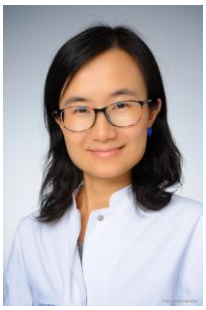
ImmunoTools *FlowISiAM* Award 2025



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Epitope-detection in monocytes as a novel liquid biopsy biomarker in personalized management of gastrointestinal cancers by using *FlowISiAM*

Background

Gastrointestinal (GI) cancers, primarily including esophageal cancer, gastric cancer, colorectal cancer, hepatocellular carcinoma, cholangiocarcinoma, and pancreatic cancer, exhibit significant heterogeneity and diverse prognostic outcomes[1]. Tumor diagnosis and staging is based on histological and serological findings as well as endoscopy and imaging[2]. GI cancers often show elevated serum levels of carbohydrate antigen 199 (CA 199), CA 125 and/or carcinoembryonic antigen (CEA)[3]. However, sensitivity and specificity are different, and not all patients have positive tumor markers available.

Liquid biopsies have recently emerged as a promising non-invasive approach for detecting and monitoring cancer[4,5]. Liquid biopsies encompass various techniques for analyzing circulating biomarkers such as circulating tumor DNA (ctDNA), circulating extracellular nucleic acids (cell-free DNA; cfDNA), circulating tumor cells (CTCs), exosomes, microRNAs and metabolomic markers, that reflect the genetic and molecular profile of tumors and their microenvironment.

Differentiated macrophages are rarely observed in peripheral blood; however, circulating macrophage-like cells that contain vesicles harboring tumor material were identified in patients with breast, pancreatic, and prostate cancer[6]. *Apo10* is an antigenic epitope of DNaseX (deoxyribonuclease) that plays a key role in cell apoptosis. The accumulation of Apo10 can be used as a marker for detecting the formation of tumor/proliferative disease. The *TKTL1* gene represents a significantly altered form of the transketolase (TKT) created in the course of vertebrate evolution[7]. The activation of the *TKTL1* gene in tumor cells leads to a fermentative, oxygen-independent

metabolism, which is accompanied by an increased intake of glucose and an increased formation of lactic acid[8].

The *FlowISiAM-* and the EDIM-technology utilizes the published epitope detection in monocytes (EDIM) technology[9]. Particularly the EDIM blood test focuses on the detection of the two biomarkers Apo10 and TKTL1 in $CD14^+/CD16^+$ activated monocytes (macrophages)[10,11]. EDIM has been reported in the diagnosis of GI cancers[12], but its ability to predict patient survival, recurrence, and response to neoadjuvant therapy remains unclear.

Experimental Design & Methods

I. Preliminary work: full ETDA blood, PBMCs from 3 patients with various tumors, both fresh and frozen at -80°C, used to compare the differences in storage methods and overcome technical challenges.

II. GI cancers cohort study

Patients Inclusion Criteria

- Age >18
- Patients must have pathologically or proven GI cancer (10 patients in each subtype, with 10 esophagus carcinoma, 10 pancreatic carcinoma, 10 hepatocellular carcinoma, 10 bile duct cancer and 10 other GI cancer types, respectively); known mixed or second original cancer is not allowed
- Able to undergo radical surgery directly or plan to undergo radical surgery after neoadjuvant therapy (excluding unresectable metastatic lesions or major vascular invasion)
- The estimate time length should be at least 3 months
- No prior systemic therapy, local therapy >6w
- If female, is not pregnant or breastfeeding
- Has adequate organ function
- Granulocytes >= 1,500/uL
- Hemoglobin >= 8.5 g/dL; patients with recent or ongoing gastrointestinal bleed may not be transfused to reach the entry hemoglobin of 8.5 g/dL; physicians should ensure patients requiring transfusion prior to registration do not have an occult or clinically apparent gastrointestinal bleed
- Platelets >= 75,000/uL
- Creatinine =< 1.5 x upper limit of normal (ULN) (or creatinine clearance calculated >= 60 cc/minute)
- Bilirubin =< 3 mg/dL
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) =< 5 x ULN
- Prothrombin time (PT)-international normalized ratio (INR) =< 1.7 (not required for patients on anticoagulation agents; patients who are being therapeutically anticoagulated with an agent such as Coumadin or heparin will be allowed to participate provided that no prior evidence of underlying abnormality in these parameters exists)

Outcomes

Primary outcomes

- The predictive value of *FlowISiAM* in patients with GI cancer undergo radical surgery (Cohort 1 and 2)

    - Overall survival
    - Recurrence-free survival
- The predictive value of *FlowISiAM* in patients with GI cancer after neoadjuvant systemic therapy (Cohort 2 only)
    - Radiological response
    - Pathological response
    - Tumor biomarker response

Secondary outcomes

- The risk factors of *FlowISiAM* in patients with GI cancer
    - Tumor types and subtypes
    - Tumor stage
    - Ageing associated changes
    - Treatment associated adverse events

Exploratory outcomes

- The dynamic changes of *FlowISiAM* in patients with GI cancer during systemic therapy (Cohort 2 only)

Flow cytometry based detection in activated monocytes/macrophages (*FlowISiAM*)

- Flow cytometry measurements using the antibody cocktail that includes anti-CD14, anti-CD16, anti-Apo10, and anti-TKTL1 from *FlowISiAM*
- Whole blood samples or PBMC from blood samples collected in EDTA tubes at the time of operations (Cohort 1 and 2), endoscopies and during systemic therapy (Cohort 2 only).

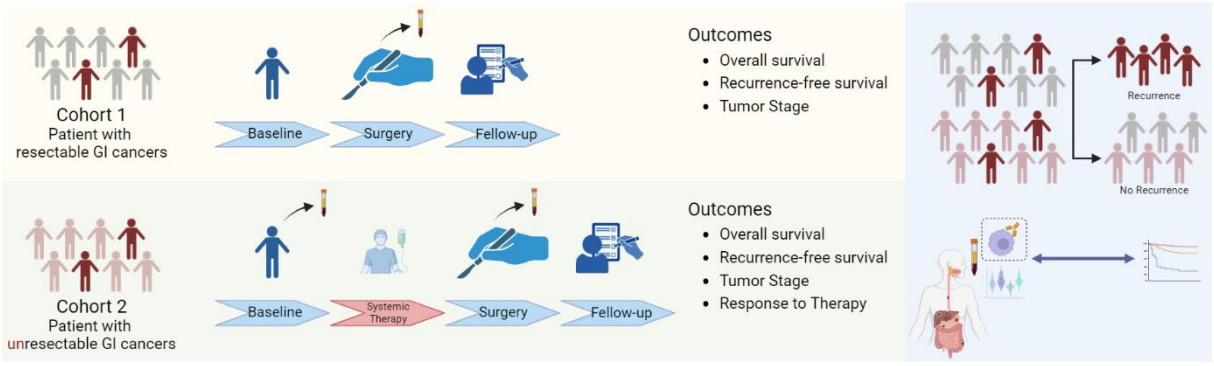
Impact

Our project aims to explore better liquid biopsy biomarkers and their potential clinical applications, providing guidance for the early diagnosis and prognosis estimation of GI cancer patients, ultimately improving the personalized care for GI cancer patients. We hope to acquire sufficient data from this pilot study for a joint research grant application.

Cooperation partner

The group of PD Dr. Yue Zhao and Dr Julia Fischer will cooperate together and collect patient samples and clinical data. PhD student ZiCheng Lv and a MTA will perform the measurement.

They will work together with ImmunoTools to adjust the experimental and instrumental set-up for *FlowISiAM* analysis. ImmunoTools will provide the reagents for *FlowISiAM* and flow cytometry analysis.



ImmunoTools *FlowISiAM* AWARD for **Julia Fischer & Yue Zhao** mainly includes antibody cocktail that includes anti-CD14, anti-CD16, anti-Apo10, and anti-TKTL1 for *FlowISiAM*, as well as other project associated antibodies if necessary; expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.

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