modeling Branch	Principal Investigator Foreign Operative Unit - Statistical methods, applications to US data, dissemination
3	Istituto Superiore di Sanita'	Servizio Informatico, Documentazione, Biblioteca ed Attività Editoriali (SIDBAE)/Settore I - Informatica	Collaborating Unit - IT support, software design and development

Project Title:
PREDICTING THE FUTURE HEALTH CARE NEEDS OF CANCER SURVIVORS IN AGEING POPULATIONS (FORECARE)

Project Code: PE-2013-02358393

Principal Investigator: De Angelis Roberta

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Istituto Superiore di Sanita'

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Carrani Eugenio	Istituto Superiore di Sanita'	Collaborating partner - IT management, supervisor of software design and development	18/03/1959

Background and Significance

Cancer survivors are a growing population that is expected to remain so in the foreseeable future. Understanding the health care needs of this growing population and the sustainability of the subsequent demand on health care systems is of increasing importance.
Future cancer burden depends on demographic changes, on cancer incidence dynamics, on prognostic improvements, as well as on competitive mortality trends for causes other than cancer. To tackle the implied societal challenges the European health research agenda increasingly aims at developing methods to assess future demand on health services.
Limited information is available from population-based registries on incidence, survival and prevalence of cancer patients. Major limitations are: i) partial population coverage ii) delayed reporting iii) limited information on cancer prevalence (overall, by phase of care, long-term and cured survivors) is derived only through ad hoc procedures.
The MIAMOD/PIAMOD approach, implemented in a software freely distributed by the PI Insitutution (ISS), is unique in providing the theoretical framework to estimate and project, both on time- and geographical- scale, all cancer epidemiological indicators. Extending the functionalities of MIAMOD/PIAMOD software to produce enhanced prevalence indicators according to flexible demographic and epidemiological projection scenarios, is both feasible and relevant to inform cancer control policies.

Specific aims

Aim 1: To develop standardized methods to forecast future health care needs of cancer survivors, according to varying demographic and epidemiological scenarios, in the conceptual framework of the MIAMOD/PIAMOD statistical approach. Specific aims are:
i) to expand the range of prevalence indicators
ii) to expand the flexibility of projection scenarios

Aim 2: To implement the new methodologies and indicators developed in Aim 1 as new functionalities of the MIAMOD/PIAMOD software, the already available software implementing both methods.

Aim 3: To apply and disseminate the methods and software developed in Aims 1 and 2.
i) to estimate the future health care needs of cancer survivors in Italy and US for major cancers
ii) to disseminate methods/results and upgraded software

Hypothesis: Predicting and qualifying cancer prevalence in terms of cure, disease duration and phase of care is important for accurately estimating the population under treatment and the morbidity costs associated with cancer. It is also important for studies on patients' quality of life that are increasingly achieved to understand the health care needs of cancer survivors.

Limited information is available from cancer registries on prevalence indicators. Observed prevalence includes a variable unknown proportion of long-term survivors. Prevalence information is often outdated or unavailable in current registries statistics in many countries.

MIAMOD and PIAMOD are statistical methods, developed by researchers of Istituto Superiore di Sanità (ISS), to estimate and project cancer incidence, prevalence and mortality in areas partially or fully covered by registration (Verdecchia 1989, Verdecchia 2003). Both methods are implemented in a software freely distributed by ISS.

MIAMOD/PIAMOD outputs include projections of cancer-specific incidence, mortality and prevalence by calendar year and age, but the software presently offers a limited control on projection scenarios and a limited range of prevalence indicators. Ad hoc procedures using MIAMOD/PIAMOD outputs were applied to project prevalence by phase of care. However since there is no standard methodology/software for these projections, it is difficult to compare and to understand the assumptions behind each study. Expanding MIAMOD/PIAMOD outputs on prevalence projections is feasible and cost-effective.

Preliminary data:

MIAMOD/PIAMOD methods are widely applied in the Italian and international context to derive cancer incidence and prevalence projections at regional and national scale (Rossi 2013, Micheli 2007, De Angelis 2009, Sanchez 2010, Yu 2013, Hermann 2013).

Comparisons of prevalence projections under constant/dynamic hypothesis on population, incidence and survival can be estimated with ad hoc procedures using different MIAMOD/PIAMOD estimation sessions (De Angelis R 2007)

Prevalence of cured survivors and by phases of care can be computed with ad hoc external procedures using MIAMOD/PIAMOD outputs (Mariotto 2006, Mariotto 2011, Yu 2013).

Materials and Methods

Population and cause-specific mortality are available from official national statistics. Cancer-specific incidence and survival data are available from population-based cancer registries. The US-SEER public use database is the reference data source for USA. The EUROCARE database is the reference data source in Europe (De Angelis 2014).
Parametric mixed cure models are used to model survival according to flexible projection scenarios. Incidence is modeled as polynomial (or spline) function of age, period, and birth cohort (APC) and derived through Poisson regression on observed cancer cases (PIAMOD) or deaths (MIAMOD). Prevalence and mortality are estimated by convolution of incidence and survival over time, and can be forward projected under varying population, incidence and survival assumptions. Projections of prevalence and mortality by disease duration allow to derive prevalence by phases of care, cured survivors, completeness of limited-duration prevalence.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: PREDICTING THE FUTURE HEALTH CARE NEEDS OF CANCER SURVIVORS IN AGEING POPULATIONS (FORECARE)
Project Code: PE-2013-02358393	Principal Investigator: De Angelis Roberta
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Istituto Superiore di Sanita'

Project Type: PE- ITALIAN RESEARCHER ABROAD

Impact and Translational Implications

Cancer survivors are a booming population in high and middle income countries and there is an increasing number of studies that aim to forecast the future cancer burden. However there is no standard methodology/software for these projections. The project aims to expand the capabilities of MIAMOD/PIAMOD methods and software to provide projections on a large number of indicators that are useful in health planning. This will improve standardisation, comparability and impact of the results.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Pancreatic ductal adenocarcinoma (PDAC): development of a new communication platform between radiologists, surgeons and pathologists based on virtual and 3D printed reconstructions of the pancreas and the tumor mass.
Project Code: PE-2013-02358887	Principal Investigator: PIETRABISSA ANDREA
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Policlinico San Matteo
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Innovative biotechnologies
Project Classification IRG: Surgical Sciences, Biomedical Imaging, and Bioengineering
Project Classification SS: Computational modeling and sciences for biomedical and clinical applications - ZRCM
Project Keyword 1: Data analysis, image construction, anatomical modeling and the modeling of therapy of diseases associated with diagnostic medical imaging.
Project Keyword 2: pancreatic ductal adenocarcinoma, tumor margin identification, tumor resectability assessment
Project Keyword 3: image analysis, image segmentation, 3D reconstruction, 3D printing
Project Request: Animals: ☐ Humans: ☐ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Marco Del Chiaro
Foreign Institution: Karolinska University Hospital
Department/Division/Laboratory: Department of Surgical Gastroenterology
City-State and Country: Stockholm, Sweden
Years of Residence Abroad: 4
Email: marco.del-chiaro@karolinska.se Phone: +46700632377

Project Title:
Pancreatic ductal adenocarcinoma (PDAC): development of a new communication platform between radiologists, surgeons and pathologists based on virtual and 3D printed reconstructions of the pancreas and the tumor mass.

Project Code: PE-2013-02358887

Principal Investigator: PIETRABISSA ANDREA

Research Type: Biomedical/Biomedica

Applicant Institution: Fondazione Policlinico San Matteo

Project Type: PE- ITALIAN RESEARCHER ABROAD

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Fondazione Policlinico San Matteo	Chirurgia Generale 2	Medical counterpart in the development and the evaluation of the communication platform. Will give the clinical support for the improvement of the image analysis tool. General coordination of the project activities.
2	Karolinska University Hospital	Department of Surgical Gastroenterology	Responsible for the clinical part of the project: will provide both radiologic and pathologic images and will perform a clinical evaluation of image analysis results.
3	DICAr, Università degli Studi di Pavia	Dipartimento di Ingegneria Civile ed Architettura (DICAr)	Engineering counterpart of the project: will develop the algorithm for pathologic image analysis and build the structure of the communication platform.

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	REALI ALESSANDRO	DICAr, Università degli Studi di Pavia	Coordination of the engineering computational part of the project: arrangement of the elaboration strategy on pathologic images and of the communication platform structure.	28/02/1977

Background and Significance

Pancreatic cancer is the fourth leading cause of cancer death in the USA. Carcinoma of the exocrine pancreas accounts for over 90% of pancreatic tumors and, among these, 95% are of PDAC type. Surgical resection represents the only curative treatment, but at presentation only 10-15% of patients can undergo surgical resection. Contrast-enhanced multi-detector computed tomography (MDCT) is the most widely used imaging technique for the diagnosis and the staging of pancreatic cancer. Despite the great improvement of MDCT technology in recent years and the development of specific acquisition protocols, it's still not easy for the radiologist to identify tumor borders and assess possible infiltrations with a high confidence level. Thus, patient inoperability is often disclosed during intervention. According to the new resection margin definition proposed by C. Verbeke [Redefining resection margin status in pancreatic cancer, HPB 2009, 11, 282-289], the pathological analysis reveals that the 80% of patients that undergo surgical resection have positive margins. Pathology provides an important amount of information that should be objectively compared to radiological evidences: such an approach cannot be found in literature. On the other hand, it's not easy for the surgeon to plan a tumor resection relying only on radiological images and annotations. No systems able to turn all the radiological hypotheses in an usable way for surgeons, such as a 3D model, are described in literature.

Specific aims

Aim 1: Create a communication platform between radiologists, surgeons and pathologists, based on the use of virtual and 3D printed models, to facilitate the information transfer among these medical fields.

Aim 2: Improve the knowledge on the diagnosis and the operability assessment of PDAC cases through a quantitative comparison between the radiological findings on MDCT images and pathological evidences on surgical specimens.

Aim 3: Improve the preoperative planning for PDAC resection, providing the surgeon with both a virtual and a 3D printed model that include all the radiological findings and assumptions made on medical images.

Hypothesis: Both the radiology and the pathology produce a big amount of information that in most cases is confined to the respective medical fields. The radiologist analyses MDCT images and relying on his own experience produces a report with all findings and hypotheses made on images. A written report along with a MDCT scan, is often not enough informative for the surgeon to let him visualize clearly in his mind the tumor morphology and location and to plan the resection. The literature shows how 3D anatomical models are increasingly employed in some medical fields for the pre-operative planning (e.g. orthopedics, dental or neuro-surgery): the use of 3D printed models is also spreading within these medical areas, thanks to the great and progressive diffusion of 3D printers. The use of 3D printings in abdominal surgery is still limited, and no cases of such employment in pancreatic surgery are reported in literature. Furthermore, the pathologist analyses surgical specimens and produces a set of images on which he defines tumor borders: the feedback of this information to the radiologist is not programmatic and depends on the specific hospital strategy. Several scanning protocols have been described and new resectability criteria have been proposed, but the precise comparison between the pathological and radiological findings is always limited to the specific study and has never been proposed as an approach to the improvement of the radiological knowledge on image analysis.

Preliminary data: We have realized an image analysis tool that elaborates MDCT scans and provides a virtual 3D model of the pancreatic parenchyma, the peri-pancreatic vessels and the tumor mass through a semi-automatic approach: the tumor is rendered preserving information on different hypodensity levels [Marconi S, Auricchio F, Pietrabissa A, (2012) 3D virtual and physical pancreas reconstruction discriminating between health and tumor tissue with fuzzy logic, Int J CARS 7 (Suppl 1):S17-S88]. The virtual model can be sent to a 3D printer to get a physical representation. Until now we have analyzed more than 20 MDCT scans e we have already provided a preliminary validation of the tool. In surgeons opinion, the virtual model allows a detailed analysis of the tumor morphology and its relation with surrounding abdominal structures, while the physical one gives the best adherence to operative reality.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Pancreatic ductal adenocarcinoma (PDAC): development of a new communication platform between radiologists, surgeons and pathologists based on virtual and 3D printed reconstructions of the pancreas and the tumor mass.
Project Code: PE-2013-02358887	Principal Investigator: PIETRABISSA ANDREA
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Policlinico San Matteo

Project Type: PE- ITALIAN RESEARCHER ABROAD

Materials and Methods

The tool will be endowed of new features to let the radiologist easily interact with the model: he will be able to modify tumor labels in each slice and add his findings through a color code (e.g. different confidence levels in assessing an infiltration should be labeled in different colors). We will develop a similar image analysis system to get a 3D model of the tumor from pathology images. The pathologist cuts the specimen in slices that are scanned into digital images on which he manually defines tumor borders and that will be processed to retrieve a virtual 3D model. The pathological and the radiological virtual models will be compared to compute differences in the tumor borders identification and in the infiltration assessment. The comparison will be quantitative: we will compute the grade of similarity between the two models, relying on the difference in the tumor volumes and in the slice per slice distance between tumor borders. Furthermore, we can also 3D print the two models.

Impact and Translational Implications

The number of patients dying during the first year after surgical resection is still high: the improvement of radiological knowledge on image analysis should lead to a more precise staging of PDAC and to a better identification of patients that should benefit from an adjuvant treatment. Virtual and physical models will help the surgeon in the pre-operative planning, leading to a better execution and to a shorter post-operative hospitalization, improving the outcome and reducing operation costs.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Sensory and autonomic markers in early diagnosis of parkinsonism. A new strategy to predict clinical evolution toward different neruodegenerative disorders.
Project Code: PE-2013-02359028	Principal Investigator: Nolano Maria
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Fondazione Salvatore Maugeri
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases
Project Classification IRG: Brain Disorders and Clinical Neuroscience
Project Classification SS: Clinical Neuroscience and Neurodegeneration - CNN
Project Keyword 1: Parkinson¿s disease and other movement disorders (Huntington¿s, Dystonias, Ataxias).
Project Keyword 2: Cutaneous innervation
Project Keyword 3: Autonomic nervous system
Project Request: Animals: ☐ Humans: ☒ Clinical trial: ☐
The project has already been presented: ☐ Project code reference:
☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit
Name and Surname: Valeria Iodice
Foreign Institution: National Hospital for Neurology and Neurosurgery, University College
Department/Division/Laboratory: Institute of Neurology, Autonomic Unit
City-State and Country: London, United Kingdom
Years of Residence Abroad: 7
Email: v.iodice@imperial.ac.uk Phone: +44 7427602555

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Fondazione Salvatore Maugeri	Department of Neurology, Skin Biopsy Laboratory	Coordination, Patients recruitment, clinical assessment, cutaneous sensory and autonomic morpho-functional evaluation.
2	National Hospital for Neurology and Neurosurgery, University College	Institute of Neurology, Autonomic Unit	Patient recruitment, Autonomic cardiovascular assessment
3	University of Naples "Federico II"	Department of Neurosciences and Reproductive and Odontostomatologic Sciences	Patient recruitment, Clinical and Neurophisiological assessment

Project Title:
Sensory and autonomic markers in early diagnosis of parkinsonism. A new strategy to predict clinical evolution toward different neruodegenerative disorders.

Project Code: PE-2013-02359028

Principal Investigator: Nolano Maria

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Fondazione Salvatore Maugeri

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Santoro Lucio	University of Naples "Federico II"	Patients recruitment, clinical assessment, neurophysiological evaluation.	15/06/1950
2	Provitera Vincenzo	Fondazione Salvatore Maugeri	Patients recruitment, clinical assessment, cutaneous sensory and autonomic morpho-functional evaluation.	11/12/1964
3	Manganelli Fiore	University of Naples "Federico II"	Neurophysiological evaluation	28/03/1972
4	De Michele Giuseppe	University of Naples "Federico II"	Patients recruitment and clinical assessment	10/06/1951

Background and Significance

The progressive neurodegenerative process underlying Parkinson disease (PD), Multiple System Atrophy (MSA), Dementia with Lewy Body (DLB) and Progressive Supranuclear Palsy (PSP) starts long before the first clinical signs that may depict a common clinical picture. The latter, has been described in patients with PD who become symptomatic, at the stage when 60-70% of neurons in substantia nigra are already degenerated, making a neuroprotective approach poorly effective. Therefore, the early diagnosis in neurodegenerative disorders can be difficult and investigation of small peripheral nerve fibre and cardiovascular autonomic function may provide vital information and improve early diagnosis. There is a growing interest in the identification of biomarkers able to early distinguish among these overlapping pathological entities. However several attempts, aimed to identify liquoral, neuroradiological or immunohistochemical markers, have been unsuccessful so far. Chronic neurodegenerative diseases can severely affect autonomic functions, resulting in chronic autonomic failure. The involvement of the autonomic and sensory nervous system has been reported, although to a different extent, in parkinsonisms. Therefore we aim to systematically study new peripheral nerve fibre markers in combination with loss of peripheral somatic and autonomic function in patients with neurodegenerative disorders in order to enable a clearer diagnostic separation of the disorders at an earlier stage.

Specific aims

Aim 1: To define early markers of peripheral sensory and autonomic nerve fibre morphology and function in patients with different neurodegenerative disorders.

Aim 2: To assess if and to what extent, sensory and autonomic impairment affect the quality of life of patients with parkinsonisms.

Aim 3: To define new behavioral, nutritional, rehabilitative and pharmacological strategies tailored on the clinical/functional/morphological profiles to prevent the complications of the later stage of the disease.

Hypothesis: The pathological involvement of peripheral sensory and autonomic nerve fibre might occur early in the disease course of neurodegenerative disorders and at a pre-clinic/pre-symptomatic stage.
Therefore, we hypothesize that patients with early parkinsonism who will develop PD, MSA, LBDor PSP, already present with a morphological and functional profile, that can be revealed by appropriate diagnostic tools.
We hypothesize that a systematically evaluation of nerve fibre function using a combination of selective

autonomic function tests, peripheral nerve fibre function tests and peripheral nerve fibre pathology (using the skin biopsy technique), will help diagnose these complex overlapping neurological disorders and will thereby advance their treatment by ensuring correct early diagnosis, and provide biomarkers for clinical trials of new therapies. An early diagnosis will also help tailoring adequate therapeutic approaches , prevent complications, ameliorate quality of life and reduce costs for the community.

Preliminary data: We found functional and morphological abnormalities of peripheral sensory and autonomic nerve system in a population of 70 patients with different parkinsonisms (unpublished data). We reported peculiar, previously undescribed, aspects of sensory involvement in PD (Nolano et al., 2008) unrelated to L-DOPA treatment (Nolano et al., 2011) and present also in patients with a short disease duration. We also demonstrated a postganglionic sudomotor involvement in MSA (Provitera et al., 2014). We have previously observed different patterns of cutaneous denervation comparing groups of PD, MSA and PSP patients (Nolano et al., 2009 abs) suggesting a disease-related progression of the neuropathic process.

Materials and Methods

100 patients with recent onset (less than one year) of signs and symptoms suggesting a diagnosis of parkinsonism will undergo:1) Clinical examination; 2) Neurophysiology study (nerve conduction study, sympathetic skin response); quantitative sensory testing; 3) sudomotor function tests icluding the termoregulatory sweat test and the dynamic sweat test; 4) Cardiovascular Autonomic Tests including Tilt Test, pressor stimuli, deep Breathing, standing test, Valsalva maneuver. Blood samples will also be collected to assess catecholamine concentrations; 5) Ambulatory 24 h BP and HR monitoring according the autonomic protocol; 6) Skin biopsy. Indirect immunofluorescence and a large panel of primary antibodies including selective cholinergic and noradrenergic markers and antibodies anti alpha-synuclein and tau protein will be applied to three mm punch biopsies to study somatic and autonomic cutaneous nerves; 6) Questionnaires specific to each of the syndromes and various autonomic domains.

Impact and Translational Implications

1) Early diagnosis would enable more specific therapeutic approaches and adequate strategies to avoid disease-specific complications.
2) Improvement of quality of life and reduction of costs for the community through the adoption of new behavioral, nutritional, rehabilitative and pharmacological strategies tailored on clinical/functional/morphological profiles
3) Improvement of population sampling for clinical trials, with the possibility to assemble more-uniform cohorts.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Development and pre-clinical validation of a soft-tethered endoscopic robot to replace colonoscopy as a screening tool for colorectal cancer
Project Code: PE-2013-02359172	Principal Investigator: AREZZO ALBERTO
Research Type: Biomedical/Biomedica	Applicant Institution: Piemonte

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in
Innovative biotechnologies

Project Classification IRG: Surgical Sciences, Biomedical Imaging, and Bioengineering

Project Classification SS: Bioengineering, Technology, and Surgical Sciences - BTSS

Project Keyword 1: Design, development and evaluation of medical devices using animal models and pre-clinical human studies, including endo-surgical procedures, catheter-based surgery, minimally invasive surgery, microsurgical procedures, and robotics.

Project Keyword 2:

Project Keyword 3:

Project Request: Animals: [X] Humans: [X] Clinical trial: [ ]

The project has already been presented: [ ] Project code reference:

[X] I declare that the object/s of this application is/are under patent copyright

Patent owner: Pietro Valdastri, Gastone Ciuti, Arianna Menciassi, Paolo Dario
Scuola Superiore di Studi Universitari e di Perfezionamento Sant'Anna

Patent number: WO2010044053 A3 & PCT/IB2009/054491 - WO2012080947 A1 & PCT/IB2011/055636

Italian Researcher Abroad – Foreign Operative Unit

Name and Surname: Pietro Valdastri

Foreign Institution: Vanderbilt University

Department/Division/Laboratory: STORM Lab

City-State and Country: Nashville, TN 37235-1592, USA

Years of Residence Abroad: 3

Email: p.valdastri@vanderbilt.edu Phone: +1 615-426-6907

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Piemonte	Department of Surgical Sciences, General Surgery I, University of Torino	PI
2	Vanderbilt University	STORM Lab	RC

Project Title:
Development and pre-clinical validation of a soft-tethered endoscopic robot to replace colonoscopy as a screening tool for colorectal cancer

Project Code: PE-2013-02359172

Principal Investigator: AREZZO ALBERTO

Research Type: Biomedical/Biomedica

Applicant Institution: Piemonte

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Valdastri Pietro		Assistant Professor of Mechanical Engineering Assistant Professor of Electrical Engineering Assistant Professor of Gastroenterology Director of the STORM Lab Engineering partner, development of prototypes	26/04/1977

Background and Significance

Colonoscopy is currently the most reliable technique for the diagnosis and management for colorectal cancer (CRC). Unfortunately, colonoscopy is an invasive exam creating discomfort and entitled with several risks such as perforation from looping. This occurs due to the design of the endoscope, which consists of a long and fairly stiff tube with a steerable head. If the colonoscope is too stiff, it will deform the lumen wall significantly at turns in the colon; yet, if it is too compliant there will be undesired buckling of the colonoscope [Loeve 2010]. The colonoscope is advanced by pushing from the distal end while the tip is aimed along the lumen center. When the intestine bends, the colonoscope pushes against the colon wall until this provides sufficient counter pressure, stretching the colon and leading to "loop" formation, thus causing patient discomfort for stretching mesentery attachments. Looping of the endoscope has been shown to be responsible for 90% of the pain experienced in colonoscopy and increases the risk of tissue damage and/or perforation [Eickhoff 2007].
We aim to develop a soft-tethered capsule to replace conventional flexible endoscopy as a screening tool. This is a derivative project of the FP6 European Project VECTOR: Versatile Endoscopic Capsule for gastrointestinal Tumor Recognition and Therapy (EU/IST-2006-033970) to which Alberto Arezzo and Pietro Valdastri both participated actively on behalf of SMIT and SSSA partners.

Specific aims

Aim 1: Assessment and optimization of the magnetic link. We need to guarantee (A) that the intermagnetic force is strong enough to manipulate the capsule, and (B) that the capsule is not dragging the intestine while moving. We will test the hypothesis that magnetic coupling is effective in manipulating the capsule inside an ex vivo colon model from at least 15 cm away. This hypothesis needs to be confirmed after modifications introduced

The main outcomes will be (1) a qualitative observation of the effectiveness of the magnetic link, and (2) a quantitative recording of the distance between the external magnet and the capsule required to achieve reliable coupling

Aim 2: Implementation of intuitive control. Real-time tracking of the endoscopic capsule position is required to prevent discoordinate motion and to guarantee safe operation. Within this aim we will (1) integrate a commercially available magnetic tracker into the capsule, (2) implement an intuitive control of capsule position, (3) assess the platform with ex vivo and in vivo animal trials, and (4) verify that the intuitive control shortens the learning curve with respect to colonoscopy

Ex vivo validation: observational study with cecal intubation rate¿, time to reach the cecum, number of loops generated¿, polyp removal (5 mm colored beads)

In vivo validation: in a porcine model, compared to standard colonoscopy, primary endpoint the farthest distance reached¿

Learning curve assessment: for the robotic platform and colonoscopy, dividing testers among attendings, fellows and novices, with primary endpoint cecal intubation rate and secondary endpoints time to reach the cecum, degree of looping (by kinematic data), intermagnetic force (to verify safety hazards)

Aim 3: Assessment of mobility in a human model. Before moving forward to clinical trials, the robotic platform must be tested in a human model by a study aiming to quantify its performance with respect to colonoscopy. Within this aim, we will assess the platform in a human cadaver model and we will optimize the safety of the robotic system in view of clinical trials

Hypothesis: The goal of this research is to reduce the risk of adverse events of colonoscopies. The robotic platform we propose has the potential to replace colonoscopy with a soft-tethered endoscopic capsule intuitive to operate and safe due to the front wheel drive

Preliminary data: The two groups are working together on this project since 2006 as clinical and engineering partners, first within the FP6 VECTOR Project, then in the development of its derivative projects. They have developed a preliminary version of a cost-effective robotic platform that uses magnetic fields to manipulate a soft-tethered endoscopic capsule.

See:
- Valdastri P, Magnetic air capsule robotic system: proof of concept of a novel approach for painless colonoscopy. Surg Endosc. 2012;26:1238-46
- Arezzo A, Experimental assessment of a novel robotically-driven endoscopic capsule compared to traditional colonoscopy. Dig Liver Dis. 2013;45:657-62

Materials and Methods

Aim 1. ex vivo and in vivo trials will be performed in a colonoscopy training simulator with fresh porcine bowel fixed to mimic human anatomy. The new features of the capsule prototype are: integration of the sensor for real time localization, use of a multi-lumen catheter, upgraded vision module, increased magnetic load

Aim 2. we aim to develop a closed-loop controller that enables the user to directly specify motion of the endoscopic device. Due to real-time pose detection, the user will know if the endoscopic device is within the appropriate spheroid and if the magnetic coupling is effective

Aim 3. 12 human embalmed cadavers will be used for three independent endoscopists: an attending, a fellow, and a novice. They will perform colonoscopy by using the robot-assisted platform and then a standard colonoscopy. Primary outcome: cecum intubation rate. Secondary outcomes: time to reach the cecum, farthest distance, inter-magnetic force, and lesion detection rate

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Development and pre-clinical validation of a soft-tethered endoscopic robot to replace colonoscopy as a screening tool for colorectal cancer
Project Code: PE-2013-02359172	Principal Investigator: AREZZO ALBERTO
Research Type: Biomedical/Biomedica	Applicant Institution: Piemonte

Project Type: PE- ITALIAN RESEARCHER ABROAD

Impact and Translational Implications

Main innovations:

- Front wheel drive of the camera, preventing looping and stretching of the colon, reducing discomfort and preventing perforations
- Soft-tethered body, with magnetic coupling to orient the camera, eliminating structural cables
- Robot-assisted driving providing steadiness and motion accuracy to increase diagnostic accuracy
- Cost-effectiveness

Magnetically driven capsule endoscopy will represent a disruptive innovation playing a major role as screening tool for CRC

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: Structural biomarkers of awareness and wakefulness in disorders of consciousness
Project Code: PE-2013-02359287	Principal Investigator: Ferraro Stefania
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Fondazione Istituto Neurologico Carlo Besta
Project Type: PE- ITALIAN RESEARCHER ABROAD	

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases					
Project Classification IRG:	Brain Disorders and Clinical Neuroscience				
Project Classification SS:	Clinical Neuroscience and Neurodegeneration - CNN				
Project Keyword 1:	Neuroimaging, functional, biochemical, and neuropathological studies to assess the onset, progression, treatment, and development of biomarkers for brain disorders.				
Project Keyword 2:	Disorder of consciousness				
Project Keyword 3:	MRI-EEG				
Project Request:	Animals: ☐		Humans: ☒		Clinical trial: ☐
The project has already been presented: ☐		Project code reference:			

☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit			
Name and Surname:	Martin M. Monti		
Foreign Institution:	University of California Los Angeles		
Department/Division/Laboratory:	Department of Psychology		
City-State and Country:	Los Angeles, California, United States of America		
Years of Residence Abroad:	10		
Email: monti@psych.ucla.edu		Phone:	+1 (310) 825-8546

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Fondazione Istituto Neurologico Carlo Besta	Neuroradiology Department	Coordination Unit. Neuroradiological assessment. Advanced MRI data analyses.
2	University of California Los Angeles	Department of Psychology	One of the leading centers in the world in the filed of Disorder of Consciousness (DOC). Advanced MRI and EEG analyses.
3	Fondazione Istituto Neurologico Carlo Besta	Neurophysiopathology Department	Neurophysiological assessment. Advanced EEG data analyses.

Project Title:
Structural biomarkers of awareness and wakefulness in disorders of consciousness

Project Code: PE-2013-02359287

Principal Investigator: Ferraro Stefania

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Fondazione Istituto Neurologico Carlo Besta

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Visani Elisa	Fondazione Istituto Neurologico Carlo Besta	Engineer Researcher. Advanced EEG data analyses.	11/12/1975

Background and Significance

Disorders of consciousness (DOC) such as coma, the vegetative state (VS) and the minimally conscious state (MCS) are among the least understood and most ethically troublesome conditions of the human brain. These conditions, typically acquired after severe brain injury, describe patients presenting an impairment of wakefulness and/or awareness.
In the absence of a substantial understanding of the neuropathology of DOC, and the mechanisms that accompany loss and recovery of consciousness after severe brain injury, there persists a ~40% mis-diagnosis rate by which MCS patients are considered VS - an error that bears clinical, legal and ethical implications.
The current submission is aimed at bridging this neuropathology gap by assessing the relationship between the damage sustained within deep structures of the brain and behavioral and electrocortical measures of awareness and wakefulness.
Based on one of 'mesocircuit hypothesis' of DOC, we will perform a theory-driven analysis relating structural damage, as observed with MRI, within subcortical areas including basal ganglia, thalamus and brainstem nuclei, and its relation to (i) awareness, (ii) electrocortical arousal and (iii) behavioral arousal.
The project is conceived to bear direct consequences in terms of (i) finding biomarkers for patient evaluation and diagnosis in the clinic, and (ii) understanding of the mechanisms underlying DOC - necessary for accurate diagnosis and for future development of interventions.

Specific aims

Aim 1: To assess the relation between structural damage in DOC patients and standard clinical measures of awareness (and therefore its suitability as a diagnostic biomarker) and etiology.

Aim 2: To assess the relation between structural damage and electroencephalographic measures of cortical arousal (i.e., EEG power spectrum analysis) as well as electroencephalographic measures of sleep-wake behavior.

Aim 3: To compare clinical (behaviorally based) diagnosis with pathology-based data-driven classification (using machine learning).

Hypothesis: HYP 1: The degree of structural damage within thalamus is significantly associated with clinical measures of conscious behavior.
HYP 2: Electrocortical profile (a global index of brain function) and measures of sleep/wake behavior are significantly associated with degree of damage in specific subcortical structures including thalamus, basal ganglia and superior brainstem nuclei originating corticopetal fibers (i.e., periacqueductal grey matter, PAG; and ventral tegmental area, VTA).
HYP 3: Pathology-based stratification might reveal subgroups of patient (within different diagnostic categories) that are not revealed by standard behavior-based stratification (i.e., diagnosis)

Preliminary data: Recent work by Monti and others have shown a significant association, in acute patients, between damage within specific areas of thalamus and patient outcome at 6 months (Lutkenhoff et al., 2013) - consistent with the mosocircuit hypothesis and the few previous studies that have assessed structural damage in small samples of DOC patients (e.g., Fernandez-Espejo, et al., 2010). Moreover, from 2011 to 2013, at FINCB, we have collected neuroimaging and electrophysiological

data in 126 patients with DOC with different etiologies. Our recent study (Rossi Sebastiano et al., 2014) confirmed the relationship between clinical scale and polysomnography in DOCs (de Biase et al., 2014). Moreover, spectral EEG properties seemed to be significantly related to DOC classes (Rossi Sebastiano et al., 2014, Varotto et al., 2013). The neuroimaging data (performed at 3T MR scanner, Philips Achieva) comprised a 3D T1-weighted, TSE T2, T2*-weighted and FLAIR imaging.

Materials and Methods

We will study a group of 126 patients with DOC and a group of 30 healthy participants. The first group comprises patients in minimal conscious state (MCS; n=44) and in vegetative state (VS; n=82) with different etiologies.
We will assess the degree of atrophy and the presence of lesions in the basal ganglia and in specific nuclei of thalamus and of the midbrain using the 3D T1-weighted images. Tissue damage will be assessed employing shape analysis (Patenaude et al., 2011) and manual segmentation for the VTA and PAG (Murty et al., 2014).
24-hour EEG and polygraphic recordings will be evaluated. Sleep will be scored according to the American Academy of Sleep Medicine rules and studied by spectral analysis. Connectivity analysis will be performed and the relative indexes derived from graph theory will be obtained. These parameters will be correlated with anatomical data.
Machine learning techniques will follow classification (Lutkenhoff et al., 2013) and k-means (MacQueen, 1967) methods.

Impact and Translational Implications

This project will have a direct translation to the clinic by evaluating the use of structural damage analysis and stratification as a basis for enhanced diagnosis - potentially decreasing the misdiagnosis problem. In addition, this project will also contribute to our theoretical understanding of DOC, by studying the (so far not much explored) pathology of DOC after severe brain injury, and its relation to current clinical methods.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Collaborazione Ricercatori Italiani all'Estero	Project Title: In vivo assessment of the role of Locus Coeruleus in the development of Alzheimer's Disease and other types of Dementia.
Project Code: PE-2013-02359574	Principal Investigator: Giorgi Filippo Sean
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Neurologic diseases

Project Classification IRG: Brain Disorders and Clinical Neuroscience

Project Classification SS: Clinical Neuroscience and Neurodegeneration - CNN

Project Keyword 1: Neuroimaging, functional, biochemical, and neuropathological studies to assess the onset, progression, treatment, and development of biomarkers for brain disorders.

Project Keyword 2: Dementia

Project Keyword 3: Locus Coeruleus

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

The project has already been presented: ☐ Project code reference:

☐ I declare that the object/s of this application is/are under patent copyright

Italian Researcher Abroad – Foreign Operative Unit

Name and Surname: Nicola Pavese

Foreign Institution: Imperial College of London,

Department/Division/Laboratory: Neurology Imaging Unit (NIU), Division of Brain Sciences

City-State and Country: London W12 0NN, United Kingdom

Years of Residence Abroad: 10

Email: nicola.pavese@imperial.ac.uk Phone: +44 020 3313 3766

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Toscana	Azienda Ospedaleiro-Universitaria Pisana, DAI di Neuroscienze, Unità Operativa di Neurologia-Neurofisiopatologia.	project coordination, selection and enrollment of patients
2	Imperial College of London,	Division of Brain Sciences, Neurology Imaging Unit (NIU)	brain PET supervision and analysis
3	Toscana	Fondazione "G. Monasterio", MRI Unit	brain MRI and analysis

Project Title:
In vivo assessment of the role of Locus Coeruleus in the development of Alzheimer's Disease and other types of Dementia.

Project Code: PE-2013-02359574

Principal Investigator: Giorgi Filippo Sean

Research Type: Clinical health care research/Clinico-assistenziale

Applicant Institution: Toscana

Project Type: PE- ITALIAN RESEARCHER ABROAD

Investigators, Institution and Role on Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Lombardo Francesco	Toscana	MRI acquisition and analysis	08/11/1962

Background and Significance

Alzheimer's Disease (AD) is mainly featured by Amyloid Plaques (AP) and neurofibrillary tangles (NFT) deposits in the brain: the phenomena triggering these alterations are still largely unknown. Mild Cognitive Impairment (MCI) amnestic domain (-a) precedes the development of AD, but the conversion to AD cannot be predicted in the single MCI-a patient, even taking into account magnetic resonance imaging (MRI), 18 F-deoxyglucose (FDG) PET, and cerebrospinal fluid (CSF) biomarkers.
Locus Coeruleus (LC) is the main noradrenergic (NE) brain nucleus, plays a relevant role in cognition, and is significantly degenerated in AD brain specimens. LC lesion potentiates AP burden in transgenic AD models mainly by increasing neuroinflammation.
LC neuromelanin has been recently detected in vivo in humans by specific T1-weighted MRI sequences, and an indirect parameter of it [Contrast ratio (CR)] has been quantified. Despite the many experimental evidences for a key role of LC in AD pathogenesis, only very recently LC-CR analysis in a small number of AD and MCA-a patients confirmed an alteration of LC vs. controls: the latter data need to be further confirmed with finer signal analysis, in better defined patients groups, and after matching patients and controls for age. Furthermore, it is not known yet also how and to which extent LC and CR-LC are affected in other Dementia types, i.e. Frontotemporal Lobe Degeneration (FTD), Parkinson's disease Dementia (PDD) , and Lewy Body Dementia (LBD).

Specific aims

Aim 1: To assess in vivo the role of LC in the onset and progression of AD and other forms of Dementia (PDD, LBD, FTD). In order to do that we will assess by ultra-high field MRI the LC-CR in patients with MCI-a or mild AD, and other forms of Dementia, and follow-up their evolution.

Aim 2: To correlate the degree of LC-CR alteration with: a) the type of dementia; b) specific neuropsychological alterations; c) specific CSF biomarkers: Aß40, Aß42, total Tau, phospho-Tau, synuclein; d) NE and metabolites CSF levels; e) pattern of brain activated microglia as assessed by PET imaging with 11C-PK11195; f) serum and CSF inflammatory biomarkers; g) MRI volumetry; h) pattern of FDG PET alteration.

Aim 3: To develop, based on the above results, an algorithm which, including the CSF data, LC-CR, MRI brain volumetry, PET and neuropsychological findings, would allow with high sensitivity and specificity: a) to predict the development of AD in MCI-a; b) to make a precise diagnosis of the type of degenerative dementia in patients with initial cognitive signs and in which classical analysis alone does not allow a clear diagnosis.

Hypothesis: The LC plays a key role in the neurodegenerative processes occurring in AD. In particular, LC impairment might represent one of the first steps of the degeneration cascades leading to AD, occurring before AP and NFT formation, which are considered as key features of its pathogenesis. Indeed, a pre-existing LC impairment might trigger/accelerate the development of those neurodegenerative events. In patients complaining for specific memory loss and classified as MCI-a, the disclosure of an impairment of LC parameters (both by MRI-CR and by CSF NE levels), associated with specific alterations of other parameters (FDG-PET pattern, MRI brain volumetry) and CSF biomarkers (Aß40, Aß42, total Tau, phospho-Tau, synuclein), might allow to predict the rate and speed of evolution to AD. In particular, we expect that: a) in patients with AD there is a significantly different LC-CR versus age-matched controls; b) in MCI-a patients with a significantly reduced LC-CR, there is a higher yearly risk of conversion to AD versus MCI-a

patients with normal LC-CR; c) LC alteration is specific for AD but not for FTD; PDD and LBD might bear a different type of LC MRI alteration; d) a direct correlation of LC impairment with PET imaging with the 11C-PK11195, a marker of activated microglia, as well as with specific CSF AD-related and neuroinflammatory markers exists; the latter phenomenon is present in AD but to a much lesser extent in FTD, PDD and LBD, in which different mechanisms are likely to be involved. The early recognition of a significantly impaired LC system could drive, in the future, the use of drugs stimulating specific noradrenergic receptors before the full-blown AD phenotype develops in the single patient.

Preliminary data: We have set up specific MRI T1-derived sequences in order to measure LC-CR; we are building our database of LC-CR in healthy controls at different ages.

Materials and Methods

Patients tested will be: MCI-a, mild AD, PDD, FTD, LBD.
All controls and patients will be submitted to:
-complete neuropsychological assessment at baseline and every 6 months (patients), or every 12 months (controls), thereafter
-3T MRI with evaluation of brain volumetry (whole brain and hippocampus) and LC-CR
-measurement of neuroinflammatory markers in serum: we will use a panel of cytokines assay including IL-1a, IL-1ß, IL-6, IL-10, IL-12, TNF-a,TGF-ß.
Subgroups of patients from MCI-a, AD, FTD, LBD, PDD groups will be also submitted to:
-lumbar puncture with CSF assay of: a) Aß40, Aß42, total tau, phospho-Tau, synuclein levels, b) levels of noradrenaline and its metabolites, c) levels of the same cytokines assayed in serum.
-Brain PET Scans with 11C-PK11195 and 18-FDG. 11C-PK11195.
Specific analysis of data will include:
-correlational analysis of LC-CR data with the other data to develop a specific algorithm for prediction of conversion to AD and early dementia type diagnosis

Impact and Translational Implications

The study of the role of LC in patients will: a) contribute in differentiating early the type of dementia; b) clarify its role in the neuroinflammation occurring in dementia; c) confirm and implementing existing data on LC-CR and evolution of MCI-a into AD. Most of the diagnostic tools applied in this study are routinely used in Memory Clinics: thus, our diagnostic algorithm will be of widespread application. These data might lead to hypothesize specific noradrenergic treatments for dementia.