BMC Cancer

STUDY PROTOCOL

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Adjuvant TRastuzumab deruxtecan plus fluoropyrimidine versus standard chemotherapy in HER2-positive gastric or gastroesophageal cancer patients with persistence of minimal residual disease in liquid biopsy after pre-operative chemotherapy and radical surgery: the multicentre, phase II randomized TRINITY trial

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Abstract

Background The standard treatment for localized/locally advanced gastroesophageal adenocarcinoma (GEA) is radical surgery and peri-operative FLOT treatment (5-fluorouracil plus leucovorin, oxaliplatin, and docetaxel), but around half patients still experience disease relapse. In gastrointestinal cancers, the presence of circulating tumor DNA (ctDNA) after surgery is associated with a high risk of relapse, and the lack of ctDNA clearance after post-operative treatment is strongly associated with early relapse. Therefore, liquid biopsy may guide the selection of patients with micrometastatic disease after preoperative chemotherapy and surgery for non-cross resistant regimens in the post-operative setting. Trastuzumab deruxtecan (T-DXd) is approved in patients with HER2-positive advanced gastric or gastroesophageal adenocarcinoma after failure of at least one prior trastuzumab-based regimen. The DESTINY-Gastric01 and 02 trials showed remarkable activity and efficacy of T-DXd, thus supporting the investigation of this agent in early-stage disease to increase the chance of achieving disease eradication. Finally,

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the DESTINY-Gastric03 trial showed the safety profile and feasibility, with preliminary promising activity results of the combination of T-DXd with a fluoropyrimidine.

Trial design TRINITY is an ongoing multicentre, randomized, open-label, interventional phase II study which will enroll approximately 46 patients with HER2-positive GEA, treated with pre-operative FLOT and radical surgery, and with the persistence of minimal residual disease detected by the Signatera™ assay in a liquid biopsy collected between 2 and 6 weeks after surgery.

The trial is designed with an observational phase enrolling patients with HER2-positive GEA eligible for standard treatment with peri-operative FLOT and surgery. Eligible patients will be randomized on a 1:1 basis to the experimental treatment arm consisting of adjuvant T-DXd (6.4 mg/kg IV on day 1) plus either capecitabine (1000 mg/sqm BID orally on days 1–14) or 5-fluorouracil (600 mg/sqm continuous IV infusion on days 1–5) Q3 W for 6 cycles, or to the control arm with standard post-operative FLOT (at the same dose used during the last pre-operative cycle) for 4 cycles. Patients non-eligible for the interventional trial will continue the standard therapy and follow-up in the frame of the observational phase with collection of exploratory longitudinal liquid biopsies.

The primary objective is ctDNA clearance at 1 year after randomization. Considering alpha- and beta-errors of 0.10 and 0.20 and hypothesizing a ctDNA clearance of 10% and 35% in the control and experimental arm, respectively, 23 patients per arm are required to prove the superiority of the experimental strategy. Secondary endpoints include disease-free survival, overall survival, metastases-free survival, patient-reported outcomes and safety. The trial also represents a translational platform, including extensive analysis of circulating, tissue, and immune biomarkers as exploratory endpoints.

Enrollment is active and ongoing.

Trial registration TRINITY is registered at ClinicalTrials.gov (NCT06253650).

Keywords Gastric cancer, Esophageal cancer, HER2, Liquid biopsy, Trastuzumab-deruxtecan

Introduction

Gastric or gastroesohageal junction (GEJ) cancer are highly aggressive diseases, figuring as some of the leading causes of cancer-related death worldwide [1]. The cornerstone of management of localized gastroesophageal adenocarcinomas (GEAs) in non-Asian countries is based on the integration of radical surgery with peri-operative systemic treatment, that aims at improving the chances of achieving cure through the increase of the rates of radical resections and the elimination of micro-metastatic foci that would eventually lead to disease relapse [2, 3]. The FLOT4-AIO trial set the current standard for the choice of peri-operative chemotherapy, supporting the use of fluorouracil, oxaliplatin and docetaxel (FLOT) combination [4]. Still, prognosis remains highly unsatisfactory, with 5-year overall survival (OS) of ~40% for patients with localized/locally advanced disease, highlighting the need to redefine the current management strategy of patients with localized gastric/GEJ cancer to increase the chances of cure. For example, it would be important to promptly identify the patients at highest risk of relapse after neoadjuvant treatment and surgery, that is those patients with minimal residual disease (MRD). MRD is indeed defined by the presence of malignant cells after a curative treatment, that is undetectable by routine diagnostic exams, but may be assessed using the liquid biopsy, that aims at identifying patients with "micro-metastatic" disease by detecting cell-free (cfDNA) or circulating tumor DNA (ctDNA). The correlation between the presence of MRD and increased risk for disease recurrence was observed across several tumor types [5–7], including gastric/GEJ cancer [8, 9], but, it is not incorporated in the international guidelines for its use in the daily practice because extensive, prospective and solid evidence-based data are scarce. To increase the chance of disease eradication, novel therapeutic strategies are also needed, that should be tailored according to patient and disease's characteristics in a personalized fashion. Of note, despite treatment of metastatic disease is guided by several molecular features, the choice of peri-operative FLOT is independent of such characteristics.

Overexpression/amplification of human epidermal growth factor 2 (HER2) is found in about one out of five patients with advanced GC/GEJC, who benefit from targeted therapy with trastuzumab (anti-HER2 monoclonal antibody, mAb) combined with the standard first-line chemotherapy, as demonstrated by the ToGA trial [10]. To further improve outcomes, combinations of anti-HER2 mAbs with novel drugs are being explored; antibody–drug conjugates (ADCs), including trastuzumab deruxtecan (T-DXd), are yielding interesting results in the advanced setting, with the potential to overcome the classic mechanisms of resistance to trastuzumab and other older anti-HER2 agents [11–13]. HER2-directed

therapy is, instead, not recommended in patients candidate to peri-operative treatment, due to lack of supporting evidence; therefore, FLOT remains the standard of care for peri-operative treatment, independently of HER2 status and type of response to the neoadjuvant therapy. However, we hypothesize that the presence of MRD after surgery may identify patients who failed the standard pre-operative FLOT plus surgery treatment and are expected to derive scant benefit from FLOT continuation. Therefore, they could be re-directed towards novel, non-cross resistant treatment strategies in the adjuvant setting for their "micro-metastatic" disease.

Moving from this rationale, we designed the proof-of-concept, randomized, phase II TRINITY study (NCT06253650), aimed at assessing the activity and safety of the combination of T-DXd and fluoropyrimidines as post-operative treatment for patients with resected GEA and presence of MRD at liquid biopsy, versus standard post-operative FLOT.

Methods

Study design

TRINITY is designed as a multicentre, randomized, open-label, interventional phase II study aimed at investigating the activity, efficacy and safety of trastuzumab deruxtecan (T-DXd) plus capecitabine or 5-fluorouracil as a post-operative treatment in patients with localized/locally advanced esophageal, gastric or gastroesophageal junction adenocarcinoma with HER2 overexpression/amplification and positive post-operative ctDNA after pre-operative FLOT followed by radical surgery.

The trial also features an Observational Phase enrolling patients with HER2-positive localized/locally advanced esophageal, gastric or gastroesophageal junction adenocarcinoma receiving standard peri-operative therapeutic management according to the best clinical practice and local guidelines at their Centre.

Study design is depicted in Fig. 1.

Observational phase

The observational phase will include patients with localized/locally advanced HER2-positive gastric or gastroesophageal junction cancer or esophageal adenocarcinoma eligible for standard pre-operative chemotherapy with FLOT for 4 cycles and radical surgery. After the signature of a specific informed consent, patients will enter the Observational Phase of the study and will undergo the molecular prescreening according to the specific algorithm described below.

Specifically, evaluation of HER2 overexpression/amplification will be locally performed (with retrospective centralized confirmation) on the archival tumor tissue collected before the start of pre-operative chemotherapy with FLOT. In details, immunohistochemistry (IHC) for HER2 will be performed according to the standard specific clinical practice guidelines:

1. in tumors with score 0 or 1 +, the HER2 status will be defined as negative
2. in tumors with score 3 +, the HER2 status will be defined as positive
3. in tumors with score 2 +, HER2 silver in situ hybridization (ISH) will be performed in order to define the case as positive (IHC 2 + and ISH amplified) or negative (IHC 2 + and ISH not amplified).

Only HER2 positive cases based on archival diagnostic tissue sample will be deemed eligible for the Observational Phase and will continue the study procedures.

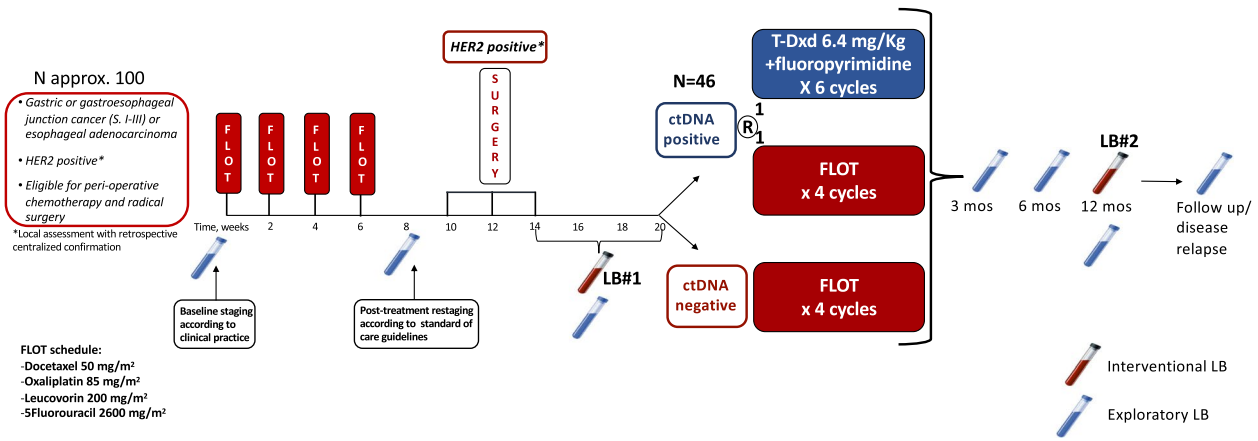

Fig. 1 Study design of the TRINITY trial, depicting main eligibility criteria, methodology and treatment arms of the experimental phase. Abbreviations. LB, liquid biopsy. ctDNA, circulating tumor DNA. T-DXd, Trastuzumab-Deruxtecan. mos, months

Patients enrolled in the observational Phase will be treated according to the national and international guidelines in the frame of the clinical practice with standard of care pre-operative treatment with FLOT for four cycles and radical surgery. In the frame of the Observational Phase, patients will undergo two exploratory liquid biopsies (LBs) and peripheral blood mononuclear cells (PBMCs) collections, before starting pre-operative FLOT and after its end (i.e., before surgery). Exploratory LBs will be used for translational analyses. Also, a copy of baseline imaging examinations will be collected. Then, at 2–6 weeks after surgery, patients will undergo a single blood draw that will serve two purposes by collecting: 1) a third exploratory LB; 2) the interventional LB which will be centrally analyzed as pre-screening test (LB#1: interventional for the potential enrollment in the TRINITY study). The window for post-operative ctDNA testing was chosen after careful evaluation of the literature data, taking into account the positivity rate for ctDNA during conventional MRD windows [14], the turn-around time of the pathology report, HER2 status and Signatera test results, and the recommended time limit for starting post-operative treatment, as the post-operative treatment ideally should start no later than 12 weeks after the date of surgery.

In details, the prescreening algorithm for enrollment in the interventional TRINITY study is the following:

- at 2–6 weeks after surgery, patients will undergo a post-operative exploratory liquid biopsy plus the post-operative interventional liquid biopsy (LB#1) that will be centrally analyzed with the tissue-informed Signatera™ CE-marked assay in order to determine the presence of post-operative ctDNA. The study requires the collection of the baseline tumor tissue before the start of FLOT treatment and the surgical sample in order to fulfill the prescreening algorithm and for exploratory analyses.
- HER2 positivity will be locally confirmed also in the surgical samples (subsequent central confirmation will be performed in a retrospective fashion).

Patients will be deemed eligible for the Screening in the Interventional TRINITY study only in case of both HER2 positivity (in both archival biopsy and surgical sample) and.

positive ctDNA at LB#1.

Of note, patients not fulfilling the Molecular prescreening Criteria will continue the standard post-operative treatment as per clinical practice and will continue follow-up within the Observational Phase with the collection of serial exploratory LBs at specific timepoints (namely, 3, 6 and 12 months from the date of pre-screening failure and then in concomitance with follow-up visits).

Inclusion Criteria of the Interventional TRINITY study:

Key inclusion criteria are: $\geq$18 years of age, Eastern Cooperative Oncology Group (ECOG) PS 0–1, radically resected (R0) GC/GEJC/esophageal adenocarcinoma after the completion of pre-operative chemotherapy with FLOT with no evidence of metastasis at postoperative radiological evaluation, locally determined HER2 overexpression/amplification on post-operative sample, absence of pathological complete response at surgery according to Becker regression criteria [15], positivity of liquid biopsy at 2–6 weeks from surgery. Key exclusion criteria are inadequate liver, kidney, cardiovascular, pulmonary and hematological functions, serious illness or medical conditions that contraindicated the treatment according to the investigators, previous anti-HER2 therapy and known dihydropyrimidine dehydrogenase (DPD) deficiency.

All patients will sign an Institutional Review Board-approved consent form (INT 233/23) before any study procedure.

Interventional TRINITY study

Patients fulfilling the molecular prescreening criteria will be screened according to the specific screening procedures between 2 and 12 weeks after the date of surgery, ensuring that the post-operative treatment in any case should start not later than 12 weeks after the date of surgery. In details, after the signature of the specific informed consent and verification of the eligibility criteria, demography, including baseline characteristics, medical history, concomitant medications will be collected and assessment of residual toxicity from pre-operative treatment and/or post-operative complications will be done. Patients will undergo full physical examination with vital signs, triplicate 12-lead ECG, Echocardiogram or MUGA, complete blood tests with virology, urinanalysis, troponin levels and pregnancy test where applicable, baseline radiological assessment including high-resolution chest-abdomen computed tomography (CT scan), ophthalmological and pneumological assessment. Moreover, exploratory liquid biopsy and PBMCs will be collected and patient-reported outcomes (PROs) questionnaires will be administered.

Patients fulfilling all the eligibility criteria for the trial will be randomized according to a 1:1 basis to:

a. the experimental arm: treatment with T-DXd (at the dose of 6.4 mg/kg intravenous every 3 weeks) plus capecitabine (1000 mg/sqm BID orally on days 1–14 every 3 weeks) or 5-fluorouracil (600 mg/m2/day

continuous intravenous infusion on days 1–5 every 3 weeks), as per Investigator's choice, for 6 cycles;

b. the control arm: treatment with post-operative FLOT for 4 cycles as per standard of care and using the doses previously adopted in the last cycle of the pre-operative phase.

Before every cycle of treatment and at the end of treatment visit, patients will undergo: targeted physical exam with vital signs, collection of concomitant medications and adverse events and treatment toxicity (grading by the Common Terminology Criteria for Adverse Events, CTCAE version 5.0), laboratory assessments and exploratory liquid biopsy and PBMCs collection. Echocardiogram or MUGA every 3 months during treatment with T-DXd and ECG before the fourth cycle of T-DXd will be performed.

After randomization, patients will undergo a chest and abdomen CT scan every 3 months for 1 year to monitor the occurrence of clinical disease relapse. During the follow-up phase, patients will undergo serial exploratory LBs at specific timepoints, at 3, 6 and 12 months from the date of randomization and during follow-up as per standard clinical practice and at the moment of disease relapse. At 12 months after randomization, during the same timepoint and through the same blood draw of the exploratory LB, the interventional LB#2 will be collected, which will be centrally analyzed to detect ctDNA and to assess the occurrence of ctDNA clearance in both arms.

The procedures for the pre-screening, screening, treatment and follow-up periods for the Interventional TRINITY study are presented in Table 1.

Endpoints

The primary objective of the study is to assess the clearance of ctDNA in T-DXd plus capecitabine/5-fluorouracil arm versus standard FLOT continuation at 1 year from randomization.

Secondary endpoints include the assessment of the efficacy of the experimental arm versus standard of care, in terms of overall survival, disease-free survival and metastases-free survival, and of the impact on patients' quality of life, through investigation of PROs, by completion of quality-of-life questionnaires, European Organization for Research and Treatment of Cancer (EORTC) QLQ -C30, EORTC QLQ-STO- 22 and EuroQol EQ- 5D- 5L.

Statistical design

Sample size has been estimated so that, hypothesizing a ctDNA clearance rate at 1 year of 10% following post-operative FLOT in patients with positive ctDNA after pre-operative FLOT and surgery, in the control arm, and a target ctDNA clearance rate at 1 year of 35% following post-operative T-DXd plus capecitabine/5-fluorouracil, in the experimental arm, a total of 46 patients (about 23 patients per arm) will be required to be able to declare the superiority of the experimental strategy, with one-sided alpha- and beta-errors of 0.10 and 0.20, respectively. The 10% estimate for null hypothesis has been chosen as per clinical expectation, since, being a disease refractory to pre-operative FLOT treatment as it showed positive post-operative ctDNA, the expectation of a ctDNA clearance with standard post-operative FLOT continuation is low, less or equal to 10%. Conversely, the 35% estimate for the alternative hypothesis has been defined for the predicted clinical relevance based upon the literature data of failure of the standard strategy [16–18].

An interim analysis for safety and feasibility is planned after the first 6 patients will complete the first 3 cycles of adjuvant treatment in the experimental arm with T-DXd plus capecitabine or 5-fluorouracil. An independent safety monitoring committee will review all the safety data of the first 6 patients to confirm the safety and feasibility of the adjuvant treatment with T-DXd plus capecitabine or 5-fluorouracil in this setting or if the indication for a study amendment will be defined for safety reasons.

All the analysis for the study endpoints will be performed in the Intention-to-Treat population, namely every subject who is randomized in the study according to randomized treatment assignment, except for the safety analysis that will be performed on the safety analysis set that will consist of all patients who received at least 1 dose of study drugs.

Discussion

By often presenting at a late stage and displaying resistance to most treatments available, GEAs are major causes of cancer-related deaths worldwide. Therefore, an early diagnosis when the tumor is still localized represents the only chance of achieving cure. To increase the likelihood of obtaining oncological radicality, guidelines from major scientific societies recommend a multimodal therapeutic approach, that may include chemotherapy, radiotherapy and radical surgery.

The MAGIC study was the first trial to demonstrate a higher rate of curative resection (79.3% versus 70.3%, $p = 0.03$) and survival advantage (5-year OS: 36% vs 23%, $p = 0.009$) with peri-operative strategies as compared to surgery only, through a regimen made of epirubicine, cisplatin and fluorouracil or capecitabine (ECF/ECX) [19]. Afterwards, the AIO4-FLOT study demonstrated the superiority of the combination of fluorouracil,

Table 1 Study plan detailing the procedures of the Interventional TRINITY study

	Schedule of assessments				
	Pre-Screening	**Screening**	**Treatment period**	**End of Treatment and response assessment**	**Follow up**
Timeline	**2–4 weeks after surgery**	**2–12 weeks after surgery**			
Informed consent					
Informed consent to the Interventional Study	X				
Pre-screening procedures					
Eligibility criteria	X				
Local evaluation of HER2 overexpression/amplification on surgery tissue	X				
LB#1 resulted as positive	X				
Study procedures in eligible patients					
ECOG performance status		X	X	X	X
Full physical exam (including height)		X			
Targeted physical exam (based on symptoms)			X	X	X
Vital signs (including weight and SpO2)		X	X	X	X
Triplicate 12-leads ECG		X			
12-leads ECG			X [0]	X	
Echocardiogram or MUGA		X	as clinically indicated	X	
Ophthalmologic assessment		X	as clinically indicated	X	
Pulmonary function tests (PFTs)			X	as clinically indicated	
Concomitant medications		< –––––– All visits ––––––– >			
Demography, including baseline characteristics		X			
Review of residual AEs from pre-operative treatment/ post-operative complications		X			
Laboratory assessments					
Serum chemistry [a]		X	X [a]	X	X
Hematology [a]		X	X [a]	X	X
Coagulation [a]		X		X	
Biomarkers: CEA, CA19.9 19.9		X		X	X
Troponin level		X		X	
Virology assessment					
HBsAg, anti-HBs, anti-HBc, anti-HCV and HBV DNA		X			
HIV		X			
Urinalysis [a]		X			
Pregnancy test [b]		X	X	X	
Tumor assessments					
Disease assessment by chest-abdomen-pelvis CT scan with intravenous contrast and HRCT[c]		X			X [d]
Chest X-ray and abdominal ultrasound					X [d]

Table 1 (continued)

	Schedule of assessments				
	Pre-Screening	**Screening**	**Treatment period**	**End of Treatment and response assessment**	**Follow up**
Other assessments and assays					
Liquid biopsy					LB#2 [e]
Exploratory liquid biopsies (blood and plasma samples)		X	X before every cycle	X	X
PBMC		X	X before every cycle	X	
Monitoring					
AE/SAE assessment		< ---------- All visits ---------- >			
Study drug administration					
Trastuzumab-deruxtecan plus 5-fluorouracil or capecitabine (Investigator's choice)			X [f]		
FLOT treatment for 4 cycles			X [f]		
PRO assessments					
EORTC QLQ-C30, QLQ-STO-22, EQ- 5D- 5L		X	X [g]	X	

All assessments on treatment days are to be performed prior to infusion, unless otherwise indicated

[0] At screening, all patients will be subjected to triplicate 12-leads ECG, afterwards only patients randomized to T-DXd will undergo 12-leads ECG prior to the fourth cycle of T-DXd, and for all patients at the end of treatment

[a] If screening laboratory assessments (including clinical chemistry, hematology, coagulation) are performed within 3 days prior to Day 1 (first infusion day), they do not need to be repeated at Day 1

[b] For women of childbearing potential only. A urine or serum pregnancy test is acceptable. Women of childbearing potential are required to have a pregnancy test within 7 days prior to the first dose of treatment and then every 4 weeks. Pregnancy test may be performed within 3 days prior to Day 1, but results must be available and reviewed by the treating physician or Investigator prior to commencing an infusion

[c] Disease assessments will be performed on images from CT with intravenous contrast of the chest, abdomen, and pelvis. Additional anatomy should be imaged based on signs and symptoms of individual patients at baseline and follow-up. Baseline assessments should be performed according to the schedule and, ideally, should be performed as close as possible to and prior to the start of treatment

[d] During follow up, radiological assessments and clinical visits should be performed according to standard clinical practice guidelines and local standards

[e] Centrally collected interventional liquid biopsy at 12 months from the date of randomization (LB#2), while exploratory liquid biopsies at 3 and 6 months from the date of randomization, and during follow up and at the occurrence of disease relapse

[f] According to the assigned study treatment arm

[g] During post-operative treatment, PROs questionnaires will be administered every other cycle in both treatment arms

oxaliplatin and docetaxel (FLOT), repeated for 4 cycles peri-operatively, versus ECF/ECX in terms of R0 resection (85% vs 78%), pathological complete response (pCR) rate (16% vs 6%) and survival (median OS: 50 months versus 35 months, respectively, p= 0.012) [4]. Differently from gastric/GEJ tumors, the management of resectable esophageal adenocarcinomas has long been controversial, as neoadjuvant chemo-radiotherapy (41.4 Gray plus carboplatin and paclitaxel, the CROSS regimen [20]) is another appealing option, other than peri-operative chemotherapy. To solve the dispute regarding the superiority between the two strategies, the ESOPEC trial was conducted: 438 patients with resectable (cT1 cN + or cT2 - 4a cN any) esophageal adenocarcinoma were randomized to receive either FLOT or CROSS as part of their multimodal treatment. The results of this study were recently published, showing the superiority of peri-operative FLOT in terms of OS (HR 0.70, 95%CI 0.53–0.92, p= 0.012), therefore supporting this strategy for most patients with locally advanced, resectable esophageal adenocarcinoma [21]. However, a significant fraction of patients still experiences disease relapse despite completing the peri-operative treatment with FLOT, posing some doubts on this "one-fits-all" approach. By also coming with the price of potentially significant toxicity, with serious adverse events reported in up to 27% cases, it is important to distinguish patients that retrieve benefit from FLOT from those who do not and are therefore at higher risk of relapse. Similarly, some patients may be cured by surgery alone and it would be important to spare possible unwanted toxicities. In this sense, to search for and validate biomarkers that can guide the physician in modulating post-operative treatment and follow-up is an unmet clinical need to develop a "tailored" strategy for specific groups of patients. In other gastrointestinal cancers, the liquid biopsy proved to be a useful tool for identifying patients at highest risk of recurrence after a curative treatment (i.e., surgery and

adjuvant therapy) due to the presence of MRD. Defined as the presence of malignant cells, undetectable by standard diagnostic means, after a treatment with curative intent, MRD eventually drives disease relapse and metastases. Therefore, patients with MRD may be considered as "molecularly metastatic" and predicted to develop macroscopic metastases at high rate. The liquid biopsy is able to detect cell-free and circulating tumor DNA (ctDNA) and can assist in identifying patients that necessitate further treatments to avoid relapse and achieve cure, i.e., those without ctDNA clearance after surgery and/or adjuvant chemotherapy. Extensive data, including prospective studies, highlighting that the absence of ctDNA clearance after a curative treatment is associated with worse outcomes in patients with early-stage colorectal cancer (CRC) [5, 22–24]. Therefore, liquid biopsy could serve as a real-time surrogate biomarker of recurrence-free status and of the efficacy of adjuvant therapy. On this basis, the first phase II liquid biopsy-driven trial was conducted: the DYNAMIC study randomized patients with stage II colon cancer (on a 2:1 ratio) to adjuvant chemotherapy decision according to ctDNA status versus standard of care, that is allocating patients to adjuvant chemotherapy according to the guideline-recommended clinic-pathological risk factors regardless of ctDNA status. The trial found that, compared to the standard, non-ctDNA informed approach, the ctDNA-guided strategy could lead to non-inferior DFS (2-year DFS 93.5% vs 92.4%, respectively) with a reduced fraction of patients receiving adjuvant treatment (15% vs 28%, respectively). The role of ctDNA is being further evaluated in several clinical trials in stage II-III CRC, with the aim to modulate the adjuvant strategy according to the predicted biological risk related to ctDNA status (NCT04259944; ACTRN/12615000381583; NCT05062889; NCT05031975). Fewer but consistent data exist in GEA [17]. A pooled analysis by Chen et al. of ~4000 patients with any-stage GC found that ctDNA-positivity was correlated to worse DFS and OS. In the non-metastatic setting, detection of post-operative ctDNA was associated to statistically significant inferior DFS (median DFS 12.5 months vs not reached for those without detectable ctDNA, respectively; $p= 0.03$) [16]. The prognostic value of ctDNA was confirmed in a prospective cohort of 125 patients affected by GEA receiving neoadjuvant chemotherapy and surgery: around 90% patients with a post-surgical positive liquid biopsy eventually experienced recurrence and had poorer DFS with respect to ctDNA-negative patients (median DFS 9.6 months vs not reached, respectively; HR 23.6; 95% CI 10.2–66.0; $p< 0.0001$) [18]. In summary, investigating the role of liquid biopsy in predicting the outcomes of patients with GEA eligible for a curative treatment is a key research question that is already an objective of existing clinical trials (NCT03957564).

The other key aspect to improve outcomes of patients with early-stage GEA is to offer new therapeutic solutions. Notably, despite the treatment algorithm of patients with advanced GEA heavily relies on molecular features, including HER2, deficient mismatch repair/microsatellite instability status (dMMR/MSI), programmed death-ligand 1 (PD-L1) expression, and Claudin18.2 over-expression, with several others (e.g., *FGFR2*amplification) being explored [25], the choice of peri-operative regimen is mostly independent of these characteristics, with peculiar aspects to consider from the prognostic and predictive point of view for dMMR/MSI patients [25]. The overexpression/amplification of HER2 is a key molecular alteration found in 15% patients with stage IV GEA, and HER2 testing is recommended in order to select the best available first-line treatment, along with evaluation of PD-L1 combined positive score (CPS). In case of HER2 positivity and PD-L1 CPS < 1%, the standard first-line treatment is doublet chemotherapy plus Trastuzumab, based on the results of the ToGa trial, where this combination improved median OS (mOS) to 13.8 months versus 11.1 with chemotherapy alone (HR 0.74; 95% CI 0.60–0.91; $p= 0.0046$) [10]. Contrarily, in case of concurrent PD-L1 CPS ≥ 1%, the KEYNOTE- 811 showed that the addition of pembrolizumab significantly improves prognosis with respect to doublet chemotherapy and trastuzumab only (mOS 20.5 months vs 15.6 months, $p= 0.0143$) in patients with advanced GEA [26]. This led to the approval of pembrolizumab, trastuzumab and platinum-based chemotherapy by EMA and FDA for first-line treatment of patients with metastatic GEA co-expressing HER2 and PD-L1 [2, 27]. Of note, many patients do not have access yet to this treatment as a standard of care, due to regulatory issues. However, most patients still inevitably progress to targeted strategies, highlighting the need to overcome primary and acquired treatment resistance. Focusing on HER2 positive tumors, previous trials combining classical anti-HER2 agents with novel compounds, beyond anti-PD-(L)1 agents, yielded disappointing results. In details, in the first-line setting, the Jacob trial found no significant survival benefit by adding pertuzumab to trastuzumab-based regimen; lapatinib plus chemotherapy also failed to significantly improve outcomes according to the results of TRIO- 013/LOGIC study [28, 29]. Similar negative results were observed also in the pre-treated population, namely the TyTAN trial, testing lapatinib with paclitaxel, and the GASTBY trial, investigating trastuzumab emtansine [30, 31]. However, more recent research efforts led to the development of antibody drug conjugates (ADCs), that combine a monoclonal antibody with a cytotoxic

payload with the aim to increase treatment accuracy and activity, and have the potential to overcome classic resistance mechanisms to anti-HER2 agents. Regarding patients with metastatic HER2 positive GEA, the Asian-based phase II DESTINY-Gastric01 study showed high activity and efficacy of Trastuzumab deruxtecan (T-DXd) as compared to physician's therapy of choice in the third-line setting (median OS 12.5 vs 8.4 months, HR 0.59; 95% CI 0.39–0.88; $p= 0.01$) [11]. The single-arm phase II DESTINY-Gastric02 trial confirmed the activity of T-DXd in the Western population, with 38% response rate, median PFS of 5.5 months and median OS of 8.1 months in pre-treated patients [12]. On this basis, the treatment with T-DXd is now standard of care in many countries after progression to a trastuzumab-based first-line therapy. Moreover, the randomized phase III DESTINY-Gastric04 study is currently ongoing, comparing T-DXd versus standard second-line therapy (i.e., paclitaxel plus ramucirumab) for HER2-positive GEA with retained HER2 positivity after progression to a trastuzumab-based first-line strategy (NCT04704934). Furthermore, the preliminary results of the multi-cohort phase Ib/II DESTINY-Gastric03 trial showed the safety and activity of the T-DXd combined with capecitabine or 5-fluorouracil in patients with advanced HER2 positive GEA. However, data regarding HER2 target therapy in the non-metastatic population are more conflicting. The phase III RTOG1010 trial did not show a significant improvement in DFS by adding trastuzumab to standard CROSS regimen followed by surgery for localized HER2 positive GEA (HR 0.99; 95% CI 0.71–1.39; $p= 0.97$). The randomized phase II/III PETRARCA study investigated the addition of trastuzumab and pertuzumab to peri-operative FLOT, finding a significant increase of the pathological complete response (pCR) rate; due to disappointing results from the Jacob trial, the study was interrupted and did not proceed to the planned phase III. However, survival outcomes were similar in the two arms, and the pCR is not a validated surrogate endpoint in clinical trials, thus potentially suggesting that trastuzumab-based regimens may not possess enough activity to eradicate MRD in the early-stage setting. Therefore, the opportunity to test new generation HER2-directed agents, such as T-DXd, in this specific setting is endowed with a strong clinical and biological rationale.

Based on these premises, we designed the TRINITY trial, a proof-of-concept, open-label, phase II trial enrolling patients with HER2 positive (defined as HER2 IHC score 3+ or score 2+ with positive ISH) GEA with ctDNA positivity at the Signatera™ liquid biopsy after standard pre-operative FLOT and surgery. The trial randomizes (1:1) patients either to continue with the standard post-operative FLOT or to receive T-DXd plus a fluoropyrimidine.

Unlike predesigned ctDNA static panels, Signatera is a personalized, tumor-informed assay that ensures that MRD can be detected with high sensitivity and specificity, powered to detect genetic variants down to 0.01% allele frequency [22, 32]. The experimental arm consists of 6 cycles of T-DXd at the standard dose of 6.4 mg/kg combined with 5-fluorouracil or capecitabine, according to physician's choice; these dosages reflect the ones used in the DESTINY-Gastric03 study (600 mg/m2/day continuous iv infusion on days 1–5 for 5-fluorouracil and 1000 mg/sq/BID oral on days 1–14 for capecitabine). Despite previous reports suggest that patients with HER2 IHC 2+/ISH positive tumors may show inferior responses to T-DXd, compared to those with HER2 IHC 3+ tumors (10–30% *versus*45–60%, respectively), these data, however, are limited, come from heterogeneous trials and are based on T-DXd monotherapy strategies. Conversely, both the combination of T-DXd with a fluoropyrimidine, and the confirmation of HER2 positivity on the surgical tissue may potentially lead to retained activity and efficacy of T-DXd in patients with IHC 2+/ISH positive tumors [12, 33]. The aim of the interventional study is to increase the rate of post-adjuvant ctDNA clearance rate, through a highly effective, personalized targeted treatment, and eventually increase the chance to truly cure patients. By including an observational phase for patients receiving standard-of-care treatment for their localized/locally advanced GEA, the study also aims at building a clinical and translational platform to elucidate biological, molecular and immune mechanisms underlying the response and refractoriness to the standard strategy.

In conclusion, the ongoing TRINITY study, by including the cutting-edge diagnostic tool of liquid biopsy and a novel, highly active drug, i.e. T-DXd, may increase the cure rate of patients with HER2-positive early-stage GEA who derive less benefit from standard approach and are at higher risk of developing disease recurrence.

Authors' contributions

V.N., A.R. and F.P. wrote the main manuscript draft and prepared Fig. 1 and Table 1. F.B., L.F., L.A., K.B., E.D.A., S.C., A.S., O.B., D.S., S.T., C.A.C., A.A., L.F., S.D.D., A.S., A.P., E.T., F.P., F.M. reviewed the draft and contributed to final manuscript. All authors reviewed and approved final version of the manuscript.

Funding

The TRINITY study is a no profit academic study sponsored by GONO (Gruppo Oncologico del Nord Ovest) and partially funded by AstraZeneca. The entire property of the data and results of the TRINITY study is held by GONO and the access to the aforementioned data could be granted upon specific and motivated and reasonable request directed to GONO. This work was supported by Fondazione AIRC per la Ricerca sul Cancro (code: IG30422) to F. Pietrantonio.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was registered on 02/02/2024 at Clinicaltrials.gov (NCT06253650) and it is currently ongoing.
The trial was submitted to CTIS and approved by the Ethics Committee "CET Comitato Etico Lombardia 4".
The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the Company policy on Bioethics and Human Biological Samples.
The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.
The names of patients will not be recorded; a sequential identification number will be attributed to each patient registered in the trial. This number will identify the patient and must be included on all eCRF. To avoid identification errors, patients' initials (maximum of 2 letters) and date of birth will also be reported on the eCRF. Investigators will guarantee that all persons involved in this study will respect the confidentiality of any information concerning the trial subject. All parties involved in this clinical trial will maintain strict confidentiality to assure that neither the person nor the family privacy of the patient participating in the trial is violated. Appropriate measures shall be taken to avoid the access of non-authorized persons to the trial data by assuring that only the principal investigator and its delegates will have the authorization to access trial data thanks to protected programs and strict organization flows. The investigator will guarantee to have a process in place to ensure that the site staff or service providers delegated by the investigator/institution can identify the occurrence of a (potential) serious breach and this will be promptly reported.
The processing of the personal data of patients taking part in the trial, and regarding data concerning consent, shall comply with local law on the privacy (D.Lg. 30 giugno 2003, n. 196, Codice in materia di protezione dei dati personali) and with the European Directive on the privacy of data, namely General Data Protection Regulation, EU regulation 2016/679 of 27 April 2016). The patient can withdraw consent whenever he/she wants and further data will not be collected, even if the already collected data will be used for the study's analyses.

Consent for publication

Not applicable.

Competing interests

FB received personal honoraria as invited speaker from Eli-Lilly, MSD, EISAI, Bristol Myers Squibb, AstraZeneca, Pierre Fabre; participation in advisory board for Servier, AAA Novartis. LF reports personal honoraria as invited speaker from Incyte, Bristol Myers Squibb, Lilly, AstraZeneca; research funding (to Institution) from MSD, Bristol Myers Squibb, AstraZeneca, Incyte, BeiGene, Astellas, Daiichi Sankyo, Roche; participation in advisory board for MSD, AstraZeneca, Incyte, Taiho, Servier, Daiichi Sankyo, Lilly, BeiGene and Astellas. AP reports personal fees for advisory boards from Takeda, GlaxoSmithKline, Daiichi Sankyo; research grant (institutional) from Amgen and GlaxoSmithKline. Travel grants from Amgen, Merck Serono, Astrazeneca FP reports personal fees from Amgen, Merck-Serono, MSD, Bayer, Astellas, Takeda, Servier, GlaxoSmithKline, Pierre-Fabre, Ipsen, J&J; and research grant (institutional) from Lilly, BMS, AstraZeneca, Incyte, Amgen, and Agenus. AR reports honoraria for speaker bureau or participation at advisory boards from Servier and MSD.
LF reports personal honoraria as invited speaker from Incyte, Bristol Myers Squibb, Lilly, AstraZeneca; research funding (to Institution) from MSD, Bristol Myers Squibb, AstraZeneca, Incyte, BeiGene, Astellas, Daiichi Sankyo, Roche; participation in advisory board for MSD, AstraZeneca, Incyte, Taiho, Servier, Daiichi Sankyo, Lilly, BeiGene and Astellas.
AP reports personal fees for advisory boards from Takeda, GlaxoSmithKline, Daiichi Sankyo; research grant (institutional) from Amgen and GlaxoSmithKline. Travel grants from Amgen, Merck Serono, Astrazeneca.
FP reports personal fees from Amgen, Merck-Serono, MSD, Bayer, Astellas, Takeda, Servier, GlaxoSmithKline, Pierre-Fabre, Ipsen, J&J; and research grant (institutional) from Lilly, BMS, AstraZeneca, Incyte, Amgen, and Agenus.
AR reports honoraria for speaker bureau or participation at advisory boards from Servier and MSD.

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Received: 23 September 2024 Accepted: 1 April 2025
Published online: 08 April 2025

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