**RESEARCH TOPIC CLI3**

# The NORMA study: ctDNA-based perioperative management in locally advanced Gastroesophageal cancers

**Research area**
Medical Area

**Clinical Unit name**
Medical Oncology Unit 2, Humanitas Research Hospital, Rozzano

**Supervisor**
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**Co- Supervisor**
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**Abstract**
Patients with locally advanced gastroesophageal adenocarcinoma (GEA) undergoing curative-intent multimodal treatment still experience high rates of failure (>40% relapses within 3 years). Although immunotherapy has improved perioperative outcomes [1], no validated clinical, pathological, or molecular biomarkers are available, thus therapy cannot be personalized, and patients are uniformly treated according to standard of care (perioperative durvalumab + FLOT) [1]. Clinicians currently lack tools to guide perioperative treatment escalation or de-escalation, leading to potential overtreatment in a substantial proportion of patients. In addition, there is a critical unmet need for scalable, non-invasive approaches capable of capturing minimal residual disease (MRD), anticipating molecular relapse (i.e., early molecular relapse EMR), defying molecular response (MR) and longitudinally tracking tumor evolution. Circulating tumor DNA (ctDNA) is a promising biomarker for real-time assessment of tumor burden and minimal residual disease (MRD) [2–3]. In GEA, ctDNA-guided strategies are not yet used in clinical practice due to limited sensitivity of single-parameter assays, lack of longitudinal integration across treatment phases, and insufficient prospective validation [4]. In this context, we designed and have been conducting since 2025 at our institution the BUTTERFLY study (PI Alberto Puccini) that is an ongoing, prospective, single-center translational project designed to evaluate the clinical utility of liquid biopsy in patients with stage II–III GEA treated with standard neoadjuvant chemotherapy, using our analytical and clinical validated, academic, tumor-agnostic, multi-modal ctDNA assay for longitudinal MRD assessment (ALMA - Agnostic Liquid biopsy Multimodal Advancement) [5-6]. BUTTERFLY integrates ctDNA analysis during all perioperative treatment phases, enabling longitudinal assessment over time. The methodological robustness of the project has been further strengthened by the addition of a validation cohort in collaboration with Memorial Sloan Kettering in New York (Dr. Yelena Y Janjigian). Preliminary data (see 1.2) generated within this project demonstrate the feasibility of serial multi-omic ctDNA profiling and support its strong association with treatment response, providing a robust biological and methodological foundation. The proposed NORMA study is conceived as the natural extension of BUTTERFLY,

leveraging existing expertise, infrastructure, and preliminary evidence to transition from translational biomarker assessment toward structured clinical decision-making across the perioperative continuum. To achieve these aims, the NORMA study has been designed around two main objectives: (i) to perform a Phase II feasibility study assessing the clinical performance of our tumor-agnostic ctDNA assay, (ii) to build a multicenter prospective cohort supporting extensive translational research to elucidate both treatment-resistance mechanisms and refine multi-parameter detection of MRD, EMR, and MR during longitudinal patient monitoring.

Study design
NORMA is a prospective, multicenter, phase II trial designed to prove the feasibility of using liquid biopsy to guide perioperative clinical management in 150 stage II–III GEA patients

Study Objectives
-Primary objective
To assess the feasibility and clinical safety of a ctDNA-based personalized perioperative decision-making strategy, defined as successful integration of longitudinal ctDNA MRD assessment into clinical workflows together with absence of signals of clinically unacceptable survival impairment.
-Secondary objectives
Association between ctDNA dynamics at different perioperative timepoints and survival outcomes.
Evaluation of Toxicity and Evalutaion of Quality of life
Translational research: for the second aim of the study, we will a) assess the predictive and prognostic value of integrating ctDNA information with genomic, molecular and immune-related signatures obtained through multi-modal profiling of FFPE tumor samples (Whole Genome Sequencing (WGS), immunohistochemistry (IHC), single cell spatial transcriptomic analysis); b) assess the mechanisms of acquired resistance to treatment through exploratory biomarker analyses on tumor samples and ctDNA; c) investigate the tumor microenvironment (TME) composition, its spatial organization and its influence on the clinical outcomes (spatially resolved transcriptomic); d) investigate the role of an artificial intelligence (AI)-derived multimodal predictive classifier of relapse and treatment resistance. Core analyses will focus on ctDNA-integrated molecular profiling and resistance mechanisms, while additional multi-omic investigations will be conducted as hypothesis-generating exploratory analyses.

Main skills requested by the applicant:
- Demonstrated expertise in clinical management and translational research of gastroesophageal cancer
- Strong background in circulating tumor DNA (ctDNA) analysis and its application in oncological research
- Documented research experience (≥6 months) in an international center of clinical and scientific excellence with a focus on upper gastrointestinal malignancies



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**Scientific references**

1. Janjigian YY, et al. N Engl J Med. 2025

2. Tie J, et al. N Engl J Med. 2022

3. Kotani D, et al. Nat Med. 2023

4. Zaanan A, et al. Nat Commun 2025

5. Paracchini L et al. The Journal of Liquid Biopsy 2025, doi: 10.1016/j.jlb.2025.100337

6. Paracchini L, et al ESMO Open 2026, https://doi.org/10.1016/j.esmoop.2026.106087

**Type of contract**

Scholarship or coordinated and continuous collaboration contract (Co.Co.Co.) amounting to at least € 35,000 gross per year, activated by Istituto Clinico Humanitas. The amount is subject to IRPEF taxation and to autonomous and full payment of social security and welfare contributions by the collaborator, directly to the relevant professional pension fund or to any other fund of their choice.

Borsa di studio o Contratto collaborazione coordinata e continuativa (cococo) pari ad almeno € 35.000 annui lordi attivato da Istituto Clinico Humanitas. Importo soggetto a tassazione IRPEF e versamento per contribuzione previdenziale ed assistenziale autonomo ed integrale a carico del collaboratore, direttamente alla cassa professionale di competenza o a qualsiasi altro Fondo dallo stesso prescelto.



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