Title:	**Targeting the lymphatic system for modulating immune rejection and promoting tolerance in vascularized composite allotransplantation**

Summary: Vascularized composite allotransplantation (VCA) is emerging as a treatment option for patients suffering from limb loss or severe disfigurement. Although good functional and aesthetic outcomes can be successfully and routinely achieved after VCA, the need for long-term and high-dose immunosuppression remains a pace-limiting obstacle to widespread application of this "life-enhancing" rather than "life-saving" approach. Unlike solid organ transplants, VCA grafts are composed of several tissues (skin, muscle, bone, etc.). Each of these tissues has a unique biology with a specific response after transplantation. Mounting evidence suggests that the lymphatic system plays an active role in modulating inflammation, autoimmune disease and the organ immune-rejection process. However, little is known about the process of lymphatic reconstitution and how this process influences graft rejection in VCA. More importantly, the possibility to use lymphatic-targeted therapies for promoting VCA tolerance is completely unexplored. Therefore, in this project we aim to specifically target lymphatic vessels and lymph nodes to reduce tissue inflammation and induce allograft tolerance. We hypothesize that lymphangiogenesis and the transfer of manipulated donor lymph nodes may be used to promote inflammation clearance, expansion and maintenance of donor-specific regulatory cells and, thus, resolution of organ-specific immunity and establishment of peripheral VCA tolerance. We will test our hypothesis in a rat model of hind-limb transplantation. After the operation, the student will monitor the animals and evaluate graft survival. Several immunological parameters (e.g., presence of donor lymphocytes, number and phenotype of T-regulatory cells, presence of donor specific antibodies, lymphatic vessel reconstitution) are analyzed by flow cytometry in peripheral blood and tissues (i.e., skin, bone marrow, spleen), and *in situ* by immunofluorescence and immunohistochemistry analysis. Lymphatic reconstitution will be monitored *in vivo* using near-infrared lymphangiography. Mixed lymphocyte is preformed in order to analyzed the induction of tolerance to alloantigen. The effect of the therapies is also evaluated by analysis of complement and immunoglobulin deposition in the tissues by immunofluorescence and by analysis of the plasma and tissue levels of inflammatory and anti-inflammatory cytokines using Luminex multiplex.

Requirements: Students selecting this module are interested both in transplantation immunology and in getting an introduction to state of the art methodologies for assessing graft survival and for characterizing the immunological response to vascularized allograft. The topic involves animal experimentation as well as organ experiments.

Literature: Olariu et al. Intra-graft injection of tacrolimus promotes survival of vascularized composite allotransplantation. *Journal of Surgical Research*, 218, 49-57
Gajanayake et al. Sci. Transl. Med. 2014; 6, 249ra110

Time-slots & # of students:	Elective module series I :	1 student
	Elective module series II:	1 student

Location: Department for BioMedical Research, Murtenstrasse 50, CH-3008 Bern

Contact: Dr. Adriano Taddeo
phone: 031 632 02 99 **e-mail**: adriano.taddeo@dbmr.unibe.ch