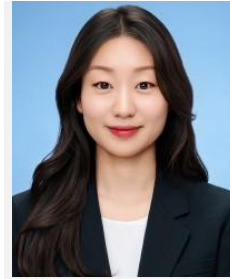


ImmunoTools *FlowSiAM* Award 2026



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Determining the role of macrophage subtypes in cell death and their significance in melanoma therapy

Background: Metastatic cutaneous melanoma responds heterogeneously to immune-checkpoint blockade. Macrophages can support anti-tumour immunity or promote immune escape and treatment resistance, but clinically useful blood indicators of their activation and cell-death regulation are lacking.

Problem statement: Current approaches rarely capture the transient intracellular programmes that define activated monocytes and macrophage precursors. Surface phenotyping cannot distinguish states that promote productive tumour-cell death from those linked to dysfunctional apoptosis, inflammatory cell death or therapy resistance. A minimally invasive functional assay is needed.

General objective: To identify, in mouse melanoma models, macrophage/monocyte signatures associated with cell-death regulation and validate the most promising blood-based markers in melanoma patients using *FlowSiAM*.

Specific objectives:

1. Define tumour, blood, and spleen myeloid subsets in patients with melanoma using CD14, CD16, CD11b, MHC-II, CD86, and CD206 to identify monocyte and macrophage states.
2. Characterize the macrophage- or tumor-derived exosomes/extracellular vesicles (EVs).

3. Measure intracellular TKTL1 (glycolytic reprogramming), cleaved caspase-3 and DNaseX/Apo10 (apoptosis), and exploratory gasdermin-D and phospho-MLKL (pyroptotic/necroptotic signalling).
4. Test whether these signatures change with melanoma growth and anti-PD-1-based treatment, and relate them to tumour-cell death and treatment response.
5. Translate the prioritized markers to a human *FlowSiAM* and *AMANDUS* panel (CD14, CD16, HLA-DR, PD-L1, CD163, CD95, CD38 and CD71)

Methodology:

- i) Peripheral blood from melanoma patients: Peripheral blood (3-5 mL EDTA) will be obtained from melanoma patients starting systemic treatment and age-matched healthy controls through an ethics-approved clinical-study/biobanking pathway with written informed consent. PBMCs will be assessed with the human panel; paired pre-treatment and early-treatment samples will test translational relevance.
- ii) Isolation of EVs: EVs will be isolated from plasma. Also, in the culture of human monocytes isolated from PBMCs, or human melanoma cells will be subjected to different modes of cell death. EVs will be characterized using established markers such as CD63, CD81, CD9, CD68, CD169, HLA-DR, ALIX and TSG101.
- iii) *FlowSiAM*-compatible intracellular staining will quantify activation, metabolic, and cell-death programmes in monocytes and tumour-associated macrophages.
 - Monocyte/macrophage subsets: CD14, CD16, CD56,
 - Apoptosis marker: CD95 (Fas)
 - Metabolic and redox markers: CD38, CD71, CD98, CD36, CD39, CD73, CD147, CD163, CD91, CD204

Expected outcomes: We expect to identify a reproducible myeloid phenotype in mouse melanoma that is linked to apoptosis dysregulation, inflammatory cell death or metabolic adaptation, and to validate its most informative blood-accessible components in patients. This staged approach will yield a translational signature for mechanistic research, risk stratification and immunotherapy monitoring. Prioritised epitopes will guide future *FlowSiAM* panels, macrophage-reprogramming studies and antibody development.

Collaboration plan: **ImmunoTools** will contribute from project initiation by providing *FlowSiAM* antibodies, supporting *AMANDUS* panel design for human surface marker, optimising fixation and permeabilisation for intracellular human epitopes, transferring robust protocols, and assisting with data analysis. INVIGATE will join

after target prioritisation to evaluate and develop antibodies against the highest-priority intracellular human epitopes. Joint reviews after assay establishment and patient validation will refine the panel and create a strong basis for joint follow-on funding.

ImmunoTools *FlowISiAM* AWARD for **Rajendra Karki** and **Ein Lee** includes antibodies for *FlowISiAM* and *AMANDUS*, 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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