CONVENZIONE

TRA

IL CONSIGLIO NAZIONALE DELLE RICERCHE,

E

LA FONDAZIONE SANTA LUCIA IRCCS

per lo svolgimento delle attività di Ricerca e Sviluppo relative al Progetto

"A multifactorial intervention for successful aging"

Il Consiglio Nazionale delle Ricerche (d'ora innanzi denominato CNR) in persona del suo Presidente, Prof. Massimo Inguscio, con sede legale in Roma, Piazzale Aldo Moro 7, cap. 00185 (codice fiscale 80054330586)

e

la Fondazione Santa Lucia IRCCS (d'ora innanzi denominata Fondazione) in persona del suo Presidente, Maria Adriana Amadio, con sede legale in Roma, Via Ardeatina 306, cap 00179 (codice fiscale 97138260589)

d'ora innanzi denominati congiuntamente le "Parti"

PREMESSO CHE

Il CNR, in base al suo Statuto, è un Ente pubblico nazionale di ricerca con competenza scientifica generale con il compito di svolgere, promuovere, diffondere, trasferire e valorizzare attività di ricerca nei principali settori di sviluppo delle conoscenze e delle loro applicazioni per lo sviluppo scientifico, tecnologico, economico e sociale del Paese, perseguendo l'integrazione di discipline e tecnologie diffusive ed innovative anche attraverso accordi di collaborazione e programmi integrati.

Il CNR ha personalità giuridica di diritto pubblico, gode di autonomia scientifica, finanziaria, organizzativa, patrimoniale e contabile in attuazione degli articoli 9 e 33 della Costituzione e si dota di un ordinamento autonomo in conformità con il decreto legislativo 31 dicembre 2009, n. 213, nel seguito decreto di riordino, con il decreto legislativo 4 giugno 2003, n. 127, con

l'articolo 8 della legge 9 maggio 1989, n. 168, e con il decreto legislativo 5 giugno 1998, n. 204, nonché, per quanto non previsto dalle predette disposizioni, con il codice civile.

Il CNR svolge, promuove e coordina attività di ricerca con obiettivi di eccellenza in ambito nazionale e internazionale, finalizzate all'ampliamento delle conoscenze nei principali settori di sviluppo, individuati nel quadro della cooperazione ed integrazione europea e della collaborazione con le università e con altri soggetti sia pubblici sia privati.

Il CNR per lo svolgimento delle proprie attività istituzionali e di ogni altra attività connessa, ivi compreso l'utilizzo economico dei risultati della ricerca propria e di quella commissionata, secondo criteri e modalità determinati con il regolamento di organizzazione e funzionamento, può:

a) stipulare accordi e convenzioni con soggetti pubblici e privati, sia nazionali che internazionali;

b) partecipare o costituire consorzi, fondazioni o società con soggetti pubblici e privati, italiani e stranieri, previa autorizzazione del Ministro dell'Istruzione, dell'Università e della Ricerca;

c) promuovere la costituzione di nuove imprese conferendo personale proprio, anche in costanza di rapporto, nel rispetto della normativa vigente;

d) partecipare alla costituzione ed alla conduzione anche scientifica di centri di ricerca internazionali, in collaborazione con analoghe istituzioni scientifiche di altri Paesi;

e) commissionare attività di ricerca e studio a soggetti pubblici e privati, nazionali e internazionali.

Le attività di ricerca, in particolare, sono focalizzate sullo studio "A multifactorial intervention for successful aging "

VISTI/VISTE

1. il Decreto Legislativo n. 127 del 4 giugno 2003 "Riordino del Consiglio Nazionale delle Ricerche";
2. il Decreto Legislativo n. 213 del 31 dicembre 2009 "Riordino degli Enti di Ricerca in attuazione dell'art. 1 della Legge 27 settembre 2007, n. 165";
3. il nuovo Statuto del Consiglio Nazionale delle Ricerche entrato in vigore il 1 agosto 2018;
4. il Regolamento di organizzazione e funzionamento del Consiglio Nazionale delle Ricerche emanato con decreto del Presidente n. provv. 14/2019, in vigore dal 1° marzo 2019;

5. il Regolamento di amministrazione, contabilità e finanza del Consiglio Nazionale delle Ricerche emanato con decreto del Presidente del 4 maggio 2005, prot. n. 25034 e pubblicato nel Supplemento ordinario n. 101 alla Gazzetta Ufficiale della Repubblica Italiana n. 124 del 30 maggio 2005;

6. vista la delibera del Consiglio di Amministrazione del Consiglio Nazionale delle Ricerche n. ….in data……

7. le collaborazioni scientifiche in essere tra il CNR e la Fondazione Santa Lucia - IRCCS

8. il Decreto Ministeriale n. .856 del 10-10-2019 (FOE 2019) che ha riconosciuto alla Fondazione per il tramite del CNR, un' assegnazione internazionale pari ad euro 1.200.000,00 finalizzata "alla partecipazione e al sostegno delle attività di ricerca di "A multifactorial intervention for successful aging", e per il cui utilizzo la Fondazione fornirà dettagliata rendicontazione amministrativo-contabile.

CONSIDERATO CHE

1. Una delle linee di ricerca portate avanti dalla Fondazione è quella che riguarda "A multifactorial intervention for successful aging";

2. Il CNR per il tramite del Dipartimento Scienze Biomediche provvederà allo svolgimento di tutte le attività di esecuzione e di coordinamento e a tutti gli adempimenti di competenza del CNR di carattere organizzativo, giuridico ed amministrativo finalizzati all'esecuzione del menzionato Progetto;

3. Le Parti intendono disciplinare i propri rapporti per l'esecuzione, in forma coordinata e congiunta, del Progetto "A multifactorial intervention for successful aging" attraverso la sottoscrizione della presente Convenzione.

LE PARTI CONVENGONO E STIPULANO QUANTO SEGUE

Articolo 1 - Premesse

1.1. Le premesse e gli allegati alla presente Convenzione (di seguito "Convenzione") costituiscono, ad ogni effetto, parte integrante ed essenziale della stessa e della disciplina contrattuale in essa contenuta.

Articolo 2 - Oggetto

2.1. Con la sottoscrizione della presente Convenzione le Parti intendono instaurare un rapporto di collaborazione teso:

a. al sostegno alle attività di ricerca svolte dalla Fondazione.

b. allo sviluppo e allo studio di quant'altro dovesse risultare connesso ai suddetti settori.

2.2. Gli interventi di cui al comma 2.1 hanno ad oggetto lo svolgimento di attività di ricerca, innovazione e sperimentazione delineate nella relazione illustrativa generale **allegato A** alla presente Convenzione. Le parti si impegnano inoltre a valutare e concordare altre iniziative congiunte su tematiche correlate a quelle sopra indicate.

2.3. Eventuali revisioni sostanziali dell'oggetto della Convenzione saranno definite con atti aggiuntivi, concordati per iscritto tra le Parti.

Articolo 3 - Durata e condizione risolutiva

3.1 La presente convenzione avrà durata annuale a decorrere dalla data di sottoscrizione della stessa e comunque fino all'erogazione del saldo di tutte le competenze maturate in virtù della stessa.

Articolo 4 - Svolgimento attività di ricerca

Per l'individuazione e realizzazione delle singole attività di ricerca affidate al soggetto contraente, con relativi finanziamenti, tempistica e modalità di realizzazione, deliverables, milestones e ogni altro connesso adempimento, si rimanda alla relazione illustrativa generale (**allegato A**) di cui alla presente Convezione che ne costituirà parte integrante e sostanziale.

Articolo 5 - Responsabile esecutivo della Convenzione

5.1. Viene individuato Responsabile Esecutivo della Convenzione la Dott.ssa Daniela Corda, Direttore del Dipartimento di Scienze Biomediche del CNR.

5.2. Detto Responsabile è incaricato della gestione operativa della Convenzione, assicurando la continuità e la proficuità della collaborazione, nonché il coordinamento degli apporti resi da ciascuna delle Parti nella realizzazione delle attività di cui al precedente articolo 2.

Articolo 6 - Risorse Finanziarie

6.1. Il CNR, fatto salvo quanto indicato al successivo punto 6.4, dell'assegnazione di euro 1.200.000,00 trasferirà alla Fondazione, per tutte le attività oggetto della presente Convenzione, l'importo di euro 1.000,000,00 e tratterrà la restante quota di euro 200.000,00 per l'attività di ricerca da ripartire tra gli Istituti dell'Ente partecipanti al progetto.

6.2 I fondi corrisposti dal CNR alla Fondazione saranno oggetto di rendicontazione scientifica e finanziaria - secondo le tempistiche e le modalità di seguito indicate - da sottoporre ai Comitati di cui all'articolo 7 che trasmetteranno i loro pareri alla Direzione Generale, al Responsabile esecutivo della Convenzione ai fini delle opportune valutazioni in merito alla corresponsione delle successive erogazioni.

6.3 Il trasferimento del finanziamento, come sopra individuato, avverrà mediante ripartizione dello stesso in tre tranches, secondo quanto di seguito convenuto dalle Parti:

1. erogazione di una prima quota pari al **50%** della totale quota finanziata, verrà trasferita alla Fondazione, come forma di anticipo, nel termine di trenta giorni dalla sottoscrizione della presente convenzione;

2. erogazione di una seconda quota pari al **40%** dell'importo concesso, da corrispondersi entro i 30 giorni successivi alla rendicontazione del primo semestre di attività, previo presentazione di una relazione scritta sullo stato di avanzamento scientifico e finanziario;

3. Il saldo del finanziamento, pari al **10%**, da corrispondersi a conclusione delle attività progettuali, previo parere positivo dei Comitati di cui all'art. 7 sulle rendicontazioni finali sia scientifiche che finanziarie. Il CNR provvederà al pagamento della quota finale entro i trenta giorni successivi al predetto parere positivo dei comitati.

4. La Fondazione si impegna a redigere e trasmettere relazione scritta finale, scientifica e finanziaria, entro 90 giorni dalla chiusura annuale del progetto.

6.4 Il trasferimento dei finanziamenti di cui ai commi precedenti si intende subordinato all'erogazione dei relativi fondi nei confronti del CNR dagli Enti preposti nazionali e/o regionali.
6.5 Le risorse, destinate agli interventi di cui sopra, sono specificatamente indicate nel prospetto finanziario del progetto, parte integrante dell'allegato A.

Art. 7 –Rendicontazione del progetto

7.1. Le disposizioni relative alle tipologie e modalità di spesa, nonché delle relative rendicontazioni sono riportate nelle Linee guida, che, condivise dalle Parti, sono parte integrante della presente Convenzione (**allegato B)**.

7.2. Con la sottoscrizione della presente Convenzione le Parti si danno reciprocamente atto che le spese, quali riportate nella Relazione illustrativa generale (**allegato A**), costituiscono l'importo massimo delle singole tipologie di spesa ammissibili e rendicontabili.

7.3. Ai fini del monitoraggio e controllo delle attività svolte dalla Fondazione, il CNR si avvarrà per tutta la durata della presente Convenzione di un Comitato di Controllo per gli aspetti amministrativi, contabili e di rendicontazione, composto da tre componenti designati dal Presidente del CNR, e da un Comitato Scientifico per la valutazione della congruità delle attività di ricerca svolte, composto da tre componenti designati dal Presidente del CNR e dal Direttore del Dipartimento di Scienze Biomediche.

7.4. Le rendicontazioni verranno inoltrate dalla Fondazione al Dipartimento di Scienze Biomediche del CNR che provvederà a sottoporle ai Comitati del CNR per l'approvazione nei termini previsti dall'art. 6.

7.5. I Comitati avranno a loro volta un termine di giorni 15 dall'inoltro di ciascuna rendicontazione, entro il quale termine far pervenire alla Fondazione le proprie eventuali osservazioni e commenti e/o richieste di chiarimenti. Eventuali integrazioni della documentazione presentata dovranno essere presentate e successivamente valutate nei medesimi termini sopraindicati.

7.6. I verbali delle riunioni dei Comitati ed i pareri relativi alle rendicontazioni esaminate verranno trasmessi alla Direzione Generale del CNR e alla Direzione del Dipartimento di Scienze Biomediche per gli adempimenti previsti.

Articolo 8 - Adempimenti per la Sicurezza

8.1. Le Parti assicurano e garantiscono il rispetto delle disposizioni in tema di sicurezza individuale e collettiva sui luoghi di lavoro dei locali, delle attrezzature e del personale utilizzato nelle attività di cui alla presente Convenzione e, in tal senso, provvederanno autonomamente alle necessarie coperture assicurative e singolarmente daranno corso ad ogni adempimento, con tempestività, secondo le competenze e responsabilità stabilite dalle norme vigenti in materia.

8.2 Il personale di una Parte che si rechi presso i centri o i laboratori di titolarità dell'altra Parte o di altri Soggetti coinvolti nel Progetto è tenuto al rispetto dei regolamenti sanitari e di sicurezza vigenti presso la parte/soggetto ospitante e dal rispetto delle prescrizioni individuate ed enucleate nel Decreto Legislativo n. 81/2008 e successive modifiche e integrazioni.

Articolo 9 Riservatezza

9.1 Le Parti si impegnano ad osservare la massima riservatezza a non divulgare né utilizzare, per alcuno scopo diverso da quello necessario se non per lo svolgimento delle attività previste, le informazioni di carattere scientifico e tecnico prodotte nell'ambito della presente Convenzione.

Articolo 10 - Diritti di proprietà

10.1 I Diritti di proprietà, intellettuale e industriale su brevetti e know–how, restano regolati dalla normativa di Legge vigente salvo diverse pattuizioni formulate per iscritto dalle Parti.

10.2 La titolarità dei Diritti di Proprietà industriale su brevetti e know-how derivanti dalle attività del Programma sarà ripartita in ragione dell'attività svolta dalle Parti in forza di accordi definiti ad hoc tra le stesse, tenuto conto dell'eventuale attività svolta da terzi.

Consiglio Nazionale delle Ricerche

Articolo 11 - Legge applicabile e Foro competente

11.1 La presente Convenzione attuativa è tesa al rispetto dei principi elencati negli Statuti degli Enti coinvolti e per quanto non espressamente specificato, si applica la legge italiana.

11.2 Tutte le modifiche apportate alla presente Convenzione dovranno essere effettuate ed approvate per iscritto. Per tutte le controversie che dovessero insorgere in merito all'interpretazione, esecuzione, validità o efficacia della presente Convenzione, le Parti procederanno per via amministrativa, dopo aver esperito e senza alcun risultato, un tentativo di bonaria composizione extragiudiziale. Nel caso in cui non si dovesse pervenire ad un accordo, sarà di competente per eventuali controversie, il Foro di Roma.

Articolo 12 - Trattamento dei dati personali e Codice Etico

12.1 Le Parti si impegnano, nell'esecuzione del presente accordo e di tutte le attività connesse che possono comportare il trattamento dei dati personali, ad agire in ottemperanza alle disposizioni di cui al Regolamento (UE) 2016/679 del Parlamento europeo e del Consiglio del 27 aprile 2016 e al D.Lgs. 196/2003, come modificato dal D.Lgs. 101/2018 ("Codice Privacy"), osservando misure organizzative e tecniche adeguate, nonché idonee a garantire la sicurezza delle informazioni relative all'attività di ognuna delle Parti sotto l'aspetto della riservatezza, disponibilità e confidenzialità dei dati personali trattati, manlevando e tenendo indenne l'altra Parte da qualsiasi conseguenza pregiudizievole derivante dal mancato rispetto di tale obbligo. Le Parti dichiarano di aver preso visione, in sede di perfezionamento del presente Accordo, dei rispettivi Codici Etici e di Condotta, così come pubblicati sui rispettivi siti web istituzionali, ai cui principi etico comportamentali si conformeranno nell'esecuzione dell'accordo stesso.

Articolo 13– Registrazione

13.1 La presente Convenzione è soggetta a registrazione solo in caso d'uso ai sensi degli artt. 5, 6 e 39 del D.P.R. n. 131 del 26 aprile 1986 e non è soggetta ad imposta di bollo come da tariffa all. A – parte I art. 2, del D.P.R. 642/72 e successive modifiche ed integrazioni. Le spese per l'eventuale registrazione sono a carico della Parte richiedente.

13.2 La Convenzione avrà piena efficacia a decorrere dalla data della sua sottoscrizione, anche a mezzo di firma digitale, ai sensi e nel rispetto del D.P.C.M. del 22 Febbraio 2013, pubblicato sulla G.U. n. 117 del 21 Maggio.

Letto, confermato e sottoscritto

Roma lì, ...

PER CONSIGLIO NAZIONALE Il Presidente	PER FONDAZIONE SANTA LUCIA IRCCS Il Presidente

Consiglio Nazionale delle Ricerche

ALLEGATO A

RELAZIONE ILLUSTRATIVA/PROGETTO

A MULTIFACTORIAL INTERVENTION FOR SUCCESSFUL AGING

INTRODUCTION

"Successful aging strategy" can be defined as a potentially modifiable characteristic or intervention that is intended to enhance the functioning of older adults who could be characterized as aging normally. While much of the focus of research on aging has been on the determinants of mortality, illness, and disability, a growing body of work has begun to assess modifiers and interventions to improve upon the usual course of aging. Most of the studies initially focused on specific phenotypes (such as lung function, grip strength or bone mineral density), searching for a correlation with molecular changes occurring in biological aging. To this purpose, centenarians represented on of the best models to investigate the molecular reasons of retarded biological ageing and longevity (Zierer et al., 2015). Among the main findings, these studies confirmed the multifactorial nature of ageing and longevity, which are the result of the balanced combination of genetic, protein, metabolic and microbiome factors that ultimately confer higher or lower survival, susceptibility to age-related disorders and extended lifespan. In this context, the development of "omic" technologies (genomics, proteomics, epigenomics) proved to be excellent tools to investigate biological aging and neurodegeneration at molecular level and identifying useful predictive biomarkers. The clinical utility of such biomarkers lies in the fact that high technology and large-scale analysis may be exploited to generate individual omic profiles in the perspective of providing early diagnosis and personalized treatment to the elderly patients (Strafella et al. 2018). This imperative comes from the fact that there are now more people who are older than the age of 65 than at any time in recorded history. In fact, two-thirds of the people who have ever reached the age of 65 are alive right now.

There is growing longitudinal evidence that actual cognitive trajectories associated with normal brain aging (a) vary widely across individuals and domains, (b) include patterns ranging from relatively sustained high levels of performance to steeply accelerating decline, and (c) are influenced by individual differences in biological, health, environmental, and lifestyle factors (Josefsson et al., 2012). Arguably, the rate and extent of individual cognitive decline may not

be inevitable or irreversible. In other words, there are things one can do to minimize loss in cognitive functioning and even to reduce the risks of pathological aging.

Much of the work on successful aging has focused on the prevention of physical illnesses and disabilities that stem from age-associated conditions, and less has focused on defining and understanding the determinants of cognitive health, specifically. In a 2006 review of 28 quantitative studies that reported an operational definition of successful aging, only 13 of the studies included cognitive functioning as a component in their definitions (Depp and Jeste 2006). Nevertheless, there are a number of reasons for increased focus on successful cognitive aging as well as a number of exciting recent findings that suggest emerging avenues to maintaining brain health in older age.

In this view, there is large evidence that pathology leading to cognitive loss begins decades prior to clinical manifestations and recent studies have revealed that the early use of imaging and biological markers can identify subjects at risk decades before the appearance of irreversible levels of neural loss and cognitive decline (Häussermann and Granet, 2018; Carlesimo et al, 2015).

It is therefore imperative to identify at-risk for cognitive decline (ARCD) individuals and act during this extended pre-symptomatic phase to promote endogenous responses in brains that still have robust levels of cognitive reserve and neural plasticity, thus determining a successful aging. Cognitive training programs, especially when targeting abilities such as memory, reasoning, and processing speed, can promote positive and durable effects on cognitive functioning in the elderly. Structured physical activity can be complementary as stimulates neurotrophic signaling (Sleiman et al, 2016) and helps to normalize vascular and metabolic risk factors (World Alzheimer Report, 2015). Furthermore, plasma biomarkers investigated by metabolomic approaches have been shown to predict phenoconversion of ARD to either mild cognitive impairment (MCI) or AD (90% accuracy) within a 2 year timeframe (Mapstone et al, 2014). Moreover, evidence suggests that lifelong nutrition might also have a direct effect on brain function. For example, longitudinal studies have identified associations between certain nutrients or dietary patterns and brain-volume loss, or brain integrity (see Scarmeas et al, 2018 for a recent review), with some clinical trials confirming these results (Smith et al, 2010). In this view, previous studies from the group involved in the present proposal have investigated the neural and cognitive effects of different nutritional factors such as homotaurine provision

(Spalletta et al, 2016), fatty acid supplementation (Cutuli et al, 2016) and deficiency (Iuliano et al, 2013) in human subject as well as animal models.

The molecular patterns of biological aging, cognitive decline and neurodegenerative mechanisms have been extensively investigated with omics technology. On this subject, genomics revealed that biological aging accounts for 20-30% of genomic factors (Khan et al., 2017). Approximately 280 SNPs have been found to discriminate centenarians with respect to younger people; among which a SNP in APOE gene has been extensively associated with longevity (Zierer et al., 2015). Interestingly, common genetic variants on the same locus have been strongly associated with cognitive decline, accelerated aging and neurodegenerative and cardiovascular disorders (such Alzheimer disease, coronary artery disesase and stroke) (Zierer et al., 2015; Strafella et al., 2018, Jacob L and Speed TP, 2018). This peculiarity fits perfectly with the complex interactions characterizing the aging and age-related conditions, reflecting thereby the polygenic nature of such processes. In this context, it would be to screen a panel of genes and/or variants associated with ageing, cognitive impairment and neurodegenerative disorders, in order to provide a comprehensive genomic picture of gene interactions underlying ageing and longevity trajectories and identify potential predictive biomarkers. Over genomics, the contribution of epigenomics has also been extensively investigated given its ability to create cross-talks between genome and environment (Cascella et al., 2018). Epigenetic modifications (DNA methylation, histone acetylation, non-coding RNAs) do not alter the nucleotide sequence but they are able to modulate gene expression switching on or off specific genes and changing the chromatin structure in response to aging and environmental (smoking, chemicals, diet) changes. In particular, epigenome-wide studies showed that a variable number of cell-types and tissues have their own “epigenetic clock” that is strictly dependent on healthy ageing (Horvath S and Raj K, 2018, Horvath S, 2013). Approximately 500 differentially methylated regions have been associated with chronological age and age-related phenotypes (including lung function, cholesterol levels and maternal longevity) (Zierer et al., 2015). In particular, DNA methylation (DNAm) levels have been utilized to develop estimators of ageing. These tools are based on the evaluation of the methylation patterns of a specific set of CpG of target genes (such as ELOVL2, PRC2-coding genes, CLOCK) which came out to be significantly correlated with positive or negative ageing acceleration (Lu at et al., 2019; Horvath S and Raj K, 2018). However, the CpG sites normally utilized for developing DNAm age estimators usually maps

near genomic sites which control the expression of genes involved in development and differentiation (such as enhancer regions and targets of PRC2). DNAm age estimators can be designed to be applied on a single tissue or multiple tissues, although multi-tissue evaluations are much more reliable and are therefore preferred (Lu at et al., 2019). Not so many proteomic studies have been performed in aging research, possibly due to the technical difficulty to analyze large set of proteins and collect large sample size. A recent study found 200 proteins robustly associated with age, although the results have to be validated on larger cohorts (Tanaka T et al., 2018). Currently, one of most interesting data are related to the increased levels of GRD15, which is known to be increased in older people in response to impaired calcium homeostasis, lower mitochondrial functioning and higher oxidative stress occurring with aging (Tanaka T et al., 2018). In addition, significantly higher levels of CHDRL1 have been detected in older individuals by proteomic analysis, that is a protein involved in neuronal differentiation, retinal angiogenesis and anterior segment eye development (Menni C et al., 2014). This protein is of particular interest since it has been correlated with higher birthweight, that is known as a healthy ageing factor. On this subject, higher circulating CHDRL1 levels have been associated with a lower susceptibility to age-related disorders (especially cardiovascular diseases) (Menni C et al., 2014). Moreover, Neurofilament light Chain (NfL) has been recently described as a promising fluid biomarker for predicting neurodegeneration and early diagnosis of Alzheimer disease (Barro et al, 2018; Preische O et al., 2019). This finding suggests that NfL may be introduced in the clinical practice in order to detect Alzheimer patients in the early phase of disease and set-up thereby, the most adequate treatment to control the disease progression.

Altogether the information collected by genomic, epigenomic and proteomic analysis can be integrated in a unique “omic” profile to be further utilized for identify the main molecular determinants for a “successful aging” strategy for the elderly patients.

Non-pharmacological single domain intervention has yielded suboptimal results (Pieramico et al, 2012), however, given the multifactorial nature of cognitive impairment, it is becoming clear that multidimensional intervention may offer better outcomes. In preventative interventions, a critical issue to be considered concerns the capability to identify, in a timely fashion, pre-symptomatic ARCD individuals.

Thus, a multidimensional tool, combining molecular (genetic and epigenetic), biological, cognitive and brain imaging markers, might offer great possibilities to catch ARCD individuals. Subsequently, a multidimensional intervention, targeting cognition, physical activity and nutrition, will impact comorbidity factors that play a crucial role in determining cognitive decline.

HYPOTHESIS AND AIMS

The hypothesis tested here is that a multidomain intervention will have great impact on final outcome (cognition, brain structure and function, disability, quality of life and neuropsychiatric symptoms). The importance of identifying methods to delay onset and/or modify progression of cognitive impairment/dementia is an urgent need. The economic and social benefits might be great since postponing cognitive decline onset by only 5 years may halve the projected prevalence of cognitive impairment in the future.

Aim 1: We will compute and apply a novel matrix (combining molecular (genetic and epigenetic), biological, cognitive and brain imaging markers) that is sensitive and specific in detecting subtle signs of incipient cognitive decline and develop a Multimodal Training (MT) program focused on cognitive stimulation, aerobic and isometric training, nutritional intervention and metabolic/vascular risk control. The matrix will be applied on a cohort of 200 healthy subjects aged 50-70 years.

Aim 2: We will evaluate and compare effects of a new set of MT (Cognitive Training + Exercise, diet and vascular risk control)] on cognitive performances and brain plasticity of: a cohort of 50 middle age ARCD individuals and 50 age-matched ARCD individuals undergoing no major changes in their daily routine.

Aim 3: We will test MT effects on several molecular biomarkers. All subjects will be assayed for BDNF and IGF-1 levels, NfL, metabolomic, proteomic and ionomic changes occurring before and after the end of training. Epigenetic modifications of BDNF, not only in the brain but also in blood cells, can be employed as marker for neurodegeneration, we will therefore investigate them in blood samples from the two study groups. “Omic” investigations will consist of three-step analyses: i) Genomics: massive screening of a panel (Table 1) of genetic variants selected considering literature data, a Minor Allele Frequency (MAF)>20%, biological

mechanisms mainly involved in neurodegenerative disorders, cognitive impairment and biological ageing;

ii) Epigenomics: evaluation of methylation patterns and miRNA expression/typing which may be associated with biological ageing, cognitive impairment and neurodegeneration,

iii) Proteomics: research of biomarkers for early diagnosis and progression of neurodegenerative disorders.

METHODOLOGIES

1) Recruitment and Screening: Participants will be selected from the database of the Neuropsychiatry Laboratory of Fondazione SantaLucia which includes more 500 middle-aged healthy subjects. We will screen 200 ARCD subjects [offsprings of people with objective cognitive impairment, having an intermediate or high risk profile according to the CAIDE risk score (Sindi et al, 2015) and individual omic profile. Inclusion criteria will be: a) age range (50-70 yrs); b) non demented (i.e. not meeting DSM-5 criteria neither for major nor for mild neurocognitive disorders); c) suitability to MR scans. ARCD will be identified as subjects with intermediate (10≤ score <15) and high (score=15) cognitive impairment risk profile. Exclusion criteria will be: a) any axis I or II psychiatric disorder; b) history of traumatic brain injury; c) relevant medical illnesses; d) history of alcohol or drug addiction; e) mental retardation. All participants will be recruited in the first 3 months of the project. ARCD will be identified subjects according to a comprehensive matrix which is constructed with the aim of disclosing individuals who show i) susceptibility to accelerated biological aging and neurodegenerative disorders on the basis of the "omic" profile, ii) modest impairment in test performances (but do lack clear symptoms as these individuals are functionally preserved and compensated), iii) signs of impoverished neural structural and functional substrates as revealed by magnetic resonance imaging. Participants will undergo: a) a comprehensive neuropsychological assessment, including tests characterized by various diagnostic power and investigating several different cognitive domains; b) a comprehensive neuropsychiatric battery able to highlight possible behavioural symptoms, in particular apathy, depression and psychosis; c) a functional assessment aimed at highlighting even subtle changes in individual's everyday life autonomy with respect to previous functioning. Internal norms for all variables will be derived from normal elderly and factor analysis employed to combine variables in weighted factors. At the

end of the multiple evaluations, a multivariate logistic regression analysis with a forward likelihood ratio method will establish a Z-score that identifies subtle pathological changes.

2) Resting state fcMRI and structural analysis. ARCD individuals will be evaluated at the time of enrolment (T0) in MT or the control program (CP), and right after the end of the training (T1). Structural and functional changes will be assessed by:

- rs-fcMRI. Data will be processed by firstly identifying, for each subject, a number of network nodes using an independent component analysis (ICA) approach, and then estimating the functional connections between these nodes, by referring to the mathematical model of Graph Theory.
- Structural 3D T1-weighted MR sequences with optimized white/grey matter contrast.
- Microstructural changes of the white and grey matter of the whole brain by using a DTI sequence with multiple b-values to permit tensor and kurtosis imaging analysis.

3) Multidimensional Training (MT)

The program, which will last for 3 months is focused on four areas of intervention which are:

A) Cognitive stimulation. Initially, neuropsychologists will provide participants with: an information brochure and an electronic device, a tablet loaded with rehabilitative software and will teach participants how to perform the training and use the electronic device. Training will be performed for the first three weeks, supervised by the neurpsychologists. Each session will include exercises, calibrated with increased levels of difficulty and set to stimulate major cognitive domains (memory, attention, language, executive functions and speed of processing). Subjects will undergo CT three times a week (30 min sessions) in the morning. Training completion will be assessed by looking at the information recorded in the electronic device.

B) Aerobic and isometric training. Training will be performed with Technogym equipment at the Fondazione SantaLucia. Subjects will be trained three times a week for 45 minutes. Aerobic training will include 15 minutes of cycling on a Bike Med and 15 minutes of walking on treadmill Run Med. As for isotonic exercises, subjects will perform strength exercises for 15 min using a Chest Press, Leg Press and Vertical Traction. All the participants will wear a MyWellness Key®, a Technogym device that automatically sets the machines to the designed set of activities and uploads, for each session, the amount of consumed calories, the intensity of the workout and compliance to the program. Control ARCD subjects will also wear the key (that includes a pedometer) in order to accurately assess their daily metabolic activities.

C) Nutritional intervention. The ARCD study group undergoing MT will also receive nutritional intervention set on international nutrition guidelines (World Health Organization, 2007) and conducted by study nutritionists (2 individual sessions and 5 group sessions). Individual sessions will tailor and customize the participant's diet. Group sessions will provide discussions and practical exercises to promote lifestyle changes.

D) Metabolic and vascular risk control. Management of metabolic and vascular risk factors will be based on international guidelines (Gerber et al, 2003). Intervention will include meetings with study physicians (at 0, 6, and 12 months) for measurements of blood pressure, weight and BMI, and hip and waist circumference, physical examinations, and recommendations for lifestyle management. Study physicians will not prescribe medication but will strongly advise participants to seek treatment if needed and contact their own physician.

4) Biomarker, Genomic/epigenomic analyses

a) BDNF and IGF-1 serum levels will be detected by sandwich ELISA, while serum NfL will be quantitively determined through Quanterix Simoa™ NF-light® kit.

b) DNA extraction, genotyping/epigenotyping analysis

Genomic DNA will be extracted by whole blood samples (7-10 ml). The extraction will be performed starting from 400µl of peripheral blood using MagPurix Blood DNA Extraction Kit and MagPurix Automatic Extraction System (Resnova), according to the manufacturer's instructions. The concentration and quality of the extracted DNA will be checked by DeNovix Spectrophotometer (Resnova). The extracted DNA will be subjected to genotyping analysis by Next Generation Sequencing (Ion Torrent technology) and high-throughput Real-Time PCR systems (OpenArray Technology, Axiom technology). The same kind of technologies will be utilized for investigating the epigenetic signatures (methylation patterns, miRNA expression and screening).

c) Biostatistical and Bioinformatic analysis of genetic and epigenetic data

The genotyping results will be subjected to biostatistical analysis. The association of the gene and variants will be measured by calculating the p-value (p). The cut-off for statistical significance will be set at a $p<0.05$. All the statistical analyses will be performed using specific software. Bioinformatic tools will be employed to assess the "in silico" functional role of the analysed variants and the target genes. In particular, predictive tools (such asMutation Taster, Polyphen-2 and ExPaSy-Prosite) will be used to predict the impact of the associated variants on

protein structure and function. Human Splicing Finder will be interrogated to assess whether the analysed SNPs could alter the splicing activity. All of these tools normally utilize a set of algorithms and database able to calculate a reliable score and predict the effect size of the variants taken into consideration. Gene enrichment tools available from DAVID (Database for Annotation, Visualization and Integrated Discovery) and KEGG (Kyoto Encyclopaedia of Genes and Genomes) will be used to investigate gene-gene interactions and their potential involvement in disease-associated and or pathways regulating the lifespan. These enrichment tools incorporate data from different public resources and provide information about the possible involvement of the genes of interest in biological and molecular pathways.

BEHAVIORAL, NEURAL AND MOLECULAR EFFECTS OF MT

Effects of MT will be evaluated at different levels. In particular, we will quantify the enhancement in cognitive performance as a function of training completion either by comparing MT and CP groups and by comparing T0 and T1 assessments. We will also quantify modifications in resting state network topography (through fMRI) and structural brain changes at the macro and microstructural levels (through volumetric MRI and DTI). Finally, we will investigate BDNF, IGF-1 and NfL levels, metabolomic, proteomic and ionomic changes occurring before and after the end of training.

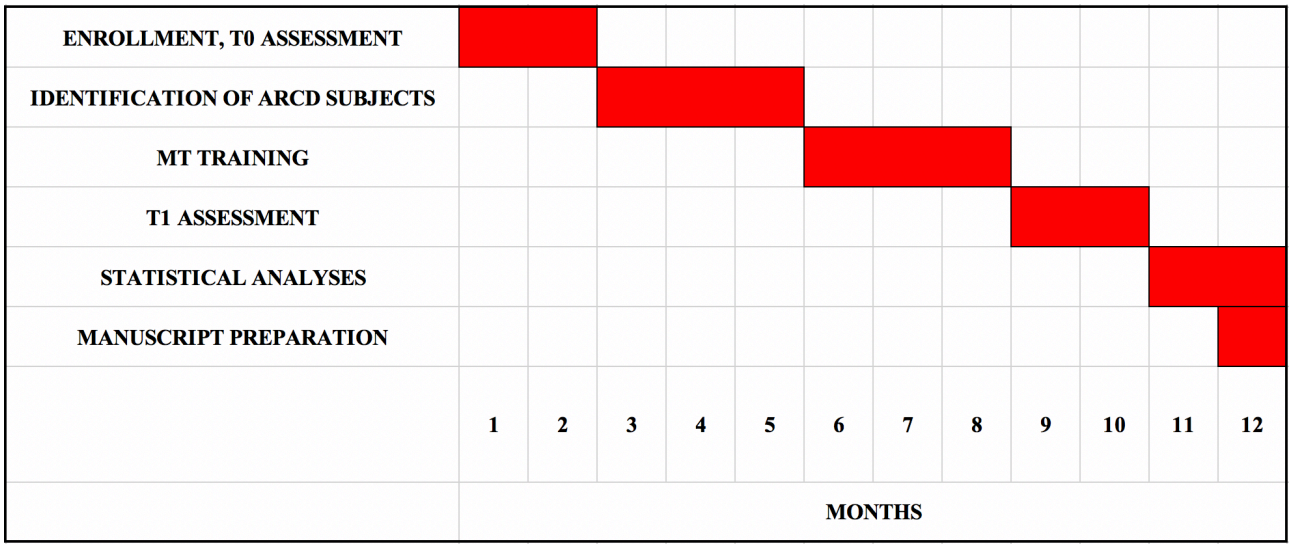
STATISTICAL ANALYSES

Statistical Analysis: All data (scores of neuropsychological and occupational tests, measures of functional and structural MRI changes, and plasma biomarkers of disease progression) will be tested for normality and homoscedasticity. Statistical Analyses will be performed using Statistica 6.0 or Statview software. Comparison among categorical variables will be done using chi-square or Fisher exact tests. Pearson's coefficient and Fisher's r to z transformation will be used to measure the correlation between continuous variables. Multivariate and Univariate Analyses of Variance (MANOVAs and ANOVAs) with pairwise post-hoc analyses (LSD or Scheffè's test) will be used to measure the difference in means of clinical and neurobiological data among groups. Repeated measure ANOVAs will be used to analyse longitudinal data on continue variables. In order to identify the best neuroimaging (at the regional level) and biological predictors (considered as independent variables) of neuropsychological, neuropsychiatric and functional scores (considered as dependent variable), we will use a series

of multivariate regression analyses (stepwise regression analysis and logistic regression analysis). These analyses will also be carried out at the voxel level by means of ad-hoc softwares (e.g. SPM, FSL).

WORKPLAN

All subjects will be recruited and assessed in the first 2 months. Individuation of ARCD subjects will follow, with a duration of 3 months. MT will start at the 6th month and will last for 3 months. T1 assessment will take place right after MT (see Gantt chart).



Gantt chart of the proposed activities

REFERENCES

Barro C, Benkert P, Disanto G, Tsagkas C et al. (2018) Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain. 2018 May 30. doi: 10.1093/brain/awy154.

Carlesimo GA, Piras F, Orfei MD, Iorio M, Caltagirone C, Spalletta G. (2015) Atrophy of presubiculum and subiculum is the earliest hippocampal anatomical marker of Alzheimer's disease. Alzheimers and Dementia. 9, 24-32.

Cascella R, Strafella C, Caputo V. (2018). Towards the application of precision medicine in Age-Related Macular Degeneration. Prog Retin Eye Res. 63:132-146.

Cutuli D, Pagani M, Caporali P et al. (2016) Effects of Omega-3 Fatty Acid Supplementation on Cognitive Functions and Neural Substrates: A Voxel-Based Morphometry Study in Aged Mice. Frontiers in Aging Neuroscience. 8:38

Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP. (2003) Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. PNAS. 100, 6940-5.

Häussermann P, Granert O (2018). Brain Functional Imaging in Preclinical Alzheimer's Disease. In Biomarkers for Preclinical Alzheimer's Disease. Springer, NY, USA.

Horvath S, Raj K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet. (6):371-384.

Horvath S. (2013). DNA methylation age of human tissues and cell types. Genome Biol. 14, R115.

Iuliano L, Pacelli A, Ciacciarelli M, Zerbinati C et al. (2013) Plasma fatty acid lipidomics in amnestic mild cognitive impairment and Alzheimer's disease. Journal of Alzheimers Disease. 36, 545-53

Josefsson, M., de Luna, X., Pudas, S., Nilsson, L. G., & Nyberg, L. (2012). Genetic and lifestyle predictors of 15-year longitudinal change in episodic memory. Journal of the American Geriatrics Society, 60, 2308-2312.

Khan SS, Singer BD, Vaughan DE. (2017). Molecular and physiological manifestations and measurement of aging in humans. Aging Cell. 16(4): 624-633.

Jacob L and Speed TP. (2018). The healthy ageing gene expression signature for Alzheimer's disease diagnosis: a random sampling perspective. Genome Biol. 19: 97.

Lu AT, Quach A, Wilson JG. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY). doi: 10.18632/aging.101684.

Mapstone M, Cheema AK, Fiandaca MS et al. (2014). Plasma phospholipids identify antecedent memory impairment in older adults. Nature Medicine. 20, 415-8

Menni C, Kiddle SJ, Mangino M. (2015). Circulating Proteomic Signatures of Chronological Age. J Gerontol A Biol Sci Med Sci. 70(7): 809-816.

Preische O, Schultz SA, Apel A. (2019). Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nature Medicine. 10.1038/s41591-018-0304-3.

Pieramico V, Esposito R, Sensi F, Cilli F, Mantini D, et al. (2012) Combination Training in Aging Individuals Modifies Functional Connectivity and Cognition and Is Potentially Affected by Dopamine-Related Genes. PLos One, 7, e43901

Scarmeas N, Anastasiou C, Yannakoulia M. (2018) Nutrition and prevention of cognitive impairment. The Lancet Neurology, 17, P1106-1015.

Sindi S, Calov E, Fokkens J et al. (2015) The CAIDE Dementia Risk Score App: The development of an evidence-based mobile application to predict the risk of dementia. Alzheimers and Dementia. 1,328-33.

Sleiman, Sama F et al. (2016). Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body β-hydroxybutyrate. eLife vol. 5 e15092. 2 Jun. 2016.

Smith AD, Smith SM, de Jager CA et al. (2010) Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. PLoS One, 5, e12244.

Spalletta G, Cravello L, Gianni W, Piras F et al. (2016) Homotaurine Effects on Hippocampal Volume Loss and Episodic Memory in Amnestic Mild Cognitive Impairment. Journal of Alzheimers Disease. 50; 807-16.

Strafella C., Caputo V, Galota MR, et al. (2018) Application of Precision Medicine in Neurodegenerative Diseases. Front Neurol. 9:701.

Tanaka T, Biancotto A, Moaddel R. (2018). Plasma proteomic signature of age in healthy humans. Aging Cell. 17(5): e12799.

Zierer J, Menni C, Kastenmüller G, Spector TD. (2015). Integration of 'omics' data in aging research: from biomarkers to systems biology. Aging Cell. 14(6):933-44.

Table 1. Proposal of genes and genetic variants to be tested.

GENE SYMBOL	GENE NAME	GENOMIC LOCATION	SNP	BIOLOGICAL FUNCTION	
APP	Amyloid Beta Precursor Protein	21q21.3	rs63750066	Neurite growth; neuronal adhesion and axonogenesis; cell mobility and transcription regulation	**AD**
BACE1	Beta-Secretase 1	11q23.3	rs638405	Formation of amyloid beta peptide from amyloid precursor protein	

PTGS2 (COX2)	Prostaglandin-Endoperoxide Synthase 2	1q31.1	rs20417	Production of inflammatory prostaglandins; immune and inflammatory response	
NAV3	Neuron Navigator 3	12q21.2	rs3803039	Axon guidance	
SIRT1	Sirtuin 1	10q21.3	rs2234975	Transcriptional regulation, cell cycle, response to DNA damage, metabolism, apoptosis and autophagy	
CLU	Clusterin	8p21.1	rs9331896	Cell death, tumor progression, and neurodegeneration	
ABCA7	Atp Binding Cassette Subfamily A Member 7	19p13.3	rs4147929	Extra- and intra-cellular transport	
PTK2B	Protein Tyrosine Kinase 2 Beta	8p21.2	rs28834970	Regulation of ion channels, activation of the map kinase signaling pathway and neural function	
APOE	Apolipoprotein E	19q13.32	rs2075650	Mediates the binding, internalization, and catabolism of lipoprotein particles.	
CD33	Cd33 Molecule	19q13.41	rs3865444	Mediates sialic-acid dependent binding to cells; induction of apoptosis	
SNCA	A-Synuclein	4q22.1	rs356219	Regulation of dopamine release and transport, fibrillization of protein tau, neuronal responsiveness to apoptotic stimuli	**PD**
LRRK2	Leucine-Rich Repeat Serine/Threonine-Protein Kinase 2	12q12	rs1491942	Regulation of neuronal process morphology in the intact central nervous system. It plays a role in synaptic vesicle trafficking and autophagy	
GBA	Glucosylceramidase Beta	1q22	rs75548401	Lysosomal activity, neuronal action potential; regarded as the most important risk factor for PD.	
S100B	S100 Calcium Binding Protein B	21q22.3	rs9722	Involved in Neurite extension, astrocytosis and axonal proliferation, inhibition of microtubule assembly, cell differentiation.	
GAK	Cyclin G Associated Kinase	4p16.3	rs6964	Vesicle-mediated transport and Clathrin derived vesicle budding.	
LMNB1	Lamin B1	5q23.2	rs1051643	Cell cycle regulation, apoptosis.	
AAK1	Ap2 Associated Kinase 1	2p13.3	rs12151791	Vesicle-mediated transport and Clathrin derived vesicle budding.	
MAOB	Monoamine Oxidase B	Xp11.3	rs1799836	Metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues.	
MAOA	Monoamine Oxidase A	Xp11.3	rs1137070	Metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues.	
COMT	Catechol-O-Methyltransferase	22q11.21	rs4680	Regulation of dopamine, epinephrine, and norepinephrine neurotransmission	
LMNA	Lamin A/C	1q22	rs1468772	Roles in cell cycle control, DNA Replication & chromatin organization.	**Aging**
COL1A1	Collagen, Type I, Alpha 1	17q21.33	rs1800012	Reduced in aging skin and bone; associated with osteoporosis	
HMGB2	High-Mobility Group Box 2	4q34.1	rs6832850	Chromatin-associated; linked to osteoarthritis	
SIRT5	Sirtuin 5	6p23	rs2253217	Inhibits gene expression profile associated with muscle ageing; linked to brain ageing	
HIST1H3E	Histone Cluster 1, H3e	6p22.2	rs811041	Replication-dependent histone; reduced gene expression in aged mice in hippocampus	
TFRC	Transferrin Receptor (P90, CD71)	3q29	rs3817672	Iron delivery to cells; previously identified as under-expressed with age	
GPC2	Glypican 2 (Cerebroglycan)	7q22.1	rs1918353	Cell surface proteoglycan; found in developing nervous system; role in cell adhesion	
RAB3A	RAB3A, Member RAS Oncogene Family	19p13.11	rs1046565	GTPase/Ca+ signaling; age-related changes in human brain	
RPP25	Ribonuclease P 25kda Subunit	15q24.2	rs34063670	Component of ribonuclease P linked to developmental brain disorders	
ADAP1	Centaurin, Alpha 1	7p22.3	rs10261233	Phospholipid binding protein; linked to Alzheimer's Disease	
BDNF	Brain Derived Neurotrophic Factor	11p14.1	rs6265	Involved in survival and differentiation of neuronal populations of the peripheral and central nervous systems. Participates in axonal growth and in the modulation of dendritic morphology.	

Consiglio Nazionale delle Ricerche

BUDGET

COSTS	DETAILS	BUDGET
Personnel	2 full time psychologists	108.000,00
	1 full time medical geneticist	54.000,00
	1 part-time radiologist	54.000,00
	3 psychologists (fellowship)	57.000,00
	1 geneticist (fellowship)	19.000,00
	1 neuroradiologist (fellowship)	27.000,00
	SUBTOTAL	**319.000,00**
Equipment	Desktop and laptop computers	30.000,00
	Cognitive testing and devices for training	30.000,00
	Stationery	10.000,00
	SUBTOTAL	**70.000,00**
Supplies	MRI scans (200 baseline +100 follow-up)	90.000,00
	DNA extraction (MagPurix Blood DNA Extraction Kit + QIAsymphony DSP DNA Kit)	9.000,00
	OpenArray Technology (TaqMan® OpenArray® Genotyping Plate, Custom Format 64/128QuantStudioTM 12K Flex - Beamer A)	22.000,00
	Axiom Technology	80.000,00
	Next Generation Sequencing (Ion AmpliSeq™ Exome RDY Kit + Sophia Genetics + Expanded Screening Igenomix)	180.000,00
	Sanger sequencing (BigDye Sequencing kit)	10.000,00
	SUBTOTAL	**361.000,00**
IT services	Software	40.000,00
	IT management	20.000,00
	SUBTOTAL	**60.000,00**
Travels	National and international scientific meetings	30.000,00
	SUBTOTAL	**30.000,00**
Publication	Scientific articles publishing fees, reprints and colour figures	30.000,00
	SUBTOTAL	**30.000,00**
Overhead	General costs directly connected to Research activity (10%)	100.000,00
	GRANDTOTAL	**1.000.000,00**

Consiglio Nazionale delle Ricerche

CONTRIBUTO CNR AL PROGETTO MUSA

INTRODUCTION

Age-related cognitive impairment and dementia are an increasing societal burden. The number of people with dementia worldwide was 35.6 million in 2010 and is estimated to increase to 65.7 million by 2030 and 115.4 million by 2050. Hence, the urgent necessity to find effective means of reducing the incidence of this disease. While dementia has a multifactorial etiology, with age being undoubtedly the major risk factor, several other factors are considered to play key roles. Among these, of particularly interest are chronic disease states and lifestyle factors. Of note, these risk factors might be modifiable even in middle or old age to promote a successful aging and to reduce dementia incidence.

Recently, the Neuroscience Institute of CNR has completed a research specifically devoted to the assessment of the efficacy of a combined cognitive and physical training, administered in a social setting, on the progression of cognitive deterioration in subjects with Mild Cognitive Impairment (MCI), considered to be at high risk for dementia. The results show a clear effect of improved cognitive status and cerebral blood flow in trained subjects (1). The available cohort of MCI subjects offers the unique opportunity to study how age-dependent changes in their cognitive status and brain health are modulated by environmental factors and how the modulatory action of the environment correlates with possible underlying molecular changes. The majority of elderly individuals with age-related cognitive disease, indeed, also suffers from vascular and neurodegenerative pathologies (2), and there is increasing evidence that chronic inflammation plays a role in brain changes leading to dementia. This may occur through cerebrovascular atherosclerosis or neuronal cell damage (3), or through the deleterious effects of inflammation on synapse function (4). Thus, the term 'inflammageing' has been recently coined to describe the relationship between low-grade chronic inflammation and aging. While reduced levels of inflammation -or protective processes against the deleterious effects of chronic inflammation- appear to favour a "healthy aging" process, genetic and environmental factors that promote inflammation confer increased susceptibility to "accelerated aging", characterized by a more pronounced functional decline (5). Several epidemiological studies indicate that multidomain interventions on modifiable lifestyle factors, such as physical exercise or stimulating cognitive activities, correlate with good brain functioning in the elderly and with reduced risk of developing dementia (6). To what extent are these beneficial effects dependent on reduced inflammatory load and whether biomarkers can be identified to predict cognitive levels and decline in elderly adults are issues which need further investigation .

HYPOTHESIS AND AIMS

Based on the available and unique Train the Brain cohort of subjects at the CNR, we will investigate how cognitive performance is modulated by an intervention based on cognitive and motor stimulation (the Train the Brain intervention) in aged, cognitively impaired human subjects (MCI subjects). In the same cohort, we will assess, in parallel with the subjects'

cognitive performance, a number of key inflammatory parameters, neurotrophin levels and markers of senescence, which will be measured in blood samples.This intervention design will shed light on the link between inflammatory, aging and cognitive decline markers, and will validate biomarkers to predict cognitive levels and decline in elderly adults with MCI.

METHODOLOGIES

At the Neuroscience Institute of CNR, 40 subjects (age> 65 and < 89) with a diagnosis of MCI (either of new recruitment, or belonging to the already available Train the Brain cohort of MCI subjects) will be subjected to cognitive screening and evaluation employing a standard neuropsychological battery and ADAS-Cog administration (1), both at the beginning of the project (T0) and immediately past the end of the Train the Brain intervention (i.e, 7 months past the beginning of training: T7). The TtB intervention will be performed as follows. 40 MCI subjects will be randomly assigned to either the training program (n = 20) or to the control arm of the program (no changes in the usual lifestyle habits). Trained subjects will be enrolled to the 7 months program of cognitive and motor training recently developed at the Neuroscience Institute of CNR (1), in a dedicated structure recently built in the CNR Research Area in Pisa fully equipped for motor and cognitive training in humans. The training program lasts 7 months and is based on 8 cycles; each cycle is composed of 18 sessions of cognitive stimulation, with exercises and activities aimed at stimulating multiple cognitive functions. Enrolled subjects are given 2 sessions of 60 min per day, 3 times a week, on Monday, Wednesday and Friday. Each cycle lasts 3 weeks, after which the same sessions of cognitive stimulation are re-started, with exercises and activities of increased complexity compared to the previous cycle. Each cycle is composed of sessions aimed at stimulating the following cognitive functions: acoustic attention, visual attention, visual memory, imagination, orientation and spatial memory, personal and temporal orientation, verbal memory, lexical abilities, memory for terms and meanings, affective memory, memory for texts, memory for faces and names, logic. In addition, Music therapy is performed once a week. Motor training consists of aerobic physical activity, three sessions per week (duration: 1h each), in collaboration with the Clinical Physiology Institute of CNR in Pisa, and in accordance with the guidelines of the American College of Sports Medicine guidelines. The program includes cycle ergometer exercise training, and exercises targeting muscular control, strength and flexibility **(IN-CNR).** Since MCI patients show poorer motor control in a variety of tasks, and given these motor deficits can be associated to an increase level of muscular fatigue, this parameter will be evaluated during the "sit-to-stand" test (STS) (7). Kinematic parameters, measured by inertial units and accelerometers, and electromyography (EMG) will be acquired in a synchronized way while the subject, instructed to sit on a chair with his back propped up against the back, the hip and knee angles at 90 ° and his arms crossed over his chest, will then be asked to sit down and get up as quickly as possible 10 times. In this way it will be possible in post-processing to derive from the inertial signal the rising and sitting phases and use this information to segment the EMG signal. The parameters related to muscle activation will be evaluated for each phase and each muscle. Patients will be monitored for cardiovascular parameters by means of non-invasive evaluation of vascular biomarkers such as endothelial function, local and global vascular stiffness, pulse wave analysis and variation in vascular reactivity to stimuli throughout the sessions **(IFC-CNR).**

Blood samples will be taken from all MCI subjects at T0 and T7. ELISA-based analysis of soluble immune mediators of inflammation will be performed. Cellular mediators will be assessed by FACS analysis on fresh heparinized blood samples for IL-6, TNFa, CCL2. FACS analysis will be performed for monocyte/macrophage, T cell (CD3/CD4/CD8) and regulatory T cell (CD4+CD25+IL7Rlo) presence. Furthermore, levels of circulating miRNAs known to be involved in mediating CNS inflammation (miR-155, miR-146a) will be quantified upon extraction from human blood samples or purification of the exosomal fraction and subsequent sequencing on the Illumina system (8) **(IN-CNR)**. Furthermore, based on the concept that homeostatic regulation of naïve and memory T and B cells dramatically changes during aging (9), the expression of senescence biomarkers expressed by T and B subcellular populations will be analyzed, together with the production of reactive oxidative species (ROS) in the peripheral blood. The immunophenotype of T and B cells will be analyzed by FACS , while quantification of serum BAFF levels and autoantibodies against self-antigens will be performed on a limited number of patients **(IRGB-CNR)**. We will also analyze by RT-PCR and Western blotting the levels of two relevant neurotrophins, Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF), known markers of neuroplasticity. Both have proved to represent useful markers in monitoring the efficacy of different non-conventional training methods designed to counteract, or delay, cognitive impairment (among these, the Quadrato Motor Training, 10) **(IBPM-CNR).** Absolute and percentage changes from baseline during the intervention period will be compared between the two arms using Student's t-test, non-parametric tests, $\chi 2$ and Fisher's exact tests. Analysis of variance (ANOVA) and regression models, controlling for confounders, will be applied to infer the effect of the intervention and its correlations and interactions with all analyzed biomarkers. Data will be handled in accordance with the General Data Protection Regulation (GDPR) **(ITB-CNR).**
A major milestone will be to determine whether the training program ameliorates cognition, cardiovascular functions and muscular fatigue, and to define possible signatures, on the basis of the parameters analyzed, to predict cognitive levels and decline in elderly adults with MCI. Finally, as a pilot activity, differentiation of iPSCs from a low number of patients into cells of the nervous system will be possibly attempted. This will lay the ground for the future establishment of a biobank, which could be used for investigating the nature of the genetic contribution to the pathology and for drug screening **(IRGB-CNR).**

WORKPLAN

TtB will start immediately (relying on the already available cohort of subjects and on Ethical Approvals already obtained). Recruitment of further MCI subjects will be performed in the first 3 months. Each class will be trained for a total of 7 months, with the entire intervention program (two classes of trained subjects, 10 subjects each) being concluded within 10 months.

REFERENCES

1. The Train the Brain consortium (2017). Randomized trial on the effects of a combined physical/cognitive training in aged MCI subjects: the Train the Brain study. Scientific Reports 7, 39471.
2. A. Viswanathan, W. A. Rocca, C. Tzourio, Vascular risk factors and dementia: how to move forward? Neurology 72, 368-374 (2009).

3. Y. Yano, S. Matsuda, K. Hatakeyama, Y. Sato, T. Imamura, K. Shimada, T. Kodama, K. Kario, Y. Asada, Plasma Pentraxin 3, but not high-sensitivity C-reactive protein, is a useful inflammatory biomarker for predicting cognitive impairment in elderly hypertensive patients. The journals of gerontology. Series A, Biological sciences and medical sciences 65, 547-552 (2010.
4. Pozzi D, Menna E, Canzi A, Desiato G, Mantovani C and Matteoli M (2018) The communication between the immune and nervous systems: the role of IL-1beta in synaptopathies. Frontiers Mol Neurosci Apr 5;11:111. doi: 10.3389/fnmol.2018.00111
5. Gabuzda D, Yankner BA. (2013) Physiology: Inflammation links ageing to the brain. Nature 497, 197-198.
6. Sale A, Berardi N, Maffei l (2014) Environment and brain plasticity: towards an endogenous pharmacotherapy. Physiological reviews 94, 189-234 (2014).
7. Roldán Jiménez C, Bennett P, Ortiz García A, Cuesta Vargas AI. Fatigue Detection during Sit-To-Stand Test Based on Surface Electromyography and Acceleration: A Case Study. Sensors (Basel). 2019 Sep 27;19(19).
8. B.-R. C. Cortez MA, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA., MicroRNAs in body fluids--the mix of hormones and biomarkers. Nat Rev Clin Oncol. 8(8), 467-477 (2011).
9. Lopez-Orin The hallmarks of aging. Cell 2013,
10. Caserta M, Ben-Soussan TD, Vetriani V, Venditti S and Verdone L (2019) Influence of Quadrato Motor Training on Salivary proNGF and proBDNF. *Front Neurosci.* 13:58.

REFERENTS:
Alessandro Sale e Michela Matteoli

BUDGET AND CONTRIBUTIONS

Dipartimento Scienze Biomediche
Overhead 5K

Istituto di Neuroscienze (IN)
Patient recruitment, organization and carrying out of training, cognitive assessment, analysis of inflammatory biomarkers and circulating miRNAs
Budget: 95K
Personnel: Alessandro Sale, Maria Luisa Malosio, Stefania Maggi

Istituto di Fisiologia Clinica (IFC)
Cardiovascular monitoring and muscular fatigue assessments
Budget: 40K
Personnel: Marco Laurino, Lorenza Pratale, Giorgio Iervasi

Istituto di Ricerca Genetica e Biomedica (IRGB)
Senescence biomarkers expressed by T and B cells, establishment of iPS (pilot)
Budget: 25K
Personnel: Barbara Cassani, Maria Carmina Castiello, Anna Villa

Consiglio Nazionale delle Ricerche

Istituto di biologia e patologia molecolari (IBPM)
Neurotrophin quantitation
Budget: 10K
Personnel: Loredana Verdone, Micaela Caserta, Patrizia Lavia

Istituto di Istituto di tecnologie biomediche (ITB)
Statistical analysis
Budget: 25K
Personnel: Fulvio Adorni, Gianluca De Bellis

Allegato B

Linee Guida Rendicontazione

Consiglio Nazionale delle Ricerche

Linee guida per la gestione e rendicontazione del Progetto:……..

PREMESSA

Le presenti Linee Guida intendono costituire uno strumento d'indirizzo per la gestione progettuale e la corretta compilazione delle tabelle di rendicontazione dei costi sostenuti durante lo svolgimento delle attività di ricerca svolte dal…….

CRITERI GENERALI DI GESTIONE

Modalità di erogazione del contributo

Le quote di finanziamento verranno erogate a ……in ragione del piano finanziario indicato nella convenzione e della erogazione del contributo da parte del MIUR in favore del CNR.

Rimodulazioni progettuali

Fermo restando il conseguimento degli obiettivi progettuali, eventuali rimodulazioni economiche di progetto nel limite del 10% della singola voce di spesa, dovranno essere unicamente e tempestivamente comunicate dal Responsabile di progetto al Dipartimento…….

Le rimodulazioni eccedenti la soglia del 10% della singola voce di spesa dovranno essere adeguatamente motivate e sottoposte al Dipartimento……..

Eventuali rimodulazioni scientifiche di progetto dovranno essere presentate al Dipartimento……., corredate di relazione illustrativa.

CRITERI GENERALI DI RENDICONTAZIONE

Il processo di monitoraggio e rendicontazione

Il Dipartimento …….convocherà semestralmente il Responsabile di progetto al fine di monitorare lo stato di avanzamento delle attività progettuali.

A tale fine, il Responsabile di progetto dovrà produrre una sintetica relazione sullo stato di avanzamento delle attività e delle spese sostenute, evidenziando brevemente i risultati conseguiti e gli eventuali scostamenti rispetto al progetto approvato.

La rendicontazione tecnico-scientifica delle attività svolte e la rendicontazione economica dovrà essere redatta secondo le tempistiche indicate nella Convenzione e secondo le istruzioni contenute nelle presenti Linee Guida e presentata al Dipartimento……, utilizzando eventualii format predisposti dal MIUR.

Impegni

I costi dovranno derivare da atti giuridicamente vincolanti (contratti, lettere di incarico, ecc.) da cui risulti chiaramente l'oggetto della prestazione o fornitura, il suo importo, la sua pertinenza al progetto.

Criterio di cassa

In linea generale i costi saranno riconosciuti solo se effettivamente sostenuti dall'Ente/Istituto/Società/ecc. cui afferisce l'unità finanziata. Varrà cioè per essi il criterio di "cassa", con le sole eccezioni degli oneri differiti per il personale. Le fatture e gli altri titoli di spesa di cui non si dia dimostrazione inequivoca dell'avvenuto pagamento alla presentazione del rendiconto contabile saranno escluse dai costi ammissibili.

Vigenza temporale dei progetti

Saranno riconosciuti solo costi attinenti allo svolgimento delle attività espressamente indicate nel progetto e sostenute nel periodo di vigenza dello stesso.

I.V.A.

I costi riguardanti le diverse tipologie di spesa dovranno considerarsi al netto di I.V.A. nel caso in cui tale imposta risulti trasferibile in sede di presentazione della dichiarazione periodica.

Dovranno considerarsi invece comprensivi di I.V.A. nel caso in cui tale imposta non sia trasferibile (è questo, ad esempio, il caso delle Università statali, degli Enti pubblici di Ricerca, delle Istituzioni ospedaliere e di tutti gli altri soggetti pubblici).

Dichiarazioni

Al fine dell'accettazione, inoltre, ogni rendicontazione dovrà contenere un'apposita dichiarazione, rilasciata dal rappresentante legale (o suo delegato) dell'istituzione beneficiaria del contributo, attestante:

- che nello svolgimento delle attività di progetto sono state rispettate tutte le norme di legge e regolamentari vigenti;
- che per le spese rendicontate, tutte effettivamente sostenute, non sono stati ottenuti o richiesti ulteriori rimborsi e/o contributi;

Documentazione

Tutta la documentazione tecnica, scientifica e contabile (fatture, ricevute, giustificativi di spesa, eccetera) a supporto delle rendicontazioni dovrà essere conservata in originale da……..per tutta la durata della realizzazione del progetto e per i cinque anni successivi alla chiusura del progetto.

CRITERI RELATIVI ALLE SINGOLE VOCI DI SPESA

Personale

Questa voce comprende il personale dipendente, sia a tempo indeterminato che a tempo determinato, in organico di……..

Il costo relativo al personale dipendente (a tempo indeterminato e a tempo determinato) è definito, per ogni persona impiegata nel progetto, in base alle ore lavorate, valorizzate attraverso le tabelle standard di costo orario.

Spese generali

L'importo della voce in oggetto è calcolato forfettariamente nella misura del 20% del costo totale del progetto.

Spese per il funzionamento dell'Infrastruttura di ricerca

Questa voce comprende le spese per Servizi di supporto all'Animal facility quali: Assistenza Tecnica, Manutenzioni, Smaltimento Rifiuti speciali, costi energetici di funzionamento, pulizia e sanificazione degli ambienti, Disinfestazione.

Consulenze scientifiche

Questa voce comprende le spese per prestazioni a carattere scientifico rese da persone fisiche o da qualificati soggetti con personalità giuridica privati o pubblici, e inerenti le attività progettuali.

Dovrà essere riportata la denominazione del soggetto erogatore della prestazione, l'attività svolta nel progetto, il numero e la data della fattura e la data di pagamento della fattura.

Altre prestazioni di terzi

Questa voce comprende le spese per prestazioni di servizi di tipo non scientifico, e legate comunque alle finalità del progetto, rese da persone fisiche o da soggetti aventi personalità giuridica.

Dovrà essere riportata la denominazione del soggetto erogatore della prestazione, l'attività svolta nel progetto, il numero e la data della fattura e la data di pagamento della fattura.

Altri costi funzionali al progetto

Questa voce comprende le spese per l'acquisto di materiale durevole, materie prime, componenti, semilavorati, materiali di consumo specifico.

Dovrà essere riportata una descrizione del bene acquistato, il numero e la data della fattura e la data di pagamento della fattura.