April 30th, 2025

Project Review Board for Serbian Clinical Immunology Fund

c/o Professor Sophie Hambleton

Newcastle University

Newcastle upon Tyne, UK

Report to the Serbian Clinical Immunology Fund Selection Panel

Project title:

Peripheral blood ILC3 as a prognostic marker for metastatic breast cancer

Principal investigator:

Marija Mojić, PhD, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia.

The **aims** of this study were:

1) To investigate the correlation between the level of ILC3 in circulation and the development of metastasis

2) To explore whether the newly synthesized AhR antagonist can suppress the activity of peripheral ILC3 in vitro.

The first of the project's aims is being successfully performed. We are nearing completion of our assessment of ILC3 levels in the blood of 15 breast cancer patients at the time of diagnosis. To date, we have collected peripheral blood from 14 newly diagnosed female breast cancer patients aged 27-60 years. Of these, seven were diagnosed with oestrogen receptor-positive breast cancer and seven with triple-negative breast cancer. Additionally, we have collected peripheral blood from 10 age-matched healthy controls without immune-related conditions or cancer. The ILC3 cells were immunophenotyped, and their levels were assessed using antibodies funded by the Serbian Clinical Immunology Fund: CD127 Monoclonal Antibody, APC-eFluor™ 780, eBioscienceTM; CD117 (c-Kit) Monoclonal Antibody, PE-Cyanine7 eBioscienceTM; CD294 Monoclonal Antibody, APC, Invitrogen. Moving forward, we will monitor breast cancer patients over the next five years to correlate their ILC3 levels at diagnosis with the subsequent development of metastases.

In addition, we assessed the levels of the other two ILC subsets, ILC1 and ILC2, and observed the redistribution of all ILC subsets in the peripheral blood of breast cancer patients. To further explore the mechanisms driving this redistribution, we utilized two animal models of breast cancer: orthotopically induced 4T1 tumours in BalbC mice (a mouse model for triple-negative breast cancer) and EO771 tumours in C57BL/6J mice (a mouse model for oestrogen receptor- positive breast cancer).

The results from breast cancer patients and mouse models were presented at the **ILCollnet**, the ILC Study Group meeting at the EFIS (European Federation of Immunological Societies), held on April 25th in Belgrade. **One research paper and one review paper from the ILC field are currently in preparation**.

We could not explore the project's second aim: testing whether the newly synthesized AhR antagonist can suppress the activity of peripheral ILC3 in vitro. The awarded funds (£1500) did not cover the cost of the IL-22 Human Uncoated ELISA Kit (Invitrogen), which is essential for this part of the study.