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BASTION - FROM BASIC TO TRANSLATIONAL RESEARCH IN ONCOLOGY

Report on the stay of Dr. Malgorzata Bajor at the Department of Oncology, Molecular Medicine Laboratories, Beaumont Hospital, Royal College of Surgeons, Dublin, Ireland within the 7PR21/BASTION/WP1 (Twinning)

Between 30th June and 13th July 2014 I had visited Laboratory of Prof. Bryan Hennessy, working at the Department of Oncology, Molecular Medicine Laboratories, Beaumont Hospital, Royal College of Surgeons in Ireland. Prof Hennessy is an international leader in the application of reverse phase protein arrays (RPPA) for quantitative protein profiling to interrogate predictive and prognostic markers in breast, colon and other cancers, and has established this technology at RCSI. Prof. Hennessy is a clinician scientist whose research team has had considerable impact on the fields of kinase signaling and 'BRCAness' research in cancers including gynecological and breast cancers. He is a consultant of the medical oncology at Beaumont Hospital, senior lecturer in the RCSI and adjunct professor in the Division of Cancer Medicine at the University of Texas, M.D. Anderson Cancer Center.

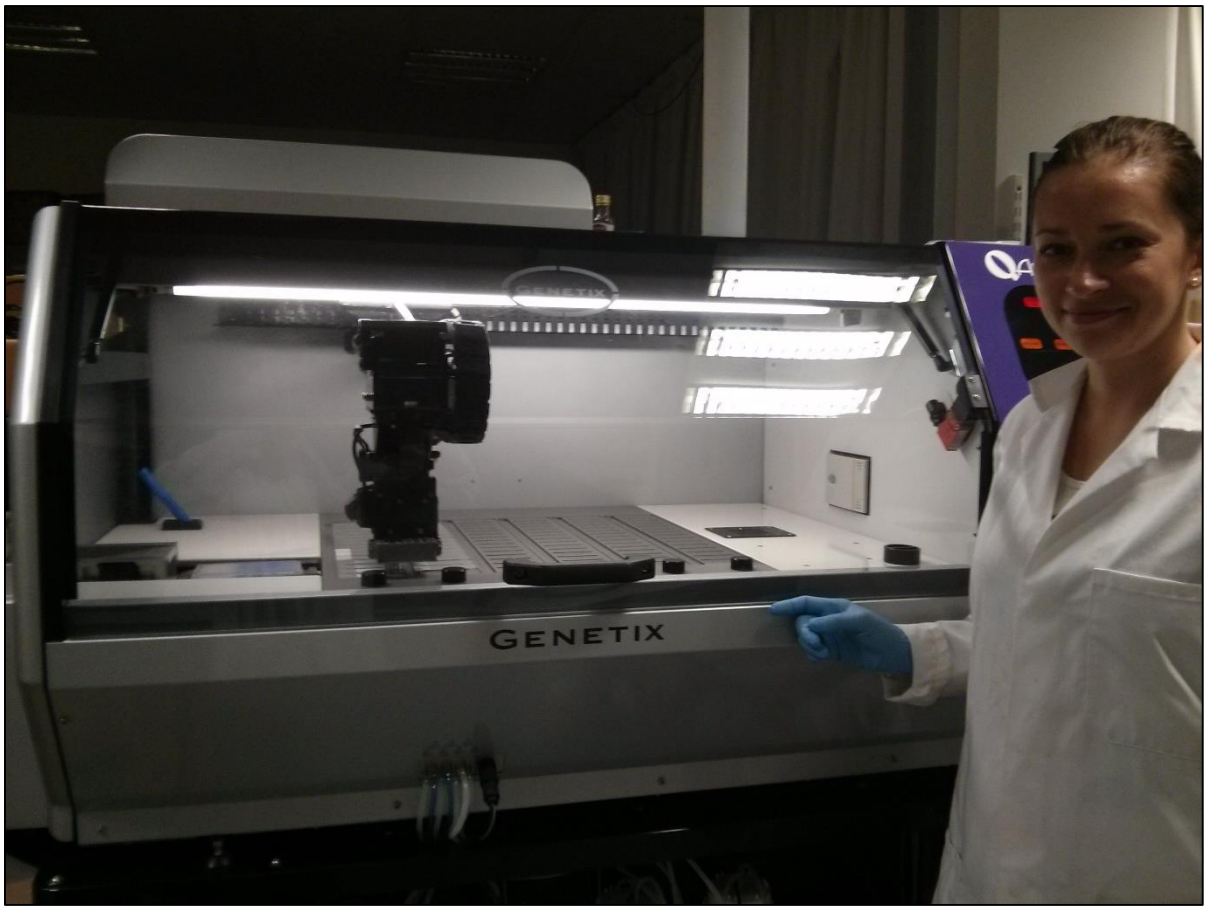
The main goal of my two-weeks stay in Dublin was to use RPPA platform to study PRDX1 and PRDX2 protein expression in panel of different type cancer cell lines.

RPPA is a high-throughput antibody based technique. In general, this technique allows for studying a large scale changes in protein levels at different stages of development and progression of the disease including cancer. The RPPA platform at the RCSI allows for analysis of >300 samples using at least 50 different antibodies. RPPA utilizes automation for increasing quality and reliability, sample preparation are similar to those of Western blot, complete assay requires only 40 μg of each sample for ≥ 50 antibodies and allows for robust quantification due to serial dilution of samples. Using RPPA approach

cellular protein activity in signaling networks may be analyzed, this technique can be applied to normal or tissue, and also cultured cells.

With help of Prof Hennessy group members, Clare Morgan and Dr Mattia Cremona, I had an opportunity to learn RPPA method, starting from sample preparation, printing denatured protein lysates on nitrocellulose-coated slides, followed by probing with appropriate antibodies to detect signal by colorimetric reaction. Moreover, I actively participated in analysis of data obtained from RPPA platform and I was able to improve my knowledge on data analysis obtained from RPPA experiments.

Using RPPA method I have determined the levels of PRDX1 and PRDX2 proteins in breast cancer cell lines. Moreover, I performed the RPPA analysis for other types of cancer cell lines including lymphoma, leukemia, myeloma, and ovarian cancer cell lines. These analyses were performed in collaboration with Dr Dominika Nowis, member of Bastion project team.



Dr Bajor at the RPPA Facility at the Royal College of Surgeons in Ireland during printing protein lysates on nitrocellulose-coated slides.