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**BASTION - FROM BASIC TO
TRANSLATIONAL RESEARCH
IN ONCOLOGY**

Report from the participation in the training-workshop “Isolation and molecular characterisation of cancer-derived exosomes”, 13 - 19 July 2014 at the University of Pittsburgh Cancer Institute in Pittsburgh, PA (United States) – Małgorzata Czystowska-Kuźmicz

Between 13th July and 19th July 2014 I took part in the training-workshop “Isolation and molecular characterization of cancer-derived exosomes” organized by the research laboratory of prof. Theresa Whiteside at the University of Pittsburgh Cancer Institute.

Professor Whiteside is a professor of Pathology, Immunology, and Otolaryngology, a member of the University of Pittsburgh Cancer Institute and a recognized expert in immune monitoring of patients with cancer. Her scientific group is on the leading edge of research in mechanisms of tumor escape from the host immune system and the development of therapies designed to eliminate tumor escape. One of the research interests of the group is the contribution of tumor-derived exosomes to tumor-induced immunosuppression and their role as potential markers of prognosis and response to therapy in patients with cancer. The group is proficient in isolating exosomes from cancer cell lines and is currently developing new methods for isolating exosomes from patients' biological material.



The main topics of the workshop were methods of exosome-isolation from biological fluids. Different optimizations of the isolating procedures for adequate recovery of exosome fractions free of contaminating larger vesicles, cell fragments and protein/nucleic acid aggregates were presented and the pitfalls and advantages discussed. Methods of exosome quality control (Western blotting, transmission electron microscopy) were presented – specially the measurement of concentration and size distribution by a



novel nanoparticle analyzing system using the qNano instrument was discussed and practically trained. Recovery losses of exosomes during isolation due to their tendency to form aggregates of varying sizes were addressed and possible problem solutions discussed. Exosome recovery from fresh versus frozen/thawed plasma or from plasma versus serum were compared and the effects of ultrafiltration and size-exclusion discussed.



Dr. Czystowska-Kuźmicz, Prof. Whiteside and some of the workshop's participants

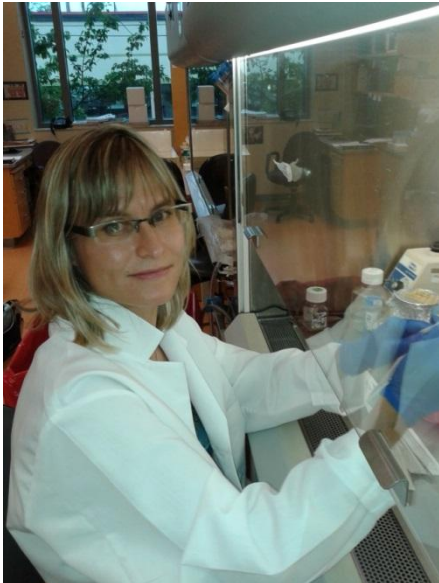
Workshop schedule:

Date	activity	Supervisor
Sunday 13.06.2014	Flight from Warsaw, arrival in Pittsburgh	
Monday 14.07.14	Presentation: "Isolation of tumor exosomes from biological fluids-pitfalls and troubleshooting." Practical training: Isolation of exosomes from human plasma – ultrafiltration, size-exclusion chromatography, ultracentrifugation	Dr. Laurent Muller
Tuesday 15.07.14	Practical training: Analyzing exosomes using the NanoSight instrument, sucrose density gradient of exosomes functional studies of exosomes: co-incubation with activated T-cells, NK cells	Dr. Laurent Muller Chang-Sook Hong
Wednesday 16.07.14	Practical training: Immunocapturing of exosomes from human plasma using antibody-coated beads	Dr. Laurent Muller
Thursday 17.07.14	Practical training: analyzing exosome-coincubated immune cell by flow-cytometry (apoptosis, activation marker)	Dr. Laurent Muller
Friday 18.07.14	Presentation: "Molecular profiling of melanoma derived exosomes and its clinical significance." Practical training: analyzing exosome-coincubated immune cell by flow-cytometry (apoptosis, activation marker) Presentation: Proteomic analysis of exosomes: protein extraction and mass spectrometry analysis	Dr. Laurent Muller, John M. Kirkwood, MD, Theresa L. Whiteside, Michael Boyiadzis

Saturday
19.07.14

Flight back to Poland

The participation in this workshop is very valuable for my future research. Since I recently received a grant on a project concerning the immunomodulatory role of tumor-derived exosomes from ovarian cancer patients (NCN grant: “Elucidation of the role of tumor-derived and exosomal arginases in avoiding immune responses by ovarian cancer”) the knowledge and skills I gained through this workshop will be very useful for me and will help me to plan my future experiments efficiently. Apart from the valuable practical training and troubleshooting tips I received from Dr. Laurent Muller, the workshop gave me opportunity to broad discussions about possible clinical significance and translational capacity of tumor-derived exosomes during presentations and meetings with other researchers from UPMC. chromatography on isolated exosomes were presented. Methods of immunocapturing exosomes from human plasma on the example of melanoma were presented and discussed. Finally, the biological activity of the isolated exosomes was practically verified. Different in-vitro functional assays with exosomes were discussed and practically trained.



Dr. Czystowska-Kuźmicz in Prof. Whiteside's laboratory



The qNano instrument for measurement of exosome concentration and size distribution