ImmunoTools *FlowISiAM* Award 2024

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Unveiling the potential of activated monocytes and glycan biomarkers as theranostic tools for cancer and glycosylation rare diseases

This project presents an innovative approach to address critical gaps in understanding and treating diseases with altered glycosylation, focusing on pancreatic cancer and Congenital Disorders of Glycosylation (CDG). By investigating immune dysregulation in these conditions and identifying therapeutic targets through monocyte analysis and molecular biomarker exploration, we aim to improve disease detection, patient stratification and ultimately, treatment outcomes.

Both pancreatic cancer and CDG represent diseases with dysregulated glycosylation and compromised immune features, posing significant challenges in diagnosis and treatment. Pancreatic cancer prevalence is rising due to lifestyle; its aggressive nature and limited treatment options urge the need for early-stage detection of its onset. Specific short sialylated O-glycan identified by us, specifically in pancreatic cancer, were associated with poor prognosis and contributes to cancer progression and metastasis. Previous observations showed that these glycans inhibited activation of monocyte-derived dendritic cells and triggered an immunosuppressive loop driving carcinogenesis through its recognition by immunosuppressive lectins such as Siglecs. Yet, clinically relevant studies using meaningful tools to investigate immune response are needed. Understanding the immunosuppressive mechanisms driven by sialylated O-glycans offers opportunities for patient stratification and target interventions.

CDG, as rare chronic diseases, also present immunological defects, particularly in PMM2-CDG, emphasizing the need for targeted therapies. Previous studies have identified altered monocyte activation in PMM2-CDG patients, indicating a potential avenue for disease monitoring and intervention. Yet heterogeneity and rarity of information underscore the necessity to investigate immune dysregulation, potentially paving the way for targeted therapies.

Traditional disease diagnostics rely on invasive procedures, posing early detection and monitoring limitations. Yet, identifying biomarkers that inform on disease onset and

progression represents a breakthrough in disease detection. In this project, we envision that coupling the monitoring of short sialylated glycans and monocyte activation can leverage and refine the detection of diseases of glycosylation, using as prototype pancreatic cancer and CDGs.

We intend first to use *FlowISiAM* technology in a cohort of patients (minimum of 150 from cancer and 20 from CDGs) whose samples come from already approved collaboration agreements with several Hospitals and following ethical procedures already signed between the institutions and NOVA. Once we gather relevant information on the relevance of monocyte activation associated with cancer and CDGs, we intend to unravel new ways for disease monitoring and/or find new therapeutical targets for these and several other diseases of glycosylation (e.g. other cancer and CDGs).

Project Workpackages

1- Establish Disease Detection Test:

- Utilize glycan biomarkers for sensitive and specific detection of immune alterations in pancreatic cancer and CDG.
- Develop robust methodologies for flow cytometry analysis of blood monocytes to enhance detection accuracy.

2- Investigate Glycan Role in Disease Progression:

- Investigate the role of specific glycans, particularly STn, in cancer pathogenesis and progression.
- Explore the potential of glycans as druggable targets for targeted cancer therapy using target antibodies.

This interdisciplinary project bridges fundamental research with clinical applications, aiming to leverage immunological biomarkers, we seek to improve disease detection, patient stratification, and therapeutic interventions, ultimately enhancing patient outcomes and quality of life.

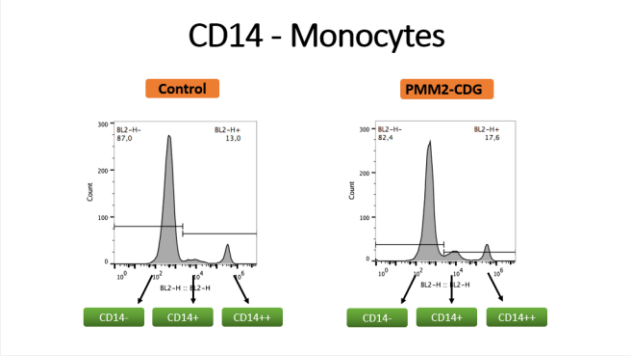

Figure legend: Monocyte phenotype of PMM2-CDG patients. Peripheral bood samples were analysed by flow cytometry to phenotype CD14 positive cells. The histogram shows an example assay (n=3) comparing blood samples of controls versus PMM2-CDG patients. Samples were stain ed with anti-CD14 antibodies, and the image shows differential distribution of $CD14^{+}$ and $CD14^{++}$ populations.

The project addresses pertinent medical problems with significant implications for public health. Pancreatic cancer, while not as prevalent as some other cancers, is one of the deadliest,

with a low survival rate and limited treatment options. Its prevalence is rising due to lifestyle factors, making early detection crucial for improving outcomes. Additionally, CDGs represent a spectrum of more than 160 rare, chronic diseases with significant unmet clinical needs. While individually rare, the collective impact of CDGs on affected individuals and families is substantial. In addition, the project focuses on two diseases of glycosylation as a prototype to investigate the correlation between disease-related glycans and monocyte activation.

The mechanisms identified, particularly related to aberrant sialylated O-glycans, may have implications for other cancers, such as breast and colon cancer, where aberrant expression of short sialylated O-glycans have also been identified (DOI: 10.1038/s41598-018-30421-w). Also, understanding the role of monocyte activation in PMM2-CDG can shed light on mechanisms relevant to other diseases exhibiting glycosylation defects, such as inflammatory diseases. Several immunologic molecules could have altered glycan content with potential functional consequences that might contribute to immune defects of PMM2 and other CDG patients (10.3389/fimmu.2024.1350101_Videira (accepted)).

It is relevant to note when studying diseases where glycosylation is altered, such as CDG and cancer; we need to carefully select the monoclonal antibodies since glycosylation may affect the epitope on the protein bound by the monoclonal antibodies, as suggested by others (DOI: 10.1186/1750-1172-8-170). The project will systematically inspect the antibodies with specific binding characteristics for the cell surface markers, regardless of its glycosylation profile.

We anticipate a substantial deepening of the innovation potential of the *FlowISiAM* technique and an enhanced alignment of the development of diagnostic and also therapy-relevant antibodies applicable to multiple diseases where glycosylation is affected.

FlowISiAM enables identifying and characterising disease-specific biomarkers with high specificity and sensitivity. By leveraging this technology, clinicians can detect and monitor diseases more accurately, leading to earlier diagnosis and better treatment outcomes.

Access to patient cohorts and blood is achievable through ongoing projects, which Paula Videira is currently leading. Namely, the InnoGlyco Project, which involves Fundação Champalimaud a leading institution in Portugal for pancreatic cancer treatment. Regarding CDG we coordinate a multinational network the CDG&Allies focused on CDG, which involves several hospitals, in Portugal such as the Centro Hospitalar Lisboa Central, Centro Hospitalar Universitário do Porto, Centro Hospitalar Universitário de Coimbra, and in Spain the Hospital Sant Joan de Déu.

Cooperation partner: ImmunoTools and INVIGATE may collaborate from the early stages of the project to provide a comprehensive panel of antibodies suitable for flow cytometric analysis. They may contribute their expertise in antibody development to support the project's goals, which can commence during the early stages of the project, particularly for the production of novel antibodies targeting disease-specific glycans.

They may also provide technical support and resources to facilitate the establishment of *FlowISiAM* in the laboratory setting. Dr. Zelia Silva, Prof. Dr. Paula Videira and Dr. Sebastian Krause (INVIGATE) intend to explore possible actions on the identification of specific markers that could facilitate early detection of pancreatic cancer by *FlowISiAM* testing, along with setting-up a tentative action plan and initial experimental evaluation. They envisage to create good preconditions for a joint research grant application.

ImmunoTools *FlowISiAM* AWARD for **Zelia Silva and Paula Videira** includes antibodies for *FlowISiAM*, 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.