Status of CIPKeBiP: Participating organisation
Title of the project: **Exosome-associated Nef released from HIV infected cells contributes importantly to the development of neuroAIDS**
Coordinator: **Dr. Boris Matija Peterlin**
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CIPKeBiP Membership

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Abstract
In the era of highly active antiretroviral therapy (HAART), neurological manifestations of AIDS remain an insurmountable problem. Clinical symptoms of NeuroAIDS include HIV-associated dementia (HAD), mild cognitive impairment (MCMD) and vacuolar myelopathy. Whereas central nervous system (CNS) of children seems to be even more vulnerable to HIV, around 10% and 30% of HIV-infected adults develop HAD and MCMD, respectively. To combat NeuroAIDS, a thorough understanding of mechanisms implicated in HIV-associated neurodegeneration is needed. Additionally, discovering biomarkers for the risk and progression of NeuroAIDS could improve the treatment of infected individuals.

It is known that HIV enters CNS early in infection via hematopoetic cells that infect neural cells. The main targets are microglia and astrocytes, capable of supporting full or only limited viral replication, respectively. In pediatric autopsied brains as well as in in vitro infected cells, the NEgative Factor (Nef) is the primary HIV protein expressed in astrocytes. It has been implicated in NeuroAIDS, but the mechanism of Nef-induced neurodegeneration remains unclear. Indeed, Nef affects the viability and metabolism of microglia, astrocytes, oligodendrocytes and neurons. It also increases the migration and recruitment of leukocytes into the brain. Previously, we demonstrated that Nef, expressed from transfected or HIV-infected primary T cells, stimulates its own export via exosomes (vesicles 40-100 nm in diameter), which causes activation-induced cell death of resting lymphocytes. Others confirmed our results and demonstrated the presence of Nef-exosomes even in the blood of HAART-treated patients.

Thus, the hypothesis of this proposal is that Nef-exosomes, released from HIV-infected microglia and astrocytes, form a pool of bioactive vesicles capable of interfering with the viability and differentiation of surrounding CNS cells. Our long-term objective is to understand this role for Nef and to assess Nef-exosomes in the cerebrospinal fluid (CSF) and/or blood as biomarkers for NeuroAIDS. In particular, we will demonstrate the clinical relevance of Nef-exosomes in the CNS. To this end we will first optimize methods for isolating exosomes. Later, we will isolate and quantify exosomes from the CSF and blood of healthy and HIV-infected people and determine their levels of Nef. For exosome quantification we will develop a new approach based on the AF4-UV-MALS-QELS-RI technique. We will also measure Nef-exosomes released from transfected or infected microglia and astrocytes. This release will be

inhibited by siRNAs targeting cellular trafficking pathways and with mutant Nef proteins. Later, we will detect Nef-exosome targets in the CNS (astrocytes, microglia, oligodendrocytes, neurons and neural stem cells) and follow their internalization by immunofluorescent microscopy . In the end, we will expose these cells to Nef-exosomes and evaluate their effect on apoptosis, proliferation and differentiation by FACS and immunofluorescent microscopy. We will use recombinant Nef proteins as a control. In the end, we will develop minimally invasive methods for following the onset and development of NeuroAIDS. To analyze the CSF for such biomarkers, we will isolate exosomes from the CSF of HIV-infected people and perform LC-MS/MS as well as miRNA expression profiling. Relevant biomarker for NeuroAIDS, identified by above approaches, will also be evaluated on blood samples corresponding to the CSF of infected people.

The findings of this proposal will contribute to the understanding of NeuroAIDS and physiological role/s of exosomes. It will also characterize effects of Nef on cells of the CNS, which will identify new strategies to inhibit and/or prevent their sequelae. It will also introduce a new method in AIDS diagnostics, where Nef-exosomes will serve as biomarkers for following this disease.