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 Cofinanziamento Industriale

INDICE PROGETTI

CODICE
CO-2013-02356242

Destinario Istituzionale
SANTA LUCIA

TITOLO PROGETTO
PALMITOYLETHANOLAMIDE TO TARGET INFLAMMATION IN NEURODEGENERATIVE DISEASES: A STUDY FOCUSED ON PERIPHERAL IMMUNE CELLS

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Biomedica	Neurologic diseases	254.508,00

CODICE
CO-2013-02356463

Destinario Istituzionale
Lazio

TITOLO PROGETTO
Genetic and functional predictors of response to biologic therapy in psoriasis.

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Immunology	Biomedica	Infectious and immunological diseases	150.000,00

CODICE
CO-2013-02357626

Destinario Istituzionale
Lombardia

TITOLO PROGETTO
Development of selective inhibitors of crizotinib-resistant oncogenic ALK kinase

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Oncology 2 - Translational Clinical	Biomedica	Oncology	235.758,00

CODICE
CO-2013-02358433

Destinario Istituzionale
SAN MATTEO

TITOLO PROGETTO
Development and validation of new assays for diagnosis and monitoring of infectious diseases using the DiaSorin Q-LAMP Technology

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Infectious Diseases and Microbiology	Biomedica	Innovative biotechnologies	150.000,00

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

Progetti Cofinanziamento Industriale

INDICE PROGETTI

CODICE
CO-2013-02358488

Destinario Istituzionale
Lazio

TITOLO PROGETTO
Tolerability and efficacy of Zinc therapy in Mild Cognitive Impairment for treatment and prevention of Alzheimer's disease: a prospective, randomized, double blind, parallel, placebo-controlled Phase II clinical trial

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Clinico-assistenziale	Neurologic diseases	224.508,00

CODICE
CO-2013-02358697

Destinario Istituzionale
Lazio

TITOLO PROGETTO
Extremely low frequency magnetic field (ELF-MF) stimulation as a neuroprotective treatment in acute ischemic stroke.

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Emerging Technologies and Training in Neurosciences	Biomedica	Neurologic diseases	150.100,00

CODICE
CO-2013-02359461

Destinario Istituzionale
Istituto Superiore di Sanita'

TITOLO PROGETTO
Identification of predictive biomarkers in Multiple Sclerosis using a proteomic approach

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Brain Disorders and Clinical Neuroscience	Biomedica	Neurologic diseases	150.000,00

CODICE
CO-2013-02359690

Destinario Istituzionale
OSPEDALE BAMBINO GESU'

TITOLO PROGETTO
Cardiac regeneration: Characterization, optimization and evaluation of force developed by engineered tissue obtained from spontaneous or scaffold based 3D culture of paediatric human adipose tissue-derived mesenchymal stem cells

Area Expertise	TIPOLOGIA RICERCA	AREA_STRATEGICA	Finanziamento Assegnato euro
Cardiovascular and Respiratory Sciences	Biomedica	Metabolic and cardiovascular diseases	184.383,00

SEGUONO ABSTRACT PROGETTI FINANZIATI

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: PALMITOYLETHANOLAMIDE TO TARGET INFLAMMATION IN NEURODEGENERATIVE DISEASES: A STUDY FOCUSED ON PERIPHERAL IMMUNE CELLS
Project Code: CO-2013-02356242	Principal Investigator: Bossù Paola
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Santa Lucia

Project Type: CO - Industrial Co-financing

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: Demyelination, neuroinflammation in Alzheimer¿s disease and Parkinson¿s disease; reactive microglia, astrocytes, macrophages, axonal damage, regeneration, and myelination/remyelination.
Project Keyword 2: palmitoylethanolamide
Project Keyword 3: peripheral myeloid cells
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

INDUSTRIAL/MANUFACTURER COFINANCING
Industry/Manufacturer: Epitech Group Srl
Address: Via L. Einaudi, 13
City-State and Country: Saccolongo (PD) Italy
Amount of Cash Co-financing: 300000
Contact Person: Name: Francesco Della Valle Email: francesco@dellavalle.info Phone: 0498016784

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Fondazione Santa Lucia	Clinical and Behavioral Neurology/Experimental neuropsychobiology	Coordination of the whole project. Cellular and molecular studies on inflammation and immune response in subjects affected by AD and PD
2	Fondazione Santa Lucia	Clinical and Behavioral Neurology/Movement Disorder Unit, "Sant'Andrea" Hospital	Selection of subjects, diagnosis of AD and PD and clinical evaluation
3	University of Messina	Department of Biological and Environmental Sciences	In vitro and in vivo studies in animal models of AD and PD

Project Title: PALMITOYLETHANOLAMIDE TO TARGET INFLAMMATION IN NEURODEGENERATIVE DISEASES: A STUDY FOCUSED ON PERIPHERAL IMMUNE CELLS

Project Code: CO-2013-02356242

Principal Investigator: Bossù Paola

Research Type: Biomedical/Biomedica

Applicant Institution: Fondazione Santa Lucia

Project Type: CO - Industrial Co-financing

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Pontieri Francesco Ernesto	Fondazione Santa Lucia	will lead the activities related to the clinical evaluation and selection of subjects, in particular PD patients. He will also be responsible for overseeing diagnostic procedures on subjects included in the study, neuroimaging examination, assessment of motor symptoms, neuropsychological and psychopathological testing	11/12/1961
2	Esposito Emanuela	University of Messina	will lead the activities related to the in vitro studies and animal models of AD and PD, including pharmacological treatment and neuropathological and inflammatory evaluations	27/08/1974

Background and Significance

Alzheimer's and Parkinson's diseases (AD, PD) are progressive and incurable neurodegenerative diseases leading to dementia and movement disorders, respectively.
Although chronic inflammation is considered a crucial participant in the pathophysiology of both AD and PD, clinical trials based on systemic anti-inflammatory therapies have so far produced disappointing results. This opened to the idea that neuroinflammation is a complex process, influenced by genetic and environmental factors, including peripheral immune activation, which, in turn, may result in either damaging or protecting effects in the CNS. GWAS studies have pointed to myeloid compartment of the immune system as a crucial component of susceptibility to neurodegenerative diseases, where monocytes and dendritic cells (DC), may be regulators/contributors of disease progression. In this context, palmitoylethanolamide (PEA), an endogenous lipid messenger produced throughout the body with peripheral and central anti-inflammatory activities, is a good candidate to oppose neurodegeneration. When applied exogenously, PEA has proven efficacy in animal models of AD and PD and reduces microglia activation. However, no data about PEA effects on blood myeloid populations have been provided in the neurodegenerative context. The elucidation of the links existing between neuroprotective and central/peripheral anti-inflammatory actions of PEA would help in discovering new and effective therapeutic approaches for AD and PD.

Specific aims

Aim 1: Assessment of the in vitro effects of PEA on patients' myeloid cell subpopulations and characterization of PEA specific mechanisms of action in these cells

Aim 2: Comprehensive evaluation of phenotypic and functional abnormalities of myeloid cell subpopulations (cultured in presence/absence of PEA treatment) in healthy subjects and patients, in relation to the clinical symptoms of neurodegeneration.

Aim 3: Confirmation and expansion of the clinical results in animal models of AD and PD: identification of in vivo PEA effects on peripheral versus brain immune cell activation, neurodegeneration and specific symptoms; characterization of PEA mechanisms of action.

Hypothesis: Given the probable role of peripheral immune cells on the progression of neurodegenerative diseases, and PEA therapeutic potential in treating neurodegeneration, we plan to study PEA anti-inflammatory effects on

different myeloid cell subpopulations from patients with AD and PD, as compared to healthy controls, and correlate such results with disease outcome. Since the implication of peripheral immune cells in brain damage is very challenging to investigate in humans, we sought to further develop human data by assessing the in vivo PEA effects on both brain and peripheral immune cells in animal models of AD- and PD-like neurodegeneration.

We hypothesize that during neurodegeneration PEA might modulate the activity of peripheral immune cells other than microglia, with consequences on AD and PD progression.

If successful, this study will provide new knowledge about anti-inflammatory signalling of PEA, offering opportunities for new therapeutic progress in neurodegeneration.

Preliminary data:

Blood borne myeloid cells may participate to immune response in neurodegeneration, modifying the disease. We and others have suggested that DC may be listed among the cell mediators of disease both in AD and PD. In fact, monocyte derived DC are modified by amyloid beta treatment and dysregulated in AD patients (Ciaramella et al., 2009; 2010 and 2013a). Furthermore, circulating DC levels, which correlate with gravity of disease symptoms , may be decreased in the blood of both AD and PD patients (Ciaramella et al. 2013b; preliminary results), possibly as an index of their recruitment to the brain. Endogenous acylethanolamides as PEA, may be protective in neurodegeneration, but their anti-inflammatory properties on different subsets of peripheral immune cells, other than mast cells, are still understudied. We have preliminary evidence that PEA is able to modulate chemotaxis of human DC in vitro, supporting the value to assess PEA effects and mechanisms of action in these cells. Since in vivo PEA can rescue behavioural impairments in AD-like conditions and protect mice against MPTP-induced neurotoxicity and motor disturbances (Esposito et al., 2012), the analysis of peripheral versus brain inflammation in the animal setting offers opportunity to confirm human data and assess the impact of PEA anti-inflammatory mechanisms on neurodegeneration.

Materials and Methods

Fifty subjects from each group (AD, PD and healthy controls, HC), will be enrolled and clinically evaluated. Blood-derived myeloid subsets, including DC and myeloid derived suppressor cells (MDSC), will be phenotypically and functionally characterized (flow cytometry, real time PCR, ELISA) in all subjects and cultured in vitro with PEA and appropriate disease-specific stimuli. Selected cannabinoid (CB1 and CB2), transient receptor potential vanilloid type-1 (TRPV1), and peroxisomal proliferator activated receptor alpha, receptor agonists and antagonists will be used to characterize PEA mechanisms of action. Triple transgenic mice over-expressing three mutant genes - APP, presenilin-1, and tau - and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice will be used as AD and PD model, respectively. After administration of PEA, neuropathological and inflammatory changes (at brain and peripheral level) will be evaluated (immunostaining, ELISA, flow cytometry, real time PCR).

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: PALMITOYLETHANOLAMIDE TO TARGET INFLAMMATION IN NEURODEGENERATIVE DISEASES: A STUDY FOCUSED ON PERIPHERAL IMMUNE CELLS
Project Code: CO-2013-02356242	Principal Investigator: Bossù Paola
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Santa Lucia

Project Type: CO - Industrial Co-financing

Impact and Translational Implications

Neurodegenerative diseases as AD and PD cause a heavy burden on the NHS. This study, aimed at evaluating the impact of the endogenous acylethanolamide PEA on peripheral immune cells and its effect on neuroprotection and disease progression, may be well exploited to design innovative strategies to combat neurodegeneration, in line with the effort to optimize public resources by reducing hospitalization days and alleviating the workload of rehabilitation services.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Genetic and functional predictors of response to biologic therapy in psoriasis.
Project Code: CO-2013-02356463	Principal Investigator: Costanzo Antonio
Research Type: Biomedical/Biomedica	Applicant Institution: Lazio

Project Type: CO - Industrial Co-financing

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in
Infectious and immunological diseases

Project Classification IRG: Immunology

Project Classification SS: Hypersensitivity, Autoimmune, and Immune

Project Keyword 1: Treatment of immune-mediated diseases: antigen specific and non-specific drug and biologic approaches to tolerance to self or foreign antigens including vaccination, gene therapy, peptide and altered ligand approaches as well as cell-based approaches; development of biomarkers of disease and related activities, and outcome assessments in clinical studies; determinants of response to therapy.

Project Keyword 2: Psoriasis

Project Keyword 3: pharmacogenetics

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

INDUSTRIAL/MANUFACTURER COFINANCING

Industry/Manufacturer: Janssen-Cilag Spa

Address: Via M. Buonarroti, 23 - 20093

City-State and Country: Cologno Monzese (Milan), Italy

Amount of Cash Co-financing: 150000

Contact Person: Name: Sebastiano Forgia Email: sforgia@its.jnj.com Phone: 02-2510512

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Lazio	Dermatology Unit, Sant'Andrea Hospital, Rome, Italy (Prof. A. Costanzo)	Coordination, Conduct clinical trials, genotyping. (team: two staff units and a researcher to be hired)
2	Istituto Superiore di Sanità	Department of Infectious, Parasitic and Immune-mediated Diseases (Dr. R. Lande)	Functional immunologic study.(team: one staff unit and a researcher to be hired)

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	lande roberto	Istituto Superiore di Sanità	Unit Coordinator, Functional immunologic study	28/11/1966

Project Title:
Genetic and functional predictors of response to biologic therapy in psoriasis.

Project Code: CO-2013-02356463

Principal Investigator: Costanzo Antonio

Research Type: Biomedical/Biomedica

Applicant Institution: Lazio

Project Type: CO - Industrial Co-financing

Background and Significance

Psoriasis affects 2-3% of individuals in Europe causing significant co-morbidities in a large number of patients. A number of studies highlight the involvement of Th17 cells and the role of IL12/IL23 and IL6 in the context of Th17 cell-dependent chronic inflammation. Polymorphisms in IL12B gene (encoding for the p40 subunit of IL12/IL23) have been recently linked to the differentiation of Th1/Th17 cells. Recently, the pathogenic role of the antimicrobial peptide LL37, overexpressed in the lesional skin has been described. These advances on psoriasis genetic and immunologic implications have led to the development of drugs specific for tumor necrosis factor-a (TNF) or the p40 subunit. However, therapeutic outcome remains variable due to the complex genetic heterogeneity of patients. Indeed, a recent study reported an association between two SNPs in the gene encoding for TNFAIP3 and the clinical outcome of anti TNF agents. Moreover, our group has previously reported that HLA-Cw6 (the most important psoriasis susceptibility marker) positive carriers have a significantly higher response rate to ustekinumab compared to Cw6-negative patients and strong preliminary data show that the skin antimicrobial peptide LL37 could play a role as T cell autoantigen in the HLA-Cw6 context.
Considering these findings, we plan to develop a patent for the combined use of genetic and functional data to generate a test for predicting the outcome of biologic therapies in psoriasis.

Specific aims

Aim 1: Confirm the role of the presence of HLA-Cw6 in predicting the clinical response to biologics in psoriasis and identify the possible role IL12B (p40) SNPs polymorphisms as additional predictors of response to biologics in psoriasis. This will be achieved through a retrospective observational clinical trial on patients under biologic therapy for psoriasis

Aim 2: To identify the molecular and pathophysiological basis of interpersonal differences in clinical response to ustekinumab and other biologics by studying the activation of T cell responses induced by LL37 autoantigen in psoriasis.

Aim 3: To set-up methods based on multiple pharmacogenetic data and a functional assay to personalize biologic therapies in psoriasis (object of the patent).

Hypothesis: Differentiation of naive T cells into Th17 cells is influenced by IL-6 and TGF beta cytokines. While amplification of Th1 and Th17 cells is promoted by IL-12 and IL-23, respectively, two heterodimeric cytokines sharing a common p40 subunit and specific p19 (IL-23) and p35 (IL-12) subunits. Interestingly, the expression of the common p40 subunit is strongly influenced by two single nucleotide polymorphisms (SNPs) that constitute a psoriasis-associated risk haplotype at the IL12B locus: the rs3212227 located in the 3¿ UTR and rs6887695 located upstream of the IL12B gene. The psoriasis predisposition haplotype (G allele of rs6887695, and its associated A allele of rs3212227) leads to increased expression of IL12B by monocytes and correlates with increased serum levels of IL-12, IFN-g and the IFN-g induced chemokine, CXCL10. Based on these data we hypothesized that IL12B gene SNPs may affect the response to biologic therapies and particularly to p40 targeting agent ustekinumab. Secondly, preliminary data generated in collaboration with Prof. Lande indicate the alarmin/antimicrobial peptide LL37 as a psoriasis autoantigen, able to be presented on HLA-Cw6 as well as on other Class I and Class II MHC. Around 60% of patients with moderate to severe psoriasis tested display strong in vitro response to LL37, inducing proliferation of Tc1, Th1 and Th17 cells.
We plan to study whether the presence of Cw6 and IL12B gene polymorphisms affect the kind of T cell response elicited by LL37. This would provide a molecular explanation for our observation of different sensitivity to IL-12/23 blocker in patients carrying HLA-Cw6 and IL12B gene polymorphisms, possibly adding prediction power to genetic data alone.

Project Title:
Genetic and functional predictors of response to biologic therapy in psoriasis.

Project Code: CO-2013-02356463

Principal Investigator: Costanzo Antonio

Research Type: Biomedical/Biomedica

Applicant Institution: Lazio

Project Type: CO - Industrial Co-financing

Preliminary data: We have conducted a pilot study on 67 patients under ustekinumab treatment. We found that the co-presence of HLA-Cw6 allele and G/G haplotype of the IL12B SNP rs6887695 was associated to: 1) a higher probability of full clinical response to ustekinumab at week 12 (PASI75 reached in 91,7% vs. 66,7% of patients) that is maintained at one year (88,3% vs. 44,4%); 2) a faster response to ustekinumab (PASI 75 at week 4 reached in 50% vs 0% patients).

Materials and Methods

1) A retrospective observational clinical trial on 300 psoriasis patients under biologics will be conducted to determine the association between Cw6, IL12B SNPs and response to therapy.
2) A smaller prospective trial will be conducted on 60-80 patients who will be screened for LL37 T cell responses and genotyped before undergoing biologics.
Genetic Characterization of HLA-Cw6 and IL12B SNPs will be performed from patients' saliva by Allele specific PCR and Taqman assys, respectively.
PBMC from healthy donors and patients will be treated in vitro with LL37, LL37-overlapping 9-mer peptides and scrambled peptides. At day 3 and 5 T cell proliferation will be measured by BrdU incorporation.Tc1/Tc17 cytokines will be measured by ELISA Kits or by intracellular staining. LL37-specific T cells will be identified by tetramer staining. After stimulation with LL37 blood cells will be stained for tetramer Cw6*02 complexed with LL37-derived peptides and co-stained for T cell specific markers.

Impact and Translational Implications

Results from our project will deliver a test based on genetic and immunologic data for predicting response to biologics (particularly ustekinumab) in patients affected by moderate to severe psoriasis, that will be subjected to patent.
Our project will ultimately lead to an optimization of therapeutic choice in psoriasis patients providing an evidence-based rationale for choosing one specific treatment in psoriasis patients.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Development of selective inhibitors of crizotinib-resistant oncogenic ALK kinase
Project Code: CO-2013-02357626	Principal Investigator: Gambacorti-Passerini Carlo
Research Type: Biomedical/Biomedica	Applicant Institution: Regione Lombardia - Direzione Generale Sanità

Project Type: CO - Industrial Co-financing

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Oncology

Project Classification IRG: Oncology 2 - Translational Clinical

Project Classification SS: Drug Discovery and Molecular Pharmacology - DMP

Project Keyword 1: New drug development and production: identification, synthesis and isolation of novel drugs and modification of existing compounds for evaluation in both in vitro and in vivo tumor model systems

Project Keyword 2: Tyrosine kinase

Project Keyword 3: Lung adenocarcinoma

Project Request: Animals: [X] Humans: [ ] 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: Università Milano-Bicocca
Università de Geneve
Università Claude Bernard Lyon 1

Patent number: Domanda Brevetto Italiano n. MI2013A001124

INDUSTRIAL/MANUFACTURER COFINANCING

Industry/Manufacturer: Galkem Srl

Address: via Italia 46

City-State and Country: Monza - Italy

Amount of Cash Co-financing: 315,000

Contact Person: Name: Jerome Bertho Email: jerome.bertho@gmail.com Phone:

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Regione Lombardia - Direzione Generale Sanità	UO ematologia - Az. Osp. S. Gerardo	Coordination and execution
2	University of Milano-Bicocca	Health Sciences	Chemical synthesis, in vitro and in vivo experiments

Project Title:
Development of selective inhibitors of crizotinib-resistant oncogenic ALK kinase

Project Code: CO-2013-02357626

Principal Investigator: Gambacorti-Passerini Carlo

Research Type: Biomedical/Biomedica

Applicant Institution: Regione Lombardia - Direzione Generale Sanità

Project Type: CO - Industrial Co-financing

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Mologni Luca	University of Milano-Bicocca	Chemical synthesis, in vitro experiments	05/07/1971
2				
3	Ceccon Monica	University of Milano-Bicocca	in vitro and in vivo experiments	24/11/1983

Background and Significance

ALK tyrosine kinase is involved in the pathogenesis of various tumors, including anaplastic lymphoma, lung cancer, neuroblastoma and others. The pathogenic role of ALK in these tumors has been confirmed by a large number of studies. Therefore, ALK represents a rational and important therapeutic target. ALK+ tumors are currently treated with crizotinib (XalkoriTM, Pfizer) an oral pharmacological inhibitor of ALK. Unfortunately, most of the patients treated with crizotinib acquired resistance to the drug and experienced a recurrence of the disease, usually as a result of point mutations within the ALK catalytic domain. Patients with recurrent disease have no option than standard chemotherapy, which involves high systemic toxicity and hospitalization. Another drug, ceritinib (ZykadiaTM, Novartis) was approved in 2014 by the FDA. Zykadia showed clinical activity on some, but not all, Xalkori-resistant tumors and itself suffered the development of resistant disease. In general, each drug brings about the problem of resistance, albeit with a different mutation profile. In the case of chronic myeloid leukemia, which represented the first example of targeted therapy, the development of resistance or intolerance to various inhibitors has has led to the approval of five different drugs. Therefore, we believe that at present there is a pressing need to develop new inhibitors of the ALK kinase

Specific aims

Aim 1: The applicant proposes to design and develop new compounds that selectively inhibit the ALK oncogenic kinase, for the treatment of ALK+ tumors. Two unrelated structural families of ALK inhibitors have been identified by the PI in the last years.The aim of this project consists in further development of these molecules into drugs able to selectively inhibit the oncogenic activity of ALK both in native and mutant (crizotinib-resistant) form, exploiting their innovative, ATP-non competitive binding mode. In the first year, lead compounds will be optimized. The molecules will be synthesized in small-scale and characterized in vitro for ALK inhibition in biochemical assays and in cells. The results will serve as feedback for subsequent preparation of optimized derivatives. The objective is to identify at least 5 preclinical candidates to be submitted to in vivo tests

Aim 2: The efficacy of the selected compounds in animal models will be studied during the second and third year. We will investigate the ability of an inhibitor to induce regression of ALK-driven xenografts in nude mice and the general toxicity caused to animals by acute administration of compounds

Aim 3: In vitro and in vivo ADMET profiles will be generated for the best derivatives. In addition, pharmacokinetics and pharmacodynamics will be studied in mice and rats, after oral administration

Hypothesis: Conventional cancer therapies have serious limitations due to their high toxicity. The success of targeted therapies in CML and other tumors indicates that even advanced disease can be effectively treated if the right target is hit. ALK+ tumors are a brilliant example of dependence on a single oncogene, as evidenced by numerous preclinical and clinical studies. However, despite efficacy of crizotinib in clinical trials, acquired resistance to the drug is a major issue, causing frequent relapses in patients. Therefore, more potent inhibitors are needed to overcome resistance. Since all compounds currently under development share a common mechanism of inhibition (i.e. ATP-competition), they will likely have overlapping resistance profiles.

Project Title:
Development of selective inhibitors of crizotinib-resistant oncogenic ALK kinase

Project Code: CO-2013-02357626

Principal Investigator: Gambacorti-Passerini Carlo

Research Type: Biomedical/Biomedica

Applicant Institution: Regione Lombardia - Direzione Generale Sanità

Project Type: CO - Industrial Co-financing

We reasoned that a structurally unrelated class of compounds with a completely different binding mode and different biochemical/kinetic properties would be beneficial to treat resistant disease

Preliminary data: We developed two series of non-ATP competitive compounds, which show higher inhibition of ALK at physiological ATP concentrations, compared to crizotinib. The current lead compounds have nanomolar IC50 values in biochemical kinase assays and in cell proliferation assays and show selectivity for ALK of approximately 10-fold. A preliminary in vivo experiment with one lead compound showed efficient tumor growth inhibition. Interestingly, the compound was able to delay the growth of a crizotinib-resistant xenograft.

Materials and Methods

Compounds will be obtained by Buchwald-Hartwig and Sonogashira sequence reactions catalyzed by palladium, using Cl-substituted precursors. Kinase assay will be performed by incubating recombinant ALK with inhibitor, ATP and peptide substrate. Phosphorylation of the substrate will be detected by ELISA using an anti-phosphotyrosine antibody. Cell growth will be assessed by 3H-thymidine incorporation assay over 72-hour incubation in the presence of inhibitors. We will use cells expressing oncogenic ALK fusions, native or crizotinib-resistant mutants. Inhibition of NPM/ALK autophosphorylation in cells will be measured by Western blot analysis with anti-phospho-ALK antibody. In vivo efficacy will be tested by injecting s.c. NPM/ALK+ cells in nude mice. Compounds will be administered orally and tumor growth will be followed using a caliper. ADMET and PK profiles will be investigated by tissue distribution, hepatic enzyme inhibition, Co, Cmax, AUClast, t¿, tmax, VD, CL parameters.

Impact and Translational Implications

We aim to develop better and more sustainable therapies in oncology. Chemotherapy causes high systemic toxicity, prolonged hospitalization and the use of additional drugs to control side effects, impacting on patients quality of life. On the contrary, targeted kinase inhibitors are usually self-administered orally at home, with low toxicity and a stark improvement in daily life. Through state-of-the-art technologies, we aim to bring high innovation and knowledge to the Italian health system

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Development and validation of new assays for diagnosis and monitoring of infectious diseases using the DiaSorin Q-LAMP Technology
Project Code: CO-2013-02358433	Principal Investigator: BALDANTI FAUSTO
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Policlinico San Matteo

Project Type: CO - Industrial Co-financing

LETTER OF INTENT - ABSTRACT

New strategies for diagnostic, therapeutic and clinical care in Innovative biotechnologies
Project Classification IRG: Infectious Diseases and Microbiology
Project Classification SS: Small Business: Non-HIV Infectious Agent Detection/Diagnostics, Food Safety, Sterilization/Disinfection and Bioremediation - SBID
Project Keyword 1: Innovations in methods or technologies for the detection or quantitation of bacteria, non-HIV viruses, eukaryotic pathogens, and prions
Project Keyword 2: viral infections in transplant recipients
Project Keyword 3: viral infections in pregnant women
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

INDUSTRIAL/MANUFACTURER COFINANCING
Industry/Manufacturer: DiaSorin S.p.A.
Address: Via Crescentino snc
City-State and Country: Saluggia, Vercelli, Italy
Amount of Cash Co-financing: € 150.000
Contact Person: Name: Ugo Gay, Vice President Italy & UK Email: ugo.gay@diasorin.it Phone: 335 6425362

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Fondazione Policlinico San Matteo	Microbiology and Virology Department	Project Coordinator

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Development and validation of new assays for diagnosis and monitoring of infectious diseases using the DiaSorin Q-LAMP Technology
Project Code: CO-2013-02358433	Principal Investigator: BALDANTI FAUSTO
Research Type: Biomedical/Biomedica	Applicant Institution: Fondazione Policlinico San Matteo

Project Type: CO - Industrial Co-financing

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1				
2				
3	Sarasini Antonella	Fondazione IRCCS Policlinico San Matteo/Microbiology and Virology dept.	development of transplantation panel	08/02/1958
4	Meroni Valeria	Fondazione IRCCS Policlinico San Matteo/Microbiology and Virology dept.	development of ToRCH panel	26/01/1953

Background and Significance

The discovery and development of molecular biology assays have been of paramount importance for diagnosis and monitoring of infectious diseases in the last 2 decades. Today, detection, quantification and typing of infectious agent(s) nucleic acids in biologic fluids using real-time PCR assays are a standard-of-care approach. The impact of molecular assays in the clinical settings is more evident in virus-associated diseases, neurologic disorders, infections during pregnancy and, in general, all those conditions related with sample volume limitations and/or difficult culturing of the infectious agent(s).
While many Diagnostic Companies are actively involved in developing wider and wider panels of molecular assays using the real-time PCR technique (Roche royalties), other molecular amplification techniques have been developed. DiaSorin is proprietary of the Q-LAMP, isothermal amplification technology. This technology has been developed by DiaSorin to offer all the benefits of isothermal LAMP (Loop Mediated Isothermal Amplification) but with the addition of real time, fluorescent, and multiplexed amplification. With multiple recognition events per reaction, DiaSorin Q-LAMP is highly specific and seems to offer sensitivity on a par with real-time PCR. Q-LAMP assays are performed on the Liason® IAM, an automated instrument developed by DiaSorin in 2012.

Specific aims

Aim 1: To set-up and validate new LAMP-based molecular assays on the Liason® IAM for detection and quantification of viral infections in transplanted patients (transplantation panel).

Aim 2: To set-up and validate new LAMP-based molecular assays on the Liason® IAM for detection and quantification of ToRCH agents (toxoplasma, rubella, cytomegalovirus and herpes, and parvovirus B19 as well) in pregnant women (ToRCH panel).

Aim 3: To set-up and validate new improved nucleic acid extraction protocols for the Liason® Ixt.

Hypothesis: Molecular diagnostics in Microbiology is a fast-evolving field, with great opportunities for technical development and steadily growing clinical demand. New molecular assays based upon the LAMP technology possess great technical and commercial potential, and a joint research and development (R&D) project with an established research group with an outstanding track record. The development of new competitive diagnostic assays will be allowed by our group experience in the performance of assays and protocols for detection and monitoring of infections in transplant recipients as well as in pregnant women.

Preliminary data: During the last 5 years, the Microbiology and Virology Dept. at Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, collaborated with DiaSorin, testing the performance of all new lots of TORCH complete assays released to customer worldwide on Liason® platform. This cooperation allowed DiaSorin to monitor the overall products quality with respect to major competitors and improve the

test performances. Furthermore, the avidity tests in the TORCH assay on Liason® XL system platform were developed and validated in a close collaboration between DiaSorin and the research group.
The Microbiology and Virology Dept is actively involved in developing new molecular assays for diagnosis and monitoring of virus infections in transplant recipients and ToRCH infections in pregnant women (Revello et al., J Clin Virol, 1999; Revello et al., J Clin Microbiol, 1999; Rovida et al., J Med Virol, 2005; Ciardelli et al., Ped Inf J, 2008; Abbate et al., J Clin Virol, 2011; Rovida et al., Diagn Microbiol Infect Dis, 2013). In addition, the Unit developed several integrated monitoring protocols in the different patients categories (Baldanti et al., J Clin Microbiol, 2000; Gerna et al., Blood, 2003; Lilleri et al., Blood, 2007; Gerna et al., J Clin Virol, 2007; Baldanti et al., Diagn Microbiol Infect Dis, 2007), and is presently recognized as an opinion-leader group in these fields (Baldanti et al., J Clin Virol, 1998; Revello and Gerna, Clin Microbiol Rev, 2002).
A wide collection of clinically characterized biologic specimens (sera, whole blood, CSF, BAL, NPS etc.) is available at the Microbiology and Virology Dept for assay development and validation.

Materials and Methods

New assays for DNA and RNA extraction from different biologic matrices with the Liaison Ixt will be developed.
The virus transplantation panel will include LAMP assays for detection and quantification of CMV, VZV, EBV, HHV-6, HSV-1 and -2, JCV, BKV, parvovirus B19, adenovirus and will be setted-up with the Liason® IAM automated instrument.
The ToRCH panel will include LAMP assays for detection and quantification of toxoplasma, VZV, parvovirus B19, rubella virus, CMV, and HSV-1 and -2 and will be setted-up for use with the Liason® IAM automated instrument.
The analytical performance of the new panel assays will be determined in specific retrospective studies using stored anonymized biologic samples from transplant recipients (whole blood, plasma, serum, CSF, BAL, urine) and pregnant women (whole blood, serum, amniotic fluid, fetal blood, newborn blood and urine) and sequential stored samples from previous prospective clinical trials in the same patient categories.

Impact and Translational Implications

The results of this R&D partnership will have an immediate transferability in improving the performance of existing commercial assays, and developing new diagnostic assays. The major advantage for the DiaSorin will be the development of highly reliable and competitive products, while the major advantage for the national healthcare system will be the availability of high performance diagnostic assays.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Tolerability and efficacy of Zinc therapy in Mild Cognitive Impairment for treatment and prevention of Alzheimer's disease: a prospective, randomized, double blind, parallel, placebo-controlled Phase II clinical trial
Project Code: CO-2013-02358488	Principal Investigator: Rossini Paolo Maria
Research Type: Clinical health care research/Clinico-assistenziale	Applicant Institution: Lazio

Project Type: CO - Industrial Co-financing

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: copper
Project Keyword 3: zinc
Project Request: Animals: [ ] Humans: [X] Clinical trial: [X]
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: Canox4Drug, SPA Patent number: Colabufo, N. and R. Squitti, P.E. European Patent Office (EPO) (RO/EP), 2012

INDUSTRIAL/MANUFACTURER COFINANCING		
Industry/Manufacturer: CANOX4DRUG		
Address: casella postale 4/A via Cristoforo Colombo sn ZI - 70010 Capurso		
City-State and Country: Bari		
Amount of Cash Co-financing: 300000 euros		
Contact Person: Name: Gaetano Canosino	Email: info@canox4drug.com	Phone: +39 080 5230306

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Lazio	Policlinico Gemelli, Istituto di Neurologia	Coordinator of all the phases and stages of the clinical trial, clinical protocols, MCI subjects recruitment. Recruitment sample collection. Corrdination of the Recruiting Units. Coordination of the sample shipments
2	Casa di cura San Raffaele Pisana	Dipartimento Riabilitazione Neuromotoria	Coordinator of all the biochemical studies. MCI subjects recruitment, sample collection, molecular analyses
3	IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia	NeuroBioGen Lab- Memory Clinic	Recruitment for trial clinic, sample collection

Project Title:
Tolerability and efficacy of Zinc therapy in Mild Cognitive Impairment for treatment and prevention of Alzheimer's disease: a prospective, randomized, double blind, parallel, placebo-controlled Phase II clinical trial

Project Code:	CO-2013-02358488	Principal Investigator:	Rossini Paolo Maria
Research Type:	Clinical health care research/Clinico-assistenziale	Applicant Institution:	Lazio

Project Type: CO - Industrial Co-financing

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Squitti Rosanna	Casa di cura San Raffaele Pisana	Biologist, she will be the responsable for all the biochemical and molecular studies of the project, and she will coordinate, in collaboration with neurologists, the MCI subjects recruitment and sample collection at IRCCS Casa di Cura San Raffaele Pisana. She will coordinate statisticians for the statistical analyses of the project.	13/04/1969
2	Binetti Giuliano	IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia	Neurologist. He will be the responsable for the recruitment of MCI subjects in IRCCS Brescia. He will coordinate the follow up of the subjects during the clinical trial and sample collection, shipment of the sample at IRCCS Brescia.	26/04/1959

Background and Significance

Milestone studies on experimental models fully demonstrate that copper not bound to ceruloplasmin (Non-Cp-Cu) is a causative factor in Alzheimer's disease (AD), since it determines a brain reduction of beta amyloid (Abeta) clearance and an increase in Abeta production in animal models of AD (Singh et al, PNAS 2013). In vitro studies in the 90's demonstrate that the hypermetallation of the Abeta peptide is at the basis of redox cycles of oxidative stress, Abeta oligomer formation and precipitation within plaques, eventually leading to neurodegeneration (Atwood et al, J Biol. Chem, 1998). Meta-analyses in living patients demonstrate that copper and Non-Cp-Cu are increased in AD (Squitti et al, JAD 2014) and associate with the typical clinical deficits of the disease (Squitti et al, Neurol 2005; Arnal et al, Brain Res 2010; James et al, Free Radic Biol Med 2012), and with Abeta and Tau in cerebrospinal fluid (Squitti et al, Neurol 2006). Non-Cp-Cu correlates with a worse prognosis of AD (Squitti et al, Neurol 2009), with the mild cognitive impairment (MCI) condition (Squitti et al, JAD 2014; Lopez et al, JAD 2013) and a 6-year longitudinal study (Squitti et al, Ann Neurol 2014) demonstrates its predictive value in MCI conversion to full AD. Therefore, re-establishing the physiological level of Non-Cp-Cu through a Zinc therapy in MCI subjects with abnormal Non-Cp-Cu can stop or delay the disease progression, offering a concrete option to reduce the economic/social burden of AD.

Specific aims

Aim 1: To demonstrate through a 24 months prospective, randomized, double blind, parallel, placebo controlled phase II clinical trial, that a Zinc Therapy of 150 mg/day in MCI subjects typified by abnormal Non-Cp-Cu (higher than 1.9 micromolar) can reduce the rate of conversion from MCI to full AD along with the clinical worsening.

Aim 2: To establish Non-Cp-Cu as a biomarker of a specific AD subtype reponding to the Zinc treatment

Aim 3: To develop a laboratory test which is widely available as a routine blood analysis

Hypothesis: Zinc is a copper competitor in intestinal absorption, and its ingestion reduces the body's capacity to absorb copper, potentiating the metallothioneins block at the intestinal level (`mucosal block'), consisting in a 25-fold

increase of the expression of methallothioneins, which bind copper and trap it into enterocytes for excretion into the stools, through mucosal cell exfoliation. During therapy with a dosage of 150 mg/day of elemental zinc, body copper balance becomes negative, reverting copper compartmentalization and distribution from the blood to organs and tissues, including the brain.

Preliminary data: This research consortium has identified an increase of Non-Cp-Cu levels in AD living patients, correlating with AD stage (Squitti et al. Neurology 2005), CSF markers of AD (Squitti et al. Neurology 2006), electroenchephalography rythms (Babiloni et al. Clin. Neurophysio. 2007) and a worse prognosis (Squitti et al. Neurology 2009). Experiments in AD living patients demonstrate that Non-Cp-Cu filters through a 10 kDa cut-off membrane, and it is 3.7 fold higher in AD than in controls (Squitti et al Neurology 2006), showing that Non-Cp-Cu is able to cross the blood-brain barrier also in AD living patients, as exemplified in Wilson's disease, the paradigmatic disease of Non-Cp-Cu accumulation. Thus in AD, increased levels of Non-Cp-Cu can react with Abeta and accelerate the amyloid disease cascade supposed to cause neurodegeneration within the brain. Additional preliminary data demonstrated that subjects with Non-Cp-Cu levels higher than normal reference range had a hazard conversion rate (50% of conversion in 4 years) that is about three times higher than those with values lower than normal reference range (less than 20% in 4 years), with a rate of progression consistently faster. Our additional preliminary data define more accurately the normal reference values for Non-Cp-Cu, measured by means of a new patented method (Colabufo, N. and R. Squitti, P.E. European Patent Office (EPO) (RO/EP), named C4D, based on a solid-phase extraction flow system to separate the low molecular weight from the protein-bound copper in serum, a coumarin fluorescent probe, which binds [Cu++], and a micro-plate device for the reading of the fluorescence signal, which allows rapid clinical diagnostic testing. This updates previous Non-Cp-Cu normative data of 0-1.6 micromolar to the new values of 0-1.9 micromolar (95% specificity).

Materials and Methods

Screening: 10 months. Double blind phase: 24 months. Statistical evaluation: 2 months. Zinc dosage: 100-150 mg/die of elemental zinc. MCI conversion to full AD will be monitored clinically at 6 months intervals. Sample size: 65 (placebo)+130 (Zinc treated) =195 MCI with Non-Cp-Cu higher than 1.9 micromolar will allow to detect differences at two-sided alpha level of 0.05 with a power of 0.80. 10% attrition is expected, therefore, the estimated sample size is 215, calculated on the basis of primary outcome Cognitive Composite 2 (CC2): in the quoted validation study (Raghavan et al., Alzheimers Dement 2013), the mean of the 2-year standardized changes of CC2 was 0.64 (SD=0.76) in MCI and we expect that Zinc therapy will reduce the cognitive impairment of 50%. Secondary outcomes: Mini-Mental State Examination, Global Deterioration Scale, Neuropsychiatric Inventory, Clinical Dementia Rating scale, Resource Utilization in Dementia, Cu, Fe, Cp, Tf, Ferr, APOE at 0, 6, 12, 18 and 24 months.

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

BANDO 2013 Progetti Cofinanziati Industria

Project Title:
Tolerability and efficacy of Zinc therapy in Mild Cognitive Impairment for treatment and prevention of Alzheimer's disease: a prospective, randomized, double blind, parallel, placebo-controlled Phase II clinical trial

Project Code:	CO-2013-02358488	Principal Investigator:	Rossini Paolo Maria
Research Type:	Clinical health care research/Clinico-assistenziale	Applicant Institution:	Lazio

Project Type: CO - Industrial Co-financing

Impact and Translational Implications

We expect that, if successful, our results will provide new opportunities for prevention/treatment of AD. We expect to demonstrate that Cu is a controllable risk factor for MCI/AD, which can be i) treated by a Zinc Therapy; ii) revealed by a simple blood test; iii) reduced, in preventive campaigns addressed to subjects with an ascertained copper dysfunction, as recently discussed in dietary and lifestyle guidelines for the prevention of AD (Barnard et al., Neurobiol Aging 2014).

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Extremely low frequency magnetic field (ELF-MF) stimulation as a neuroprotective treatment in acute ischemic stroke.
Project Code: CO-2013-02358697	Principal Investigator: Di Lazzaro Vincenzo
Research Type: Biomedical/Biomedica	Applicant Institution: Lazio

Project Type: CO - Industrial Co-financing

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 treatments for brain injury and diseases including neuroprotection, immunotherapeutics, cell transplantation, nanotechnology and deep brain stimulation.
Project Keyword 2: Extremely low frequency magnetic fields (ELF-MF)
Project Keyword 3: Neuroprotection
Project Request: Animals: [ ] Humans: [X] Clinical trial: [X]
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: © 2011 IGEA S.p.A. Via Parmenide, 10/A - 41012 CARPI (MO) ITALY Patent number: Provisional Patent (USA); numero della domanda: 61/953,447; data di deposito: 14 Marzo 2014

INDUSTRIAL/MANUFACTURER COFINANCING
Industry/Manufacturer: © 2011 IGEA S.p.A.
Address: Via Parmenide, 10/A
City-State and Country: 41012 CARPI (MO) ITALY
Amount of Cash Co-financing: 160000
Contact Person: Name: Cadossi Ruggero Email: r.cadossi@igeamedical.com Phone: +39 059 699600

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Lazio	Policlinico Universitario Campus Bio-Medico, Neurology	Principal Investigator

Investigators, Institution and Role in the Project

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

Project Title:
Extremely low frequency magnetic field (ELF-MF) stimulation as a neuroprotective treatment in acute ischemic stroke.

Project Code: CO-2013-02358697

Principal Investigator: Di Lazzaro Vincenzo

Research Type: Biomedical/Biomedica

Applicant Institution: Lazio

Project Type: CO - Industrial Co-financing

Background and Significance

Thrombolysis is the only approved treatment for acute stroke and so, there is great interest in the development of alternative therapies. For the last two decades, several neuroprotective drugs have been investigated in clinical trials but none has proved efficacious. This project aims at providing an innovative neuroprotective strategy, in which unconventional non-invasive brain stimulation will be tested as an alternative approach to drugs. Recent evidence suggests that extremely low frequency magnetic fields (ELF-MF) might influence human brain activity. These findings are supported by neurophysiological studies revealing measurable changes in brain electrical activity following ELF-MF exposure. Moreover, the experimental data at cellular level, showing the effects on cell membrane receptors and intracellular signaling, suggest possible mechanisms for ELF-MF action on the brain. In particular, the modulation of neurotransmitters receptors such as adenosine and glutamate could represent possible substrates for the effect of ELF-MF in acute ischemic stroke (Di Lazzaro et al.2013). Preliminary results from a small feasibility study (clinicaltrials.gov:NCT01941147), running in our center, suggest that ELF-MF stimulation is safe in acute stroke patients and reduce the ischemic infarct size measured by brain MRI. Thus, this project is justified especially given the low cost and non-invasive nature of the treatment and the promising evidence arising from animal and human studies.

Specific aims

Aim 1: To evaluate the safety and tolerability of pulsed ELF-MF for acute ischemic stroke treatment, by measuring the incidence of adverse events throughout the follow-up period.

Aim 2: To evaluate the efficacy of pulsed ELF-MF for acute ischemic stroke treatment in terms of functional clinical scales (Barthel, Modified-Rankin, NIHSS).

Aim 3: To evaluate the effect of pulsed ELF-MF for acute ischemic stroke treatment on the size of ischemic damage (as measured by brain MRI).

Hypothesis: Pulsed ELF-MF stimulation is a non-invasive, safe and effective tool to promote recovery in acute ischemic stroke patients because its documented effect on the main mechanisms of brain ischemic damage and regeneration.

Preliminary data: Recent evidence suggests that ELF-MF could be effective in acute ischemic stroke therapy because of their effects on the main mechanisms of brain ischemic damage and regeneration. Indeed, in vitro studies have shown that ELF-MF promote neurite outgrowth (McFarlane et al. Bioelectrochemistry. 2000), reduce apoptosis (Oda et al. Neurosci Lett. 2004), facilitate neuronal differentiation of neural stem cells (Piacentini et al. J Cell Physiol. 2008) and increase BDNF production (Di Loreto et al. J Cell Physiol. 2009). In addition, ELF-MFs delivered in pulsed mode can modulate glutamate (Wieraszko Bioelectromagnetics. 2004) and adenosine (Varani et al. Bioelectromagnetics. 2011) receptors. The neuroprotective potential of pulsed ELF-MF has been also confirmed in animal models of brain ischemia. Grant et al. (Bioelectromagnetics. 1994) have evaluated the effects of pulsed ELF-MF on cerebral damage in a rabbit model of transient focal ischemia. They found that pulsed ELF-MF exposure, immediately after the onset of ischemia, attenuated cortical edema on Magnetic Resonance Imaging (MRI) and reduced neuronal damage on histological examination. These results have been recently confirmed in mice by Pena-Philippides et al. (Transl Stroke Res. 2014) who evaluated the effect of pulsed ELF-MF on ischemic infarct size and post stroke inflammation. They found that pulsed ELF-MF reduced the infarct size and changed the profile of inflammatory cytokines, thus unveiling anti-inflammatory and anti-apoptotic effects. Recent evidence suggests that ELF-MF might influence human brain function. In particular, Capone et al. (J Neural Transm. 2009) demonstrated that pulsed ELF-MF can influence cortical excitability and do not produce side effects in healthy volunteers. Based on these data, we planned a small size open label

study (clinicaltrials.gov: NCT01941147) to evaluate the safety and efficacy of pulsed ELF-MF in acute ischemic stroke. Preliminary data from this running study (unpublished data), suggest that ELF-MF do not produce side effects in acute stroke patients and reduce the ischemic infarct size.

Materials and Methods

A randomized double-blind sham-controlled study will be carried out. Two cohorts of 30 patients with first, acute, monohemispheric ischemic stroke will undergo real and placebo ELF-MF stimulation respectively, delivered daily for 5 consecutive days, starting within 48h from the onset of stroke. Patients will be re-evaluated at different time intervals (5, 30, 90 and 180 days after the end of treatment). For pulsed ELF-MF administration, we will adopt the system presented in Capone et al. (J Neural Transm. 2009). The ischemic hemisphere will be stimulated for 240 min daily, for 5 consecutive days, with a pulsed signal (75 Hz, 1.8 mT). The safety endpoint will be evaluated by the incidence of AEs and mortality throughout the 6-month follow-up. Survival and AE profile will be compared with the control group. The efficacy endpoint will be evaluated by comparing the changes (before and after ELF-MF treatment) in clinical and radiological scores observed in the treatment and control groups.

Impact and Translational Implications

The National Health Service could greatly benefit of the rapid translational application of the results of this project in clinical practice, in order to increase the efficiency of the therapeutic procedures both in terms of costs for the Health Service and benefits for the patients. Italy could also gain a very high visibility within the scientific community as a provider of an innovative strategy for the treatment of acute stroke.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Identification of predictive biomarkers in Multiple Sclerosis using a proteomic approach
Project Code: CO-2013-02359461	Principal Investigator: Margutti paola
Research Type: Biomedical/Biomedica	Applicant Institution: Istituto Superiore di Sanita'

Project Type: CO - Industrial Co-financing

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:
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

INDUSTRIAL/MANUFACTURER COFINANCING
Industry/Manufacturer: Merck Serono S.p.A.
Address: Via Casilina 125 |
City-State and Country: Rome Italy
Amount of Cash Co-financing: 150000,00 euro; one hundred and fifty thousands euro
Contact Person: Name: Andrea Visconti Email: andrea.visconti@merckgroup.com Phone: +39 3474518018

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Istituto Superiore di Sanita'	Cell Biology and Neuroscience	MVs purification, proteomic analysis and phage bio-panning selection
2	Università "La Sapienza"	Neurological Science	Selection patients, collection of blood and CSF, clinical assessment

Project Title:
Identification of predictive biomarkers in Multiple Sclerosis using a proteomic approach

Project Code: CO-2013-02359461

Principal Investigator: Margutti paola

Research Type: Biomedical/Biomedica

Applicant Institution: Istituto Superiore di Sanita'

Project Type: CO - Industrial Co-financing

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	Francia Ada	Università "La Sapienza"	selection of patients and clinical assessment	07/05/1951
2	Pontecorvo Simona		selection of patients, collection of blood and CSF and clinical assessment	12/12/1978
3	Camerini Serena	Istituto Superiore di Sanità	proteomic analysis	31/05/1973
4	Flego michela	Istituto Superiore di Sanità	construction antibodyphage display library and bio-panning selection	17/09/1969

Background and Significance

Multiple Sclerosis (MS) is an immune-mediated, inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) and the most common cause of chronic neurologic disability in young adults. The heterogeneity of pathophysiological processes in MS contributes to the highly variable course of the disease and unpredictable response to therapies. MS typically presents with a relapsing-remitting course (RRMS), characterized by clinical relapses followed by subsequent partial or total improvement. In most of patients, RRMS can be preceded by a condition called clinically isolated syndrome (CIS), characterized by a single neurological episode suggestive of MS, with a high probability to convert to clinically definite MS (CDMS). Furthermore, RRMS evolves over a variable period of time into a slowly progressive form of neurological dysfunction, called secondary progressive MS (SPMS). The ability to predict the evolution of such a complex disease as MS is a major challenge. For many years, intensive efforts have been directed to identify biomarkers in bodily fluids, such as cerebrospinal fluid (CSF) or blood. However, currently there are no clearly established MS biomarkers. The availability of reliable biomarkers could provide clearer guidance on the management of MS at critical phases of the disease spectrum, allowing intervention strategies that may prevent evolution into long-term neurological disability.

Specific aims

Aim 1: The goal of this prospective case-control study is to identify biomarkers to predict the response to first-line disease-modifying therapy and the development and progression of MS, by proteomic characterization of CSF- and blood-derived microvesicles (MVs) purified from MS patients.
For this purpose, MVs will be purified from CSF and blood of MS patients and controls, and their proteomic profiles will be performed. MS patients will be selected and stratified into different stages of the disease. Biomarkers will be identified by a comparative proteomic approach.

1) 1) identification of biomarkers predicting the response to first-line disease-modifying therapy
Candidate biomarkers will be identified by comparing with controls the proteomic profiles of CSF- and blood-derived MVs from RRMS patients over a follow-up time of 1 year after the start of treatment.

Aim 2: 2) Identification of biomarkers predicting the development and disease progression of MS.

a) Biomarkers predicting conversion from CIS to CDMS.
Candidate biomarkers will be identified by comparing the proteomic profiles of CSF- and blood-derived MVs from patients with CIS who convert to CDMS with patients who remain CIS over a follow-up time of 2 years.

b) Biomarkers predicting disease progression
Candidate biomarkers will be identified by comparing the proteomic profiles of CSF- and blood-derived MVs from

RRMS patients at high risk of conversion from RRMS to SPMS over a follow-up time of 1 year with SPMS patients and untreated RRMS patients in relapse phase.

Aim 3:

c) Phage bio-panning selection of high-affinity recombinant antibody fragments specifically targeting novel candidate biomarkers, to develop a highly sensitive and specific immune-detection assays.

Hypothesis: Recent proteomic investigations suggest that MVs, released by cells during pathological conditions, may dynamically reflect the status of disease and constitute a yet-unfathomed source of circulating biomarkers. Furthermore, in MS, CNS-derived MVs can enter the bloodstream by drainage of CSF into the venous blood and/or blood-brain barrier breakdown.

Preliminary data: MVs were purified from the blood of 12 MS patients (7 untreated RRMS in active phase, 4 RRMS in remitting phase and 1 SPMS) and matching healthy controls using a Sephacryl S-500 gel filtration column and ultracentrifugation. The purified MVs were analyzed by electron microscopy and showed a 50-500 nm diameter range. Their proteomic profile was obtained by LC-MS/MS. In addition to a significant enrichment in proteins involved in synaptic transmission, peripheral and CNS-derived proteins unique to different stages of the disease have been detected in MVs from SPMS patient and RRMS patients in active phase, but not in remitting-phase patients and healthy controls.

Materials and Methods

Purification and proteomic analysis of MVs
CSF- and blood-derived MVs will be purified by size applying the samples to a Sephacryl S-500 gel filtration column and then the MV-containing fractions will be concentrated by ultracentrifugation. Purified MVs will be analyzed by LC-MS/MS. Qualitative and semi-quantitative proteomic analyses of MVs will be carried out through pre-fractionation of MV proteins by one dimensional SDS-PAGE followed by LC-MS/MS using an LTQ-XL instrument (Thermo).
Selection of recombinant antibody fragments against candidate biomarkers
High-affinity recombinant antibody fragments will be selected from human semi-synthetic, naïve, and immune antibody phage display libraries. A panel of scFvs against candidate biomarkers will be obtained by an affinity enrichment process, consisting of repeated absorptions and elutions of a antibody phage display library to target immobilized antigens.
Statistical methods
One-way analysis of variance (ANOVA) and Student¿s t-tests

Impact and Translational Implications

Early therapeutic interventions in MS have the potential to prevent or delay neurological disability. Therefore, biomarkers predicting conversion to CDMS would have an enormous impact on treatment decision-making. With the establishment of a definite MS, a central question pertaining to future disability and ongoing treatment is how to best detect disease activity. Thus, biomarkers predicting the future course of the disease would be valuable in monitoring individual response to treatment.

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2013 Progetti Cofinanziati Industria	Project Title: Cardiac regeneration: Characterization, optimization and evaluation of force developed by engineered tissue obtained from spontaneous or scaffold based 3D culture of paediatric human adipose tissue-derived mesenchymal stem cells
Project Code: CO-2013-02359690	Principal Investigator: Amodeo Antonio
Research Type: Biomedical/Biomedica	Applicant Institution: Ospedale pediatrico Bambino Gesù

Project Type: CO - Industrial Co-financing

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: Cardiovascular Sciences Small Business SECS
Project Keyword 1: Cell therapy and novel approaches to tissue repair/regeneration
Project Keyword 2: Cardiac regeneration
Project Keyword 3: Stem cells
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

INDUSTRIAL/MANUFACTURER COFINANCING
Industry/Manufacturer: Conad
Address: via Michelino 59
City-State and Country: Bologna, Italy
Amount of Cash Co-financing: 225000
Contact Person: Name: Alberto Moretti Email: alberto.moretti@conad.it Phone: 3351670078

Operative Units

	INSTITUTION	Department/Division/Lavoratory	Role in the project
1	Ospedale pediatrico Bambino Gesù	Dipartimento Medico Chirurgico di Cardiologia Pediatrica	Bioptic specimen of subcutaneous and/or mediastinal sample from selected pediatric patients.In vivo porcine model of ischemic heart failure and implantation of pediatric human adipose mesenchymal stem cells (ADMSCs).
2	Istituto Superiore di Sanità	Technology and Health Department Dipartiment	In vitro and in vivo biomechanical testing of pediatric human adipose tissue derived mesenchymal stem cells (ADMSCs) encapsulated in 3D systems (Biomaterials/scaffolds)
3	University of Rome "Sapienza"	Department of Medical-Surgical Science and Biotechnologies	Isolation, characterization and expansion of pediatric human adipose tissue derived mesenchymal stem cells (ADMSCs)

Project Title:
Cardiac regeneration: Characterization, optimization and evaluation of force developed by engineered tissue obtained from spontaneous or scaffold based 3D culture of paediatric human adipose tissue-derived mesenchymal stem cells

Project Code: CO-2013-02359690	Principal Investigator: Amodeo Antonio
Research Type: Biomedical/Biomedica	Applicant Institution: Ospedale pediatrico Bambino Gesù

Project Type: CO - Industrial Co-financing

Investigators, Institution and Role in the Project

	Key Personnel	Institution/Org./Pos.	Role in the project	Birth Date
1	DANIELE CARLA	Istituto Superiore di Sanità	Biomechanical testing of pediatric human adipose tissue derived mesenchymal stem cells ADMSCs encapsulated in 3D systems (Biomaterials/scaffolds)	19/08/1963
2	De Falco Elena	University of Rome "Sapienza"	Isolation, characterization and expansion of pediatric human adipose tissue derived mesenchymal stem cells (ADMSCs)	07/06/1974
3	Marullo Antonino	University of Rome "Sapienza"	In vivo porcine model of post ischemic heart failure ,development and implantation of pediatric human adipose tissue derived mesenchymal stem cells (ADMSCs) encapsulated in a 3D system	23/10/1966

Background and Significance

The incidence of paediatric heart failure (pHF) has increased in the recent years and the outcome of children with cardiomyopathies is poor, with a 5-year risk for death or trasplantation of 50%. The fate of pHF and shortage of donors requires new strategies.Over the years great excitement have been growing for the potential of stem cell-based strategies to restore heart function. However, results have been mixed, with benefits ranging from absent to transient and, at most, marginal and evidence underlining major limitations related not only to the different precursor but also to the low efficient delivery strategies. The integration with tissue engineering, to obtain clinically translatable bioengineering systems, seems to be appealing and helpful in addressing stem cells transdifferentiation and engraftment to the cardiac tissue.Recently human adipose tissue-derived mesenchymal stem cells (ADMSCs) emerged as adult multipotent progenitor cells with distinctive abilities to both generate multiple cell types, including cardiac phenotype, and to reveal biomechanical properties greater than other stem cell populations, making them eligible candidates for regenerative medicine applications.We propose to design anenhanced engineered approach based on a combination of stem cells and 3D biomimetic scaffolds aiming to develop complex tissues as the heart by exploring in vitro and in vivo biomechanical properties, behaviour and function of pediatric ADMSCs seeded in 3D systems.

Specific aims

Aim 1: To expand and characterize in vitro heterologous/autologous ADMSCs derived from subcutaneous/mediastinal tissue of pediatric patients w/w/o HF,to identify the ideal window for their collection and potential correlations with donor clinical parameters.We will also assess whether and how hypoxia culture might affect their cardiac commitment,paracrine potency and 3D assembling properties (see step 2).

Aim 2: To characterize and optimize spontaneous or scaffold-based 3D culture systems for ADMSC cultures to select the most promising approach for therapeutic purposes and to develop engineered cardiac tissues. To provide suitable biomechanical characteristics to the graft immediately upon implantation, different loading regimes will be investigated for each type of 3D graft. According to Zimmermann et al., engineered heart muscle should adapt the contraction cycle to the stretch cycle so as not to contract actively while being distended, resembling isotonic

contractions. Hence, ad hoc loading procedures will be created, with real-time feedback, in order to measure the force developed by the engineered tissue, and adapt the loading scheme accordingly, in view of the optimal conditioning without deterioration of the graft's metabolic status. Standard material characterization of the engineered tissues will be also carried out, after in vitro preconditioning

Aim 3: Development of a prediction model of the engineered heart tissue implantation outcome.Local contractile properties of engineered tissue will be assessed during in vivo experiments, using our recently developed porcine model of ischemic heart, by means of real-time measurements of muscular fiber length.The latter signal will be provided by ultrasound crystals, placed near the apical region of the left ventricle.The contractility of the grafted heart will be correlated with the clinical outcome.This study will enable to investigate the minimum performance that the engineered cardiac tissue must provide,aiming to give the implantation a favorable outcome.

Hypothesis: Clinical translation of stem cell-based engineered cardiac tissue in pediatric patients has yet to be defined.The combination of ADMSC plasticity and cardiac-like commitment with their known mechanotransducer properties and the concurrently control of the 3D biomaterial scaffold properties will allow to design children's custom made engineered heart tissue

Preliminary data: We have already standardized the ADMSC isolation/characterization(Siciliano et al.2013),currently demonstrating their potential to transdifferentiate into a cardiac/endothelial/muscular-like phenotype if subjected to cardiac progenitor endogenous paracrine action(Siciliano et al 2014 under revision CT1307)and reported the design and application of a novel porcine experimental model of closed chest chronic ischemia suitable for biomedical research(Biondi-Zoccai et al. 2013).The in vitro/in vivo combined expertise and the further addition of novel 3D bioysystems will provide the foundation of the whole project.

Materials and Methods

ADMSCs isolated from pediatric patients(10) w/wo HF as we described and seeded in 3D bioconstructs(CollagenI/III and alginate).Fluorescent viable dyes used for live monitoring of cells encapsulated in 3D systems.Hypoxia effects on ADMSC growth/scaffold seeding analyzed by preconditioning with low oxygen tension. Cytokine pattern eleased in media and cardiac commitment screened(array analysis and RTPCR).Force developed by engineered ADMSCs/3D system measured to adapt the latter's loading scheme with flexible feedback-driven loading procedures,optimizing conditioning w/o deterioration of the graft's metabolic status.Porcine model(20)of HF performed as we reported.Local contractile properties assessed by means of real-time measurements of fiber length.Latter signal will be provided by ultrasound crystals,placed at the ends of the engineered tissue,after implantation on the ischemic heart.Correlation of grafted heart contractility with functional outcome(transthoracic echocardiograpy)

Impact and Translational Implications

We expect that this study will give important information relative to functional and engraftment properties of ADMSCs derived from subcutaneous and/or mediastinal adipose tissue of pediatric patients. Investigation of an in vivo functional graft based on a combination of ADMSCs and a 3D biomimetic system will provide novel insights to improve cardiac regenerative therapy in pediatric patients and possibly open a new scenario in exploiting heterologous mesenchymal precursors banking.