ImmunoTools *FlowISiAM* Award 2026

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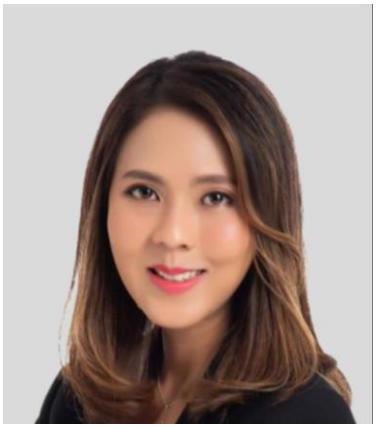

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Elucidating the intracellular proteins in activated circulating monocytes as potential immune regulatory biomarkers for the differential diagnosis of osteoarthritis and osteosarcoma – Immunophenotyping using *FlowISiAM* and ImmunoTools *AMANDUS-PrexRegenOA* platform

Osteoarthritis (OA) and osteosarcoma (OS) are distinct musculoskeletal disorders that often present with overlapping clinical features, making early and accurate diagnosis challenging. While OA is a degenerative joint disease characterized by chronic inflammation and cartilage breakdown, OS (i.e. proximal tibial OS) is a highly aggressive malignant bone tumour requiring prompt intervention. Current diagnostic approaches rely heavily on imaging (i.e., radiographs) and histopathology, which may not always enable timely differentiation, particularly in early or ambiguous cases. Although OA predominantly affects older individuals, osteosarcoma OS is more common in adolescents and young adults; overlapping clinical presentations such as joint pain and functional impairment can complicate initial clinical assessment, particularly in atypical cases. Therefore, there is a critical need for minimally invasive and reliable biomarkers to improve diagnostic precision.

Circulating monocytes play a pivotal role in immune regulation and inflammatory responses, and their activation states reflect underlying pathological conditions. Intracellular protein staining within these cells may provide valuable insights into disease-specific immune signatures. This study aims to elucidate the intracellular protein profiles of activated circulating monocytes as potential immune regulatory biomarkers for distinguishing OA from OS. By employing advanced immunophenotyping techniques, including *FlowISiAM* and ImmunoTools *AMANDUS*, this research aims to identify novel circulating cellular biomarkers that can enhance differential diagnosis and contribute to more targeted clinical decision-making. Establishing robust biomarker frameworks could transform OA and OS clinical management and improve patient outcomes in orthopaedics. The blood samples collected will be analyzed using the *FlowISiAM* AT-test and ImmunoTools *AMANDUS-PrexRegenOA* platform (precision regenerative medicine Orthopaedics Advanced profiling tool for Orthopaedic diagnosis).

Experimental Design

Ethical approval from the UMMC Medical Research Ethics Committee (MREC) will be granted. We will analyze a cohort of patients (n = 120) from our Orthopaedic clinic with a diagnosis of osteosarcoma (OS) and osteoarthritis (OA). A total of 3 ml of whole blood will be collected in EDTA blood collection tubes from the consented subjects. Non-OA and non-OS subjects from the Sports Orthopaedics Clinic will be recruited as the control group. The whole blood will be analyzed using *FlowISiAM* AT-test and ImmunoTools *AMANDUS-PrexRegenOA* platform, with the flow cytometer.

Fragments of TKTL1 and DNaseX (Apo10) in circulating monocytes and macrophages will be detected using the *FlowISiAM* AT-test. Alterations in these biomarkers may indicate early metabolic reprogramming, malignant transformation, or imminent disease progression.

The immune cell subsets will be characterized via immunophenotyping analysis using the ImmunoTools *AMANDUS-PrexRegenOA* platform. Changes in these cell subsets reveal different

clinical conditions, i.e. OA and OS, as well as progression of these disease conditions. The results obtained will be most accurate compared with the final diagnosis from the diagnostic lab. Moreover, tissue and further blood samples will be processed and stored in the NOCERAL for subsequent analyses on further solid and liquid biomarkers.

Impact: This study will provide novel insights into whether phagocytosed epitopes from pre-selected biomarkers can be detected by *FlowISiAM* analysis and how they correlate with the diagnosis and prognosis of OS. In addition, we look forward to validating single-cell analysis results of OA-related markers by *FlowISiAM* in peripheral blood monocytes/macrophages. These validation findings will serve as a foundation for a novel blood-based test concept for early diagnosis of OA.

Cooperation partner: Dr Sik-Loo Tan of the NOCERAL research group will work together with ImmunoTools and INVIGATE to adjust the experimental and instrumental setup to conduct *FlowISiAM* analysis. Furthermore, ImmunoTools *AMANDUS* will support some mAbs to antigens specific for cancer, musculoskeletal tissues, mesenchymal stem cells (MSCs), as well as other antibody reagents for *FlowISiAM* analysis. Dr. Sik Loo Tan intends to intensify the findings from this study and further expand the work on the development of optimized monoclonal antibodies for the detection of OA and OS, as well as inflammation-related molecular signatures within the *FlowISiAM* setting.

ImmunoTools *FlowISiAM* AWARD for

Sik-Loo Tan, Seow-Hui Teo, Nor Faissal Yasin and Azlina Amir Abbas includes antibodies for *FlowISiAM* and *AMANDUS*-*PrexRegenOA*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.

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