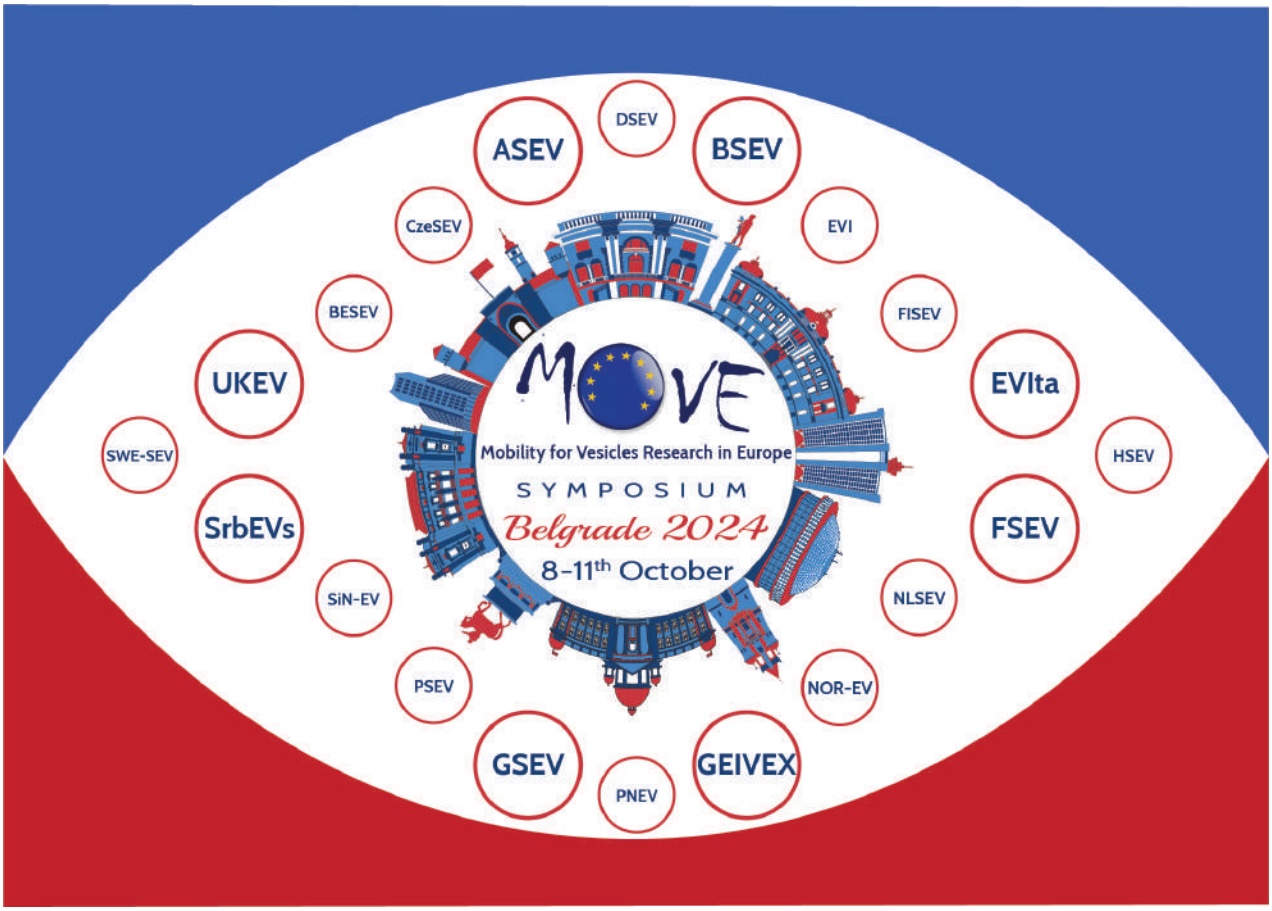
2nd MOVE Symposium



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European National Societies for Extracellular vesicles

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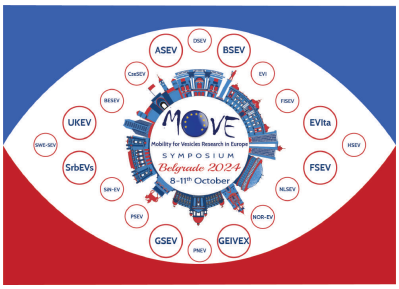

2nd MOVE Symposium

8-11 October 2024, Belgrade, Serbia

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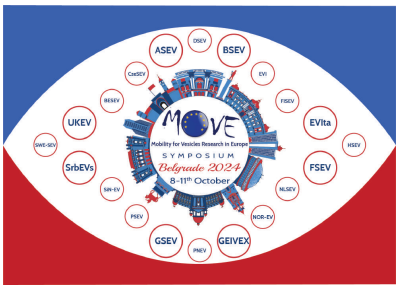

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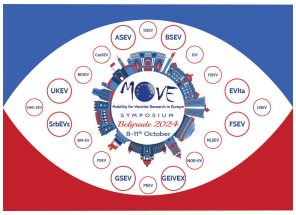

O-N-4

Isolation of extracellular vesicles from resistant tumor cells using nanobodies-based immunoaffinity approach

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Introduction: Extracellular vesicles (EVs) are an important contributing factor to drug resistance in cancer. In order to study their features and elucidate their molecular composition. To that extent, we have decided to use two pairs of multi-drug resistant (MDR) cancer cell lines (non-small cell lung carcinoma NCI-H460/R and glioblastoma U87-TxR) and their sensitive counterparts (H460 and U87, respectively ion and contribution in drug resistance, EVs need to isolated in an efficient manner and sufficient quantities. Broadening new possibilities in EV-based diagnostics requires innovative, adaptable, and affordable methods for the scalable isolation of high-purity EVs from different sources. This study aims to adapt high-performance immune capture chromatography based on nanobody technology for EVs isolation from cell culture media of MDR cancer cells and their sensitive cells.

Methods: The nanobodies utilized in this study were selected from a heavy-chain only-VHH library by direct panning against EVs and generated in *E. coli* with eGFP and a 6xHis tag. To isolate EVs, purified VHHs-GFP were immobilized on polymethacrylate polymer to create immunoaffinity capture. Isolated vesicles have been characterized by a set of biochemical and instrumental techniques (colorimetric sulfophosphovanilin-SPV assay, BCA assay, Flow cytometry, and Nanoparticle tracking analysis).

Results: The combined analysis of proteins, lipids, and flow cytometry analysis of three tested biomarkers (CD9, CD63, and CD81) showed that we successfully isolated EVs from both pairs of cancer cell lines. The detergent control (TRITON X-100) for biomarkers analysis showed reduced signal, thus confirming the presence of lipid-origin structures. The NTA analysis showed that MDR cancer cells produced EVs with a bigger diameter.

Conclusion: This study demonstrates the application of spherical porous methacrylate-based polymer coupled with VHHs for the purification of EVs from MDR cancer multi-drug-resistant cells. This inexpensive, relatively fast, and easy-to-perform method has great potential for the isolation of different classes of EVs from various biological sources.

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