ImmunoTools *FlowISiAM* Award 2026

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Endothelial senescence and dysfunction as a promoter for monocyte activation (inflammageing) in dementia

Background

With age, brain endothelial cells (BECs) undergo molecular changes associated with senescence, sustaining chronic inflammation by secreting pro-inflammatory cytokines (IL-6, IL-1β, TNF-α- a part of senescence-associated secretory phenotype (SASP)), losing tight junction proteins, and exhibiting heightened transcytosis, resulting in a leaky barrier that allows immune cells and toxins to enter the brain [1-3]. In addition to the established markers of endothelial dysfunction, recently the expression of Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1B (DYRK1B) was also found to increase *in vitro* induced senescence of ECs by localizing to the adherens junctions and disrupting monolayer permeability and proper ECs orientation [4]. In this way, vascular endothelial cell ageing and dysfunction transform the blood-brain barrier (BBB) from a protective barrier into a source of low-grade inflammation via peripheral monocyte infiltration, potentially serving as a starting point for the development of dementia [1, 5]. Depending on the tissue signals (emitted SASP from senescent ECs), the immune response can be polarised towards inflammatory or anti-inflammatory during the development of dementias [6]. The pro-inflammatory phenotype leads to the accumulation of Aβ plaques and hyperphosphorylation of tau protein, which disrupts neuronal communication - a key pathological feature of dementias [7]. In contrast, monocytes with an anti-inflammatory profile exhibit increased phagocytic activity and can

engulf senescent endothelial cells via efferocytosis [8, 9]. The removal of senescent ECs may prevent disease and improve health and well-being. In this context, the discovery of novel early biomarkers that effectively identify senescent endothelial cells is particularly important.

Objectives: *The FlowISiAM* methodology enables the detection and quantification of internalized biological materials in peripheral monocytes, thereby clarifying the effectiveness of efferocytosis of senescent endothelial cells during the pathogenesis of dementia.
FlowISiAM and AMANDUS method will be used to:

- Quantify the pro-inflammatory type of monocytes – levels of CCR2
- Quantify the anti-inflammatory type of monocytes (phagocytic activity) – levels of CD163 and CD33
- Quantify the intracellular levels of CD31, vWF and DYRK1B
- Correlate the functional readouts with clinical measures (biochemical tests and Neuropsychological tests) of disease severity

Experimental Design & Methods:

Using the *FlowISiAM* technique in collaboration with INVIGATE and ImmunoTools *AMANDUS* we will use:

Dementia patients

A group of 20 patients with vascular and degenerative dementia from Hospital "Ivan Rilski", Sofia, Bulgaria, who have completed neuropsychological tests, MRI, and neurochemical assessments, will be examined for increased pro-inflammatory or anti-inflammatory/phagocytic-differentiated monocytes in peripheral blood. The blood samples will be used for *FlowISiAM* and *AMANDUS* analysis of CCR2, CD163 and CD33 and internalized CD31, vWF and DYRK1B.

Impact

The intended investigations will provide new insights into the relationship between endothelial senescence and dysfunction, on the one hand, and inflammaging (monocyte activation), on the other, which collectively lead to neuroinflammation and the progression of dementias of different origins.

Furthermore, we look forward to identifying reliable early markers of endothelial senescence and monocyte activation using *FlowISiAM* and *AMANDUS* in peripheral bloodmonocytes/macrophages.

The research will help provide a non-invasive, reliable blood-based test for the early diagnosis of dementia.

Cooperation Partners:

The groups of Prof. Tzoneva and Prof. Oreshkova will work with ImmunoTools to adjust the experimental and instrumental setup for conducting *FlowISiAM* and *AMANDUS* analysis.

References

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ImmunoTools *FlowISiAM* AWARD for

Rumiana Tzoneva and **Tsvetelina Oreshkova** includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against some specific biomarkers from INVIGATE, engagment regarding development of specific *AMANDUS*, and expert assistance in evaluating the results obtained, and integration into the international ImmunoTools *FlowISiAM* network.

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