Final Report Short-term Research Fellowship

I. Type of Fellowship, duration and location.
   - **Type of Fellowship:** short-term research Fellowship.
   - **Title of the project:** “Targeting rheumatoid arthritis with monoclonal antibodies derived from single synovial B cells: how to improve diagnosis and therapy”.
   - **Research Fellow:** Dr Elisa Corsiero, PhD
   - **Host supervisor:** Dr Ilaria Puxeddu, MD, PhD
   - **Duration:** 3 months (01/04/2017 – 30/06/2017)
   - **Location:** Clinical Immunology and Allergy Unit, Department of Clinical and Experimental Medicine, University of Pisa, Pisa (Italy)

II. What questions were addressed and why?
In my previous work (1), I have demonstrated that NETs can be the source of citrullinated antigens (i.e., histones H2A and H2B) targeted by recombinant monoclonal antibodies (rmAbs) generated from single synovial tissue B cells from RA patients. However, only 40% of the RA-rmAbs recognize NETs-derived antigens. Thus, the purpose of this project was to address whether, in addition to neutrophils extracellular traps (NETs), other cells can be the source of autoantigens in rheumatoid arthritis since for 60% of the RA-rmAbs the reactivity was unknown.

III. What was the nature of the research?
Preliminary data revealed that the RA-rmAbs can target RA fibroblast-like synoviocytes (FLS) (data not shown). The project aimed to characterise the fine epitope specificity and affinity of the RA-rmAbs towards a specific protein named calreticulin (CRT) – a protein expressed in RA-FLS, as a potential novel autoantigen in RA. In particular, during the Research Fellowship, the main aim was to screen all the RA-rmAbs by means of immunoenzymatic assays (i.e., ELISA tests) using both the native and citrullinated form of CRT as well as native/citrullinated peptides spanning the whole CRT protein.

IV. What was the results?
First, commercial recombinant CRT was citrullinated in vitro using rabbit skeletal muscle PAD enzyme and the level of citrullination was checked by Western blot analysis with an anti-citrulline detection kit (data not shown). Both the native and citrullinated peptides were synthetized by EspiKem Srl (Italy). Thus, all the RA-rmAbs (n=71) were screened in ELISA towards native and citrullinated CRT protein and peptides. As shown, in Fig.1, a small number of RA-rmAbs showed a reactivity towards CRT protein in a dose-dependent manner. Such
reactivity was increased towards the citrullinated form of CRT (Fig.1). Regarding the reactivity towards CRT peptides, preliminary data showed that the RA-rmAbs have a preferential binding towards the N-terminal part of CRT protein (data not shown). Further tests need to be performed in order to identify the specific peptides that will be used for affinity binding analysis by means of surface plasmon resonance test using a Biacore platform.

![Fig.1. CRT ELISA assay with the RA-rmAbs.](image)

V. How will the findings impact future research?
The aim of this project was to characterise new possible autoantigens in RA, thus potentially this work could translate basic research into novel diagnostics and in the long-term into new therapies for RA patients.


Overall, although the time spent in the Department of Clinical and Experimental Medicine hosted by Dr Puxeddu was limited, this experience has been extremely useful and rewarding as I had the chance to work in a highly reputable and internationally recognised environment and setup all the conditions in order to achieve all the objectives set in the application and to work independently. Moreover, these three months paved the way for further collaboration with the group who hosted me. Last but not least, I would like to thank the European Academy of Allergy and Clinical Immunology (EAACI) for supporting my research with this short-term Research Fellowship.