Allegato 2 – Tematiche di ricerca Spoke n. 7

Obiettivo generale: Lo Spoke 7 propone di sviluppare una piattaforma multiomica cellulare e molecolare per studiare i meccanismi immunitari e la neuroinfiammazione nelle malattie del sistema nervoso, e per comprendere le interazioni tra il sistema nervoso e quello immunologico. Queste attività, tramite l'identificazione delle vie molecolari e cellulari alla base delle interazioni tra infiammazione, risposta immunitaria e funzione neurale, consentiranno lo sviluppo di nuovi biomarcatori diagnostici per condizioni neurologiche causate dall'infiammazione e di identificare nuovi bersagli terapeutici per le malattie neuroinfiammatorie.

Tematiche di ricerca oggetto del bando:

	TEMATICA	Agevolazione minima di ogni proposta progettuale	Agevolazione massima di ogni proposta progettuale
A	**Genetic variability and gene-environment interactions in neurological autoimmunity**	**240.000,00 €**	**300.000,00 €**
B	**Identification of risk factors, biomarkers and new therapeutic targets for cerebral amyloid angiopathy-related inflammation**	**120.000,00 €**	**150.000,00 €**
C	**Role of cytotoxic T cell subsets in neurodegenerative diseases**	**120.000,00 €**	**150.000,00 €**
D	**Characterizing molecular and cellular interplays in advanced in vitro systems mimicking neuroinflammation and nervous system dysfunction**	**200.000,00 €**	**250.000,00 €**
E	**Characterization of regulatory neutrophils in central nervous system diseases**	**160.000,00 €**	**200.000,00 €**
F	**Advanced immunophenotyping of innate and adaptive immunity cells in brain inflammatory diseases**	**160.000,00 €**	**200.000,00 €**
G	**Autoantibodies against neuronal and glial proteins in neuroimmunological disorders: laboratory diagnostic, pathogenic mechanisms, and discovery of novel targets**	**120.000,00 €**	**150.000,00 €**
	TOTALE	**1.120.000,00 €**	**1.400.000,00 €**

La tabella sottostante riporta le tematiche il cui finanziamento è espressamente riservato agli Organismi di Ricerca che svolgeranno l'attività in sedi operative nel sud Italia:

	TEMATICA	Agevolazione minima di ogni proposta progettuale	Agevolazione massima di ogni proposta progettuale
H	Using genomics and trascriptomics to uncover novel mechanisms in B cells and determine their relevance to the causal biology of brain autoimmune diseases	240.000,00 €	300.000,00 €
I	Translational investigation of peripheral and central immune biomarkers in neuroinflammatory diseases	240.000,00 €	300.000,00 €
L	Study of the brain areas controlling the inflammatory reflex involved in the modulation of immune responses during cardiovascular diseases: a role for neuroimmune cardiovascular interface.	160.000,00 €	200.000,00 €
	TOTALE	640.000,00 €	800.000,00 €

Di seguito si riporta per ogni tematica una descrizione analitica:

A. Genetic variability and gene-environment interactions in neurological autoimmunity

The role of environmental factors such as the Epstein Barr virus (EBV) in the development of autoimmunity in multiple sclerosis (MS) is well recognized and the interaction between human genetic predisposition and the genetic variability of EBV is considered a major driver of the autoimmune processes leading to MS. The analysis of the interaction between human and viral genetic variability may provide key information to the understanding of disease mechanisms and to the identification of therapeutic targets in MS.

This call includes the following objectives:

1. Bioinformatics analysis of the interaction between disease-predisposing human genetic variability (in MS and in other immune-mediated conditions) and "environmental" (i.e. non-heritable) risk factors, including EBV

2. Whole genome sequencing of EBV infecting patients and controls for a complete identification of viral genetic variability that associates with the disease

3. Identification and validation of the most relevant interactions between human and viral genetic variability and prioritization of therapeutic targets.

B. Identification of risk factors, biomarkers and new therapeutic targets for cerebral amyloid angiopathy-related inflammation

Cerebral amyloid angiopathy-related inflammation (CAA-ri) is a potentially reversible, autoimmune encephalopathy associated with the downstream negative effects mediated by autoantibodies against brain-deposited amyloid-β protein (autoAbs) in the CSF of patients with cerebral amyloid angiopathy and comorbid Alzheimer's disease.

The current lack of validated fluid biomarkers makes CAA-ri an exclusion diagnosis, challenged by the expert reading of MRI images and the complex interpretation of their heterogeneous presentations. Innovative biomarkers' assays such as the CSF testing for autoAbs are emerging as promising tools to overcome current limitations of MRI in the diagnosis and response to treatment monitoring of outcome. In this framework, the main objectives of this call are:

1) To characterize the autoAbs profile along the course of CAA-ri

2) To provide biological evidence on the natural history of CAA-ri and on the autoimmune and neuroinflammatory mechanisms and risk factors

3) To validate fluid biomarkers enabling improved diagnostic and management accuracy for CAA-ri.

C. Role of cytotoxic T cell subsets in neurodegenerative diseases

This call aims to analyse the role of CD4+T-cells in central nervous diseases and investigate their secretion of pro- or anti-inflammatory cytokines in response to disease-relevant antigens.

The project is expected to provide information on the relative abundance of CD4+T-cell subsets with cytotoxic and/or regulatory functions in the CSF, and of their TCR antigen specificities in different neurodegenerative diseases. The experimental strategy should include both cellular and molecular approaches, like stimulation of CD4+T-cells with disease-relevant antigens and generation of single cell RNA and TCR sequencing data from the CSF of patients with neurodegnerative diseases, in order to analyse gene expression in TCR clonotypes. In addition, public available datasets on CD4+T-cells in the CSF of patients with neurodegenerative diseases should also be exploited with cutting edge bioinformatic tools. It is expected that CD4+T-cell subsets that are activated by disease-relevant antigens in different neurodegenerative disease will be identified.

Main objectives of this call are:

1. Analysis of disease-relevant TCR antigen specificities of cytotoxic and/or regulatory CD4+T-cell subsets in neurodegenerative diseases *versus* healthy controls

2. Identification of frequencies and phenotypes of cytotoxic and regulatory CD4+T-cell subsets in the CSF of neurodegenerative diseases

3. Identification of gene signatures of CD4+T-cell clonotypes in the CSF of patients with neurodegenerative diseases

D. Characterizing molecular and cellular interplays in advanced in vitro systems mimicking neuroinflammation and nervous system dysfunction

Neuroinflammation represents a key factor in the pathogenesis of Alzheimer's disease (AD), but its cellular and molecular mechanisms are still unclear. For this call, the role of neuroinflammation will be analyzed in AD in the context of organoids containing neurons, astrocytes and microglia from induced-pluripotent stem cells (IPSC) from healthy or mutated donors. Cellular interplays upon exposure to inflammatory hints such as amyloid oligomers will be also analyzed in terms of glial reactivity and cytokines production. Analysis will include confocal and superresolution microscopy to investigate neuronal network integrity, astrocyte/microglia morphological changes and synapsis engulfment, as well as ultra-sensitive detection of biomarkers. Research activities will cover two main objectives:

1. Study the influence of mutations associated with AD in terms of glial reactivity and neuronal survival.

2. Investigate the impact of drugs on AD-related neuroinflammatory mechanisms in human iPSC-derived neural cells using cells carrying mutations associated with AD and after exposure to amyloid oligomers.

E. Characterization of regulatory neutrophils in central nervous system diseases

Traditionally seen as uniform effectors of the innate immune system, neutrophils are now acknowledged for their functional heterogeneity. Neutrophils are involved in autoimmune disease progression, including neuroinflammatory diseases, due to their role as effectors of the innate immune system. Suppressor functions have, however, also been described and this call is aimed at characterizing neutrophils in both human and experimental neuroinflammation focusing on suppressor subpopulations.

Main objectives are:

1. Identification and kinetic of neutrophils with regulatory functions during multiple sclerosis and neuromyelitis optica and in the corresponding mouse models.

2. Characterization of modulatory and immune-suppressive pathways triggered by regulatory neutrophils in human and experimental neuroinflammation.

3. Identification of main cell targets for regulatory neutrophils in human and experimental neuroinflammation.

F. Advanced immunophenotyping of innate and adaptive immunity cells in brain inflammatory diseases

Neuroinflammatory and neurodegenerative diseases of the central nervous system (CNS) are complex pathologies characterized by heterogeneous immune cell phenotypes acting in concert at peripheral and central levels during disease development. The project of this call is expected to obtain and integrate large amounts of data obtained in the context of neurodegenerative and neuroinflammatory diseases by using advanced technologies and bioinformatics. Particularly, it is expected that research activities will include advanced flow cytometry and multiomics studies on circulating immune cell populations, which will be integrated with clinical data to identify new disease signatures and biomarkers. In addition, the phenotype of migrated innate and adaptive immune cells will be also studied on tissue samples from patients with neurodegenerative and neuroinflammatory diseases at single-cell level by using spatial biology (RNA sequencing and proteomics). Advanced neuropathological analysis may include spatial cell phenotyping, cell-cell interactions, cellular neighborhoods, tissue architecture and networks, biomarker identification.

The call will have the following main objectives:

1. Determine and integrate the immunophenotype of circulating innate and adaptive immune cells by using advanced flow cytometry and multiomics studies to identify new pathological immune signatures

2. Integrate proteomics and transcriptomics analysis at single cell level on tissue samples from patients with neurodegenerative and neuroinflammatory diseases

G. Autoantibodies against neuronal and glial proteins in neuroimmunological disorders: laboratory diagnostic, pathogenic mechanisms, and discovery of novel targets

Autoantibodies directed against neuronal or glial proteins can be relevant diagnostic markers in several neurological disorders of the central and peripheral nervous system. In addition, current evidence suggests that many neuroglial antibodies can be pathogenic, and that patients with specific clinical phenotypes might harbor autoantibodies yet to be discovered. Research studies for this call will include the following objectives:

1. Standardization of the complex laboratory diagnostic for neuronal and glial autoantibodies

2. Characterization of the pathogenic properties of neuronal antibodies in animal and in-vitro models

3. Identification of novel antibody targets in specific subgroup of patients

Tematiche Bando SUD:

H. Using genomics and trascriptomics to uncover novel mechanisms in B cells and determine their relevance to the causal biology of brain autoimmune diseases

B cells play a crucial role in the pathogenesis of brain autoimmune diseases such as multiple sclerosis (MS). However, the specific contribution of B cells to MS onset and the mechanisms underlying drugs targeting B lymphocytes in MS need to be better understood.

The project aims to identify cellular subtypes, genes, and pathways implicated in the disease pathogenesis focusing on B cells. This will be accomplished through advanced experimental methods, encompassing genome and transcriptome sequencing analyses (including the use of single-cells RNAseq techniques and B-cell receptor repertoire profiling) in both healthy individuals and patients. Validation of findings will be carried out through functional studies.

Research studies for this call will include the following objectives:

1. Identify and prioritize the cellular populations affected by genetic variants associated with brain autoimmune diseases.

2. Characterize the specific transcriptional profile of disease-associated B cells and identify target genes and pathways associated with the pathology.

3. Validate the causal action on transcriptional profiles of risk-associated genetic variants through functional studies.

I. Translational investigation of peripheral and central immune biomarkers in neuroinflammatory diseases

Dysregulated immune responses characterize neuroinflammatory and neurodegenerative pathologies such as multiple sclerosis (MS) and Parkinson's disease (PD). The immunoprofiles in these disorders show great differences between males and females and sex represents a strong bias in both MS and PD also based on the following main evidence: a) woman are more frequently affected by MS than men, but men with MS often show worse disability progression; b) men have a higher risk to develop PD than women. This call will have the following objectives:

1. Study the effect of sex effect on the peripheral and central nervous system dysregulation of immune responses in PD and MS patients and in animal models of these diseases.

2. Determine the sex effect on time-dependent damage to brain barriers in neuropathological models of PD and MS.

3. Characterize the influence of genetic factors in immune dysregulation in MS and PD patients.

These objectives will be reached by using flow cytometry, RNAseq, ELISA assays, immunofluorescence and confocal microscopy analysis, genome-wide association studies (GWAS).

L. Study of the brain areas controlling the inflammatory reflex involved in the modulation of immune responses during cardiovascular diseases: a role for neuroimmune cardiovascular interface

The brain influences and modulates peripheral immune and inflammatory responses, but how it perceives the disturbances of peripheral homeostasis - the phenomenon called "interoception" – to organize coordinated neuroimmune reflex actions is still object of investigation in several diseases, including cardiovascular disease.

Tissues and organs of the cardiovascular system have been shown to establish neural bidirectional circuits with the brain. However, the function of these circuits in health and disease is still partially unknown. Typically, cardiovascular disease has been investigated to dissect the pathophysiological mechanisms underlying alterations of cardiovascular system itself. Looking into the multiorgan connections – particularly into the brain-cardiovascular system - might offer new perspectives and the main objectives of this call will be:

1) Identifying the brain areas that control the inflammatory reflex involved in the modulation of immune responses relevant to cardiovascular disease

2) Tracking and characterizing the neuronal ensembles that perceive challenges exerted on the cardiovascular system

3) Utilizing tools of bioelectronic medicine to modulate neuroimmune cardiovascular interfaces with therapeutic purposes