ImmunoTools *FlowISiAM* Award 2024

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Carbonic Anhydrase IX as a diagnostic and prognostic marker for chemoresistance onset in gastric adenocarcinoma cancer

Background

Gastric cancer (GC) represents the fifth most frequently diagnosed cancer accounting for more than 1,000,000 new cases per year and about 760,000 deaths worldwide. Even though surgical resection with D2 lymphadenectomy is still the mainstay of curative therapy, the patient's prognosis remains overall poor due to the high incidence of advanced and inoperable disease at diagnosis, and the high percentage of progression after surgery. In this context, multimodal approaches and new diagnostic tools to achieve an earlier diagnosis are urgent to be developed.

Chemotherapy (CT) before surgery (perioperative CT, pCT) significantly increases the chance for curative resection, and sterilizes micro-metastasis, generally improving patients' overall survival. To date, in Europe, the FLOT regimen (5-FU, Folinic acid, Oxaliplatin, and Docetaxel) rapidly become the gold standard of care for patients with resectable GC who can tolerate a perioperative three-drug combination regimen. However, the development of drug resistance and the subsequent tumor relapse still represent the main cause of death in advanced GC patients. Recently, our group identified the Carbonic Anhydrase IX (CAIX) as a marker of chemoresistance in GC. As shown in our last study (*doi: 10.1016/j.canlet.2023.216338*), GC patients who accessed the cure of Azienda Ospedaliero Universitaria Careggi (AOUC) in Florence, were grouped into responder and non-responder based on the tumor regression grade (TRG) after the FLOT regimen they were administered before surgery. We demonstrated that CAIX expression significantly increases in the non-responder group and correlates with FLOT resistance. Even in our in vitro experimental setting, we observed that CAIX is overexpressed in FLOT-resistant cells compared to sensitive cells and that its expression correlates with the loose of therapy response. Overall, our findings demonstrated that CAIX is involved in the survival and proliferation of GC cells and its expression is mandatory for cell adaptation in the road to chemoresistance acquisition. Thereby, CAIX could be a good candidate target for monitoring

therapy response in GC patients during the pCT regimen and for early detecting any tumor relapse.

Aims

With this project, we propose to determine the reliability of CAIX as a liquid biomarker of therapy resistance in GC patients.

Experimental Design & Methods

A preliminary *in vitro* study phase is expected where we will resemble what we would perform with the *FlowISiAM* technology in GC patients. In more detail, we will use *in vitro* activated monocytes by stimulating PBMCs for 6 days with GM-CSF and M-CSF. Naïve M0 and polarized M1 or M2 macrophages with IFN-γ + LPS or IL-4/IL-10/IL-13, respectively, will be co-cultured with sensitive and FLOT-resistant GC cell lines, already generated and available in our laboratory. At different co-culture time points, macrophages will be tested for intracellular CAIX levels through the *FlowISiAM* technology.

Following this starting phase, we will collect activated monocytes of GC patients of AOUC in Florence at different time points (i.e., before pCT, after pCT-before surgery, and after surgery) and subsequently determine the CAIX levels via the *FlowISiAM* technology. As reported in our last study (*doi: 10.1016/j.canlet.2023.216338)*, GC patients will be grouped into responders and non-responders based on the TRG value determined by the pathologist board of the AOUC in Florence. Parallelly to the *FlowISiAM* technology, serum levels of CAIX will be detected by ELISA (as protein) and droplet digital PCR (as DNA and mRNA). Such analysis will be also accompanied by the immunohistochemical detection of CAIX in solid biopsies collected during surgery. Clinical history and follow-up data of GC patients will be stored and paired with the *FlowISiAM* results together with the IHC and serum CAIX analysis. The collection of patients' samples and clinical data for this project will be guaranteed by the already established collaboration with the Directors of AOUC Oncology and Gastroenterology Units.

Impact

Our project hopefully will develop and deliver a reliable diagnostic tool for the analysis of chemoresistance in GC, thereby aiding the choice of personalized CT regimens. GC patients could benefit from this technology as the early detection of chemoresistance onset will allow the oncology board members to rapidly change the therapy saving precious time for patients and offering them more chances to be cured from cancer.

Cooperation Partners

The research group of Dr. Elena Andreucci and Dr. Alessio Biagioni will cooperate with the Gastroenterology and Oncology Units of the AOUC in Florence for the collection of patient samples and clinical data. They will work together with ImmunoTools to adjust the experimental and instrumental set-up for *FlowISiAM* analysis. ImmunoTools will provide the reagents for *in*

vitro macrophage polarization and flow cytometry analysis. ImmunoTools and INVIGATE will develop antibodies for *FlowISiAM* experiments and share specific know-how for computer-aided scoring from raw data for optimal test outcomes.

Dr. Elena Andreucci and Dr. Alessio Biagioni together with Dr. Sebastian Krause (INVIGATE) intend to further expand the work on the development of optimized monoclonal antibodies for detection and intend to create proof-of-principle results for a joint research grant application.

ImmunoTools *FlowISiAM* AWARD for

Elena Andreucci and **Alessio Biagioni** 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.