ImmunoTools *FlowISiAM* Award 2026

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Identification of Monocyte-Derived Biomarkers for Early Detection, Proton Therapy Prognosis, and Treatment Stratification in Childhood Neuroblastoma

Background

Neuroblastoma is the most common outside-the-brain solid tumor in children, making up about 8–10% of all childhood cancers and causing around 15% of cancer deaths in kids under five [1]. It originates from neural crest progenitor cells and exhibits a wide range of clinical and biological differences, from spontaneous regression in infant disease to rapid, fatal progression in high-risk metastatic cases. [2]. Current diagnosis depends on imaging, histopathology, and bone marrow biopsy. Risk assessment follows the International Neuroblastoma Risk Group Staging System (INRGSS), which uses clinical features and, as its only molecular markers, MYCN amplification, 11q chromosomal changes, and DNA ploidy [3]. Despite undergoing comprehensive multimodal treatment including induction chemotherapy, surgery, high-dose consolidation therapy, and anti-GD2 immunotherapy, more than half of patients with high-risk disease still do not survive five years [2, 3]. There is a pressing and unmet need for minimally invasive, blood-based biomarker methods that can detect neuroblastoma early, predict how treatment will go, and help guide personalized treatment choices.

A key characteristic of the neuroblastoma microenvironment is the extensive presence of macrophages and myeloid-lineage cells, whose functional polarization profoundly influences both tumor progression and response to response [4]. Tumor-associated macrophages in neuroblastoma mainly assume immunosuppressive M2 phenotypes. These are characterized by high expression levels of CD163, CD206, or MRC1, MerTK, and intracellular IL-10. They actively support processes such as angiogenesis, extracellular matrix remodeling, and immune evasion. [4]. Conversely, M1-polarized macrophages that express CD80, HLA-DR, and pro-inflammatory cytokines can directly mediate anti-tumor cytotoxicity. High infiltration of M2-TAMs is linked to poor event-free and overall survival in neuroblastoma [4, 5]. Importantly, these polarization dynamics are not limited to the tumor site. Circulating peripheral blood monocyte subsets, including classical CD14-positive CD16-negative, intermediate CD14-positive CD16-positive, and non-classical CD14-low positive CD16-positive, undergo both quantitative and functional changes. These changes reflect the systemic immune dysregulation seen in pediatric cancer patients, making circulating monocytes and macrophages useful surrogates for studying the tumor microenvironment.

Liquid biopsy techniques using cell-free DNA, circulating tumor DNA, circulating tumor cells, and exosomes have been tested in neuroblastoma. They show increasing potential for noninvasive disease monitoring and relapse detection [1]. However, technical complexity, low analyte concentrations, and the severely limited blood volumes obtainable from pediatric patients present significant barriers to widespread clinical use. The Epitope Detection in Monocytes (EDIM) principle offers an innovative alternative: after phagocytosis of tumor-derived material, circulating $CD14^{+}/CD16^{+}$ monocytes and macrophages exhibit disease-specific intracellular antigens that can be detected by flow cytometry using only 100 microliters of whole blood [6, 7]. Stagno and colleagues were the first to demonstrate that this approach is feasible in pediatric neuroblastoma. They detected the neuroblastoma-associated disialoganglioside GD2 within blood macrophages, along with TKTL1, a marker of Warburg-effect glycolytic reprogramming, and Apo10 or DNaseX, an epitope reflecting dysregulated apoptosis. This was achieved using the EDIM method in a pilot cohort of children [6]. GD2 is prominently expressed on the surface of neuroblastoma cells, making it a proven therapeutic target and an accessible tumor-associated antigen suitable for EDIM-based detection [8]. These data provide compelling proof-of-concept for a macrophage-centered liquid biopsy strategy in childhood neuroblastoma.

Building on this foundation, the present study will implement the *FlowISiAM* platform, which stands for Flow cytometry-based Intracellular Staining in Activated Monocytes or Macrophages. This platform is an advanced, customizable evolution of the EDIM principle developed by ImmunoTools GmbH. Its goal is to establish a comprehensive biomarker panel for monocytes and macrophages tailored for three urgent clinical applications. The first is early and non-invasive detection of neuroblastoma in children who are newly diagnosed. The second involves monitoring treatment response and stratifying prognosis during proton beam radiotherapy. The third aims to stratify biological risk by correlating circulating immune

phenotypes with established molecular markers such as MYCN, 11q, and ploidy. Proton therapy has become increasingly preferred for pediatric neuroblastoma because of its superior dose conformality and reduced dose to healthy, developing tissues. However, there is currently no blood-based biomarker available to guide adaptive treatment decisions or predict outcomes in children receiving proton therapy [5, 9]. This study will directly address that gap.

Experimental Design

Peripheral whole blood (3 mL per collection, EDTA anticoagulation) will be collected from three groups at Taipei Medical University Hospital (TMUH). These include a healthy pediatric control group of age-matched individuals, a newly diagnosed neuroblastoma group of patients sampled before any treatment to evaluate early detection sensitivity and specificity and for risk group stratification, and a longitudinal proton therapy group of children undergoing curative-intent proton beam radiotherapy. The latter will be sampled at four time points: before treatment, midway through treatment, at the end of treatment, and three months after. All participants will provide ethical approval and written informed consent from guardians before sample collection. Samples will be processed within six hours of venipuncture following the *FlowISiAM* standard operating protocol provided by ImmunoTools. The ImmunoTools *AMANDUS* panel will provide some integrated immune phenotyping of monocyte and macrophage subsets alongside the AT-test, using a multiparametric panel targeting: CD14, CD16 (monocyte subset classification), CD163 and CD206/MRC1 (M2-polarisation markers), CD68 (pan-macrophage), CD33 (myeloid lineage), CD38, CD95/Fas, and CD71/TfR1 as ROS-sensitive and activation-state markers, together with intracellular IL-10 as a readout of immunosuppressive macrophage programming. Polarization indices (M1:M2 ratio; $CD163^{+}CD206^{+}$ monocyte frequency; $IL\text{-}10^{+}$ monocyte proportion) will be calculated and correlated with: INRGSS risk group, MYCN amplification status, 11q chromosomal aberration, tumor ploidy, proton therapy dose-volume histogram parameters, RECIST-based or MIBG response assessment at end-of-treatment, event-free survival, and overall survival. This integrated analytical framework will enable the identification of predictive biomarker signatures linking circulating immune phenotypes to treatment outcomes.

Expected Outcomes

This study aims to define a minimally invasive, blood-based biomarker panel capable of distinguishing neuroblastoma patients from healthy controls at diagnosis, differentiating biological risk groups, and dynamically monitoring treatment response to proton therapy using only 100 microliters of peripheral blood. We expect that intracellular GD2 positivity in CD14-positive and CD16-positive monocytes will serve as a sensitive and specific marker of

active neuroblastoma burden, with levels correlating with disease stage and MYCN amplification status. The combined increase of TKTL1 and Apo10 in circulating monocytes is anticipated to reflect tumor metabolic activity and immune evasion capabilities, providing additional prognostic information beyond current molecular markers. An immunophenotype at diagnosis characterized by a high frequency of CD163-positive and CD206-positive monocytes and elevated intracellular IL-10 is expected to be associated with high-risk INRGSS classification and poorer treatment response, consistent with the established role of immunosuppressive tumor-associated macrophages in neuroblastoma biology [4].

For children undergoing proton beam therapy, we expect that sequential *FlowISiAM* profiling will reveal dynamic immune signatures that precede conventional radiological assessment: declining GD2, TKTL1, and Apo10 signals during treatment will serve as early indicators of effective tumor cell kill, while persistence of an M2-polarized monocyte phenotype post-treatment will identify patients at elevated risk of relapse who may require treatment intensification. Ultimately, this work will establish the first clinically validated *FlowISiAM*-based liquid biopsy framework for childhood neuroblastoma, providing a translational roadmap for developing a pediatric neuroblastoma monitoring kit and for integrating immune phenotyping into risk-adaptive treatment protocols.

Research Team and Complementary Expertise

Professor Lucas A. Lane, who leads the development of the ImmunoTools *FlowISiAM* platform and biomarker analytics at TMU, has internationally recognized expertise in nanomedicine, cancer diagnostics, and the biology of tumor-associated macrophage reprogramming. He earned his PhD in Bioengineering from Georgia Tech in 2014 and completed postdoctoral work in advanced nanoparticle engineering. In 2022, Professor Lane joined Taipei Medical University, where his lab develops innovative biosensing platforms, including Surface-Enhanced Raman Scattering nanotags, and stimuli-responsive nanocarriers. These tools aim to enable ultra-sensitive detection of intracellular cancer biomarkers and to support targeted cancer therapy involving macrophages. His recent research focuses on macrophage polarization as a key target for cancer diagnosis and treatment. He published a strategy that combines CRISPR/Cas9 and cuproptosis to exploit anticancer macrophage polarization via biomimetic nanocarriers, as well as an siRNA gene-silencing approach to improve photothermal cancer therapy. Professor Lane's extensive experience in intracellular target detection in immune cells, along with his active grants from the National Science and Technology Council on nano-biosensing and nanocarriers, positions him well to lead the establishment and enhancement of the *FlowISiAM* platform at TMU. He is also developing new antibody conjugate strategies to improve intracellular antigen detection in pediatric patient samples.

Dr. Hsin-Lun Lee is Co-Principal Investigator leading the clinical cohort and bringing expertise in proton therapy. He is an Associate Professor in the Department of Radiology and serves as the Director of the Division of Radiation Oncology at Taipei Medical University Hospital. Additionally, he works as a treating physician at the TMU Proton Center. Dr. Lee earned both an MD and a PhD in Translational Medicine from Taipei Medical University. He completed specialist fellowships in particle beam therapy at the National Institute of Radiological Sciences in Chiba, Japan; the Fox Chase Cancer Center in Philadelphia, USA; and the Hyogo Ion Beam Medical Center in Japan, which is one of the world's leading centers for proton and heavy-ion therapy. His current research focuses on translating radiosensitizing strategies into proton therapy. His published work includes studies on radiosensitizing nanoparticles for proton therapy, nanoradioenhancers targeting tumor metabolism in radioresistant cancers, and the combination of hyperthermia with proton therapy to address radioresistance. Dr. Lee directs a dedicated proton therapy program at TMUH, one of Taiwan's top cancer centers. This program provides the only essential resource for our project: a well-characterized, prospectively recruited cohort of pediatric neuroblastoma patients receiving standardized proton beam radiotherapy, with clinical, dosimetric, and outcome data. His translational focus, expertise in proton therapy, and access to pediatric oncology patients make him an invaluable clinical partner for this project.

Together, Lane and Lee form a complementary team that combines advanced immune biosensing technology with frontline pediatric proton therapy. This intersection is vital to the scientific and clinical goals of our study and is unique within the *FlowISiAM* network.

Collaboration Plan

The studies will be carried out with collaborative support from ImmunoTools GmbH and its partner INVIGATE in Friesoythe & Jena, Germany. This collaboration will provide validated antibody panels for establishing the *FlowISiAM* AT-test, including antibodies against TKTL1, Apo10/DNaseX, GD2, CD14, and CD16. It will also include an *AMANDUS* panel, technical training, protocol and know-how transfer, and guidance on selecting biomarker-specific antibodies for pediatric neuroblastoma targets. INVIGATE will support assay development, optimize the *FlowISiAM* AT-test protocol for pediatric whole blood samples, and assist in interpreting multiparametric flow cytometry data. Data analysis and integration into the ImmunoTools *FlowISiAM* network will be done jointly, aiming to contribute a pediatric oncology use case to the growing international research network. This partnership is intended to facilitate future international co-funded grant applications and support the development of a neuroblastoma-specific *FlowISiAM* testing kit that can be translated into clinical use in pediatric oncology centers.

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