**PROJECT 1**

DoS:    Georgia Fousteri

Title:    **Role of follicular helper T cells in the humoral immune alterations in patients with mutations in the Wiskott Aldrich Syndrome protein before and after hematopoietic stem cell transplantation or gene therapy**

Curriculum:    BAIO

Link to OSR/UniSR personal page: https://research.hsr.it/en/institutes/diabetes-research-institute/regulation-of-adaptive-immunity.html

**Project description** (*Number of characters, including spaces*: 2.000 – 3.000):

Mutations in Wiskott-Aldrich Syndrome (WAS) gene lead to an X-linked primary immunodeficiency (PID) with cellular and humoral defects, increased risk of autoimmunity (AI) and lymphomas (1). Pathogen clearance and humoral immunity require B cell differentiation into memory cells and long-lived plasma cells (PCs) in the germinal centers (GCs) where T follicular helper cells (Tfh) are the master providers of B-cell help (2). WAS protein (WASp) deficiency alters the development of GC B cells and Tfh in WAS patients and Was-/- mice (3).However, little it is known about the underlying cellular and molecular mechanisms. Our goal is to identify the role of Tfh cells in the regulation of humoral immunity in patients with WAS mutations. This will provide new biomarkers of disease course prior and after hematopoietic stem cell transplantation (HSCT) or gene therapy (GT) and identify novel therapeutic targets to manipulate Tfh cell function to improve the clinical outcome in the affected patients. More specifically, with this proposal we aim to:

**Aim-1: Analyze the pool of circulating memory Tfh and Tfr cells in WAS patients and their capacity to promote and control B cell responses, respectively.**

The questions (Q) we are going to address in this aim are:

Q1: What is the frequency and number of the major circulating Tfh subsets: Tfh1, Tfh17, Tfh2, highly functional Tfh, activated Tfh and Tfr cells in patients with WASp deficiency and do they change longitudinally as the disease progresses?

Q2: Do circulating Tfh cells and subsets and their ratio to Tfr cells associate with WAS severity and how do they change after HSCT or GT?

Q3: Do plasma CXCL13 levels associate with GC activity in WAS patients and could they be used as biomarker to predict disease progression or remission pre and post HSCT or GT?

**Aim-2: Dissect the cellular and molecular mechanisms by which WASp controls GC responses in vivo by employing selective mouse models.**

The questions (Q) we are going to address in this aim are:

Q1: How does WASp control the differentiation program of Tfh and Tfr cells in vivo and if so by which mechanism?

Q2: How do WASp-defective Tfh cells affect the development of GC B cells and the production of PCs during the GC response?

**Skills to be acquired by the student:**

The PhD student will develop concrete scientific knowledge and in the field of clinical and experimental immunology. More specifically he/she will acquire proficient knowledge in genetics of primary immunodeficiency, in T cell immunology and particularly the mechanisms that drive and control antibody responses. The technical skills to be developed are: staining and analysis of single cells by flow cytometry (FC). FC-based sorting of cells that will be used in in vitro experiments and for gene expression profile analysis. ELISA and Bioplex assays for the detection of cytokines and chemokines in the patients' sera and in culture supernatants. Mouse models. Histology (multiple immunofluorescence) of secondary lymphoid organs for the detection of GCs. NGS data analysis and validation. He/she will acquire the ability to clearly and forcefully articulate his/her ideas-in person and in writing. He/she will learn to analyze data, interpret the results, present data at conferences and write scientific manuscripts and research proposals.

**References** (max. 3)

1) Catucci, Front Immunol, Autoimmunity in wiskott-Aldrich syndrome: an unsolved enigma. 2012 18;3:209

2) Vinuesa, Annu Rev Immunol. Follicular helper T cells. 2016 34:335-68.

3) Zhang, Blood. Abnormalities of follicular helper T-cell number and function in Wiskott- Aldrich syndrome. 2016 23;127(25):3180-91