GASTHER2: Efficacy of adding trastuzumab to standard chemotherapy in patients with advanced HER2-negative gastric cancer and HER2 positive expression in circulating tumor cells

Study Protocol and Statistical Analysis Plan

authors: Mauro DS Donadio[1], Victor Hugo F de Jesus[1], Francisca Giselle R Moura[1], Ângelo B Brito[1], Marcos PG Camandaroba[1], Newton Augusto[1], Celso A Mello[1], Tiago C Felismino[1], Virgílio Souza[1], Milton JB Silva[1], Michelle LR Dias[2], Ludmilla TD Chinen[3], Rachel SP Riechelmann[1].

Departaments: 1- Clinical Oncology; 2- Radiology; 3- International Research Center

08 / November / 2019

Version 2 November 2019

SUMMARY:

RESUME: BACKGROUND: Gastric cancer is one of the most common cancers and is one of the most deadly cancers. Most patients have advanced disease and should receive first-line trastuzumab-associated chemotherapy when the biopsy is positive for immunocytochemical expression and / or HER2 gene amplification. A study conducted by our group noted that there may be disagreement in HER2 expression between circulating tumor cells (CTCs) and tumor tissue. However, the effectiveness of using anti-HER2 treatment when only CTC express HER2 is unknown. The present study aims to evaluate the expression of HER2 in patients with relapsed or metastatic gastric cancer and what would be the efficacy of adding trastuzumab to chemotherapy when tumor tissue is negative for HER2, but there is expression of this gene in CTCs. OBJECTIVES: The primary objectives are to evaluate HER2 expression in circulating tumor cells of relapsed or metastatic gastric cancer patients with negative HER2 expression on tissue biopsy and response to standard treatment with combined anti-HER2 chemotherapy in this population. Secondary objectives are to assess the prognostic impact of HER2 positivity on circulating tumor cells in advanced gastric tumors and to evaluate HER2 expression in CTCs at the time of treatment progression. METHODS: The investigators will prospectively evaluate HER2 expression in CTC and its response to treatment with standard chemotherapy and addition of trastuzumab in patients with relapsed or metastatic gastric cancer with positive expression of HER2 only in CTC. HER2 expression in tissue and in CTC will be evaluated by immunocytochemistry. Descriptive statistics will be used to report the results of categorical and continuous variables, and respective dispersion measures. Time-to-event variables will be reported in Kaplan Meyer medians and curves. EXPECTED RESULTS: Upon completion of the study the investigators expect to show the frequency of HER2 expression in this specific population, higher radiological response rate with trastuzumab combination compared to chemotherapy alone, determine the prognostic impact associated with HER2 expression in CTCs and show the frequency of HER2 expression in CTCs at the time of study treatment progression. This study may open a new opportunity for anti-HER2 treatment for gastric cancer patients.

Keywords: gastric cancer, HER2, trastuzumab, circulating tumor cells

INTRODUCTION

Gastric cancer is one of the most common cancers worldwide. Its incidence varies according to its geographic region, being more common in East Asia, Eastern Europe and South America. About 70% of gastric neoplasms occur in developing countries. (1) Globally, it is estimated that in 2018 over 1 million cases were diagnosed with about 780,000 deaths, making gastric cancer the 5th in frequency and 3rd in mortality. (2,3) In Brazil, 13,540 new cases were diagnosed in 2018, being the third leading cause of cancer death in Brazil in males. (4) Most patients already have inoperable, advanced or metastatic disease at diagnosis, thus requiring palliative treatment. (5)

Over 90% of stomach tumors are adenocarcinomas and, in terms of histology, Lauren's classification subdivided gastric cancer into diffuse (undifferentiated) or intestinal cancer (6) and both types have distinct clinical and pathological features. The diffuse type occurs in all age groups with equal gender distribution, involves the body or the entire stomach and has a greater tendency to invade the gastric wall (resulting in plastic linitis) and to metastasize, as well as a faster disease progression and worse prognosis. (7) On the other hand, the intestinal type predominates in men and the elderly, occurring predominantly in the gastric antrum and notch. Although both subtypes are related to *H. pylori* infection, in the intestinal type the evolution of sequential preneoplastic alterations is observed, namely: atrophic gastritis / intestinal metaplasia; dysplasia and adenocarcinoma. Such a sequence may not occur in diffuse type. (7,8) There is also a rare genetically related type called hereditary diffuse gastric cancer (HDGC) that is not related to H. pylori infection. Diffuse-type adenocarcinoma is the major histological subtype of HDGC. (9) In addition to diffuse hereditary gastric cancer syndrome, gastric cancer is present in the description of several other hereditary syndromes, such as Lynch syndrome, juvenile polyposis syndrome, Peutz-Jeghers syndrome, Familial Adenomatous Polyposis, ataxia-telangiectasia syndrome, Li-Fraumeni and Xeroderma Pigmentosa. (10-14)

Regardless of association with genetic syndrome or even histological type, it is necessary to investigate the amplification of the human epidermal growth factor 2 (HER2) gene in the case of recurrent or metastatic gastric cancer. Today of well-defined importance in gastric cancer, HER2 was initially recognized 30 years ago as an amplified oncogene in over 20% of breast cancers and, after the genomic revolution, has recently been seen to

amplify in many other cancers. (15) HER2 is a tyrosine kinase-like receptor that crosses the cell membrane of cells, with the extracellular half not coupling to ligands, but designed to recognize ligand-activated forms of family members as the factor of human epidermal growth (EGFR), HER3 or HER4, leading to dimerization with these receptors. The cytoplasmic part of HER2 and its partners contains a kinase domain that triggers HER2 kinase activity. The end result is phosphorylation at various tyrosine residues and recruitment of second messenger proteins for these phosphotyrosines. When the gene is amplified in cancer cells, the result is massive overexpression of HER2, which causes continuous activation of the various biological pathways that promote cell proliferation. (15) For gastric cancer, positivity for HER2 expression, as assessed by immunohistochemistry (IHC) or in situ hybridization immunofluorescence (FISH), may vary between 8 and 36% between studies in the literature; However, those studies that considered only intestinal histology found positive expression of HER2 between 20 and 30% in patients' biopsies. (16) A study conducted at AC Camargo identified 12% HER2 expression and 8% amplification in gastric tumors, with greater association with intestinal subtype and poor survival. (17)

Because it has a worse prognosis and has a target drug, treatment of tumors with HER2 amplification requires HER2-targeted agents, often in combination with chemotherapy or hormone therapies. (15) Trastuzumab-associated chemotherapy (fluoro-uracil and platinum) is the first-line standard treatment for HER2-positive metastatic gastric cancer based on the phase III ToGA study (Trastuzumab for Gastric Cancer). This study recruited 594 patients and showed a median overall survival gain of 13.8 months for the combination versus 11.1 months in the chemotherapy arm alone (hazard ratio [HR], 0.74; 95% CI, 0.60 to 0.91; P = 0.0046). Survival gain was even more significant in the HER2 overexpressed subgroup (IHC 3+ or IHC 2+ with positive FISH), which achieved a median overall survival of 16 months. (5)

Regarding the second line, however, phase III studies failed to demonstrate the benefit of using anti-HER2 therapy. In the TyTan study, the addition of lapatinib in the second line was not superior to chemotherapy alone. (18) In the GATSBY study, the use of trastuzumab entansine was also not superior to taxane chemotherapy. In this study, 77% of

patients received first-line anti-HER2 agents. (19)

Several clinical reasons may explain these disappointing results; One of these is resistance acquired after use of anti-HER2-based therapy. Another reason is the loss of HER2 expression, as reported in a study by Pietroantonio et al that demonstrated loss of HER2 positivity and overexpression in 32% of the tissue samples analyzed after anti-HER2 therapy. (20) It is already known that in selective breast cancer treatment pressure can eradicate HER2-expressing clones and lead to proliferation of those HER2-negative clones, a phenomenon that can occur in gastric cancer because of the marked heterogeneity that exists in stomach tumors. (21,22) In addition, it is known that HER2 expression discrepancy may exist between primary and metastatic or recurrent breast tumors (probably due to sample variability). (23) In discordant cases, HER2-positive metastases from negative primary tumors are more frequent than the opposite. (20) Therefore, it is recommended that metastatic disease on first relapse be biopsied as part of the investigation and HER2 status should be reevaluated. (24) In cases of gastric cancer, this issue has not been widely explored.

In this sense, the GASTHER 1 study investigated the role of HER2 expression reassessment in stomach cancer at primary, metastatic or recurrent sites in patients whose primary tumor was initially negative for HER2 expression. The results showed a positive rescue of 8.7%, confirming the relevant heterogeneity of HER2 status. (25).

This heterogeneity, however, may also be associated with the HER2 status assessment method. A prospective study in patients with localized gastric adenocarcinoma and treated with perioperative chemotherapy at our institution found 69.4% agreement for HER2 expression in primary tumor tissue biopsy and in paired circulating tumor cells (CTCs). HER2 in CTCs showed higher positivity compared to tumor tissue (43% x 11%). (26) The positivity in HER2 CTCs was 60% for HER2-negative localized gastric cancer patients treated with perioperative chemotherapy whose disease recurred. Also, HER2 expression in CTCs correlated with progression-free survival, but in the tumor tissue the same relationship was not found.

BACKGROUND

Analysis of CTCs from the blood of patients with gastric adenocarcinoma may be useful to better understand the behavior of the disease, as well as patients more likely to respond to treatment, and may offer a less invasive way of investigating tumor dynamics. In addition, prospective evaluation of HER2 expression in CTCs has not been evaluated in metastatic gastric cancer, and the frequency with which this expression changes after first-line treatment with standard regimen with trastuzumab at the time of disease progression. Thus, this study is necessary to evaluate the frequency with which these phenomena occur and thus expand the knowledge of the dynamics of gastric cancer tumor biology.

Hypothesis:

Primary:

1- There is disagreement in HER2 expression positivity between diagnostic tissue biopsy (preferably metastasis) and circulating tumor cells in metastatic intestinal cancer.

2- Positivity for HER2 expression in circulating tumor cells in relapsed or metastatic gastric cancer may predict response to standard treatment with trastuzumab combination chemotherapy.

Secondary:

1- HER2 expression may have prognostic effect when positive in CTCs.

2- HER2 expression in CTCs may be modified following treatment with anti-HER2 therapy.

Objectives:

Primary:

1- Evaluate HER2 expression in circulating tumor cells of patients with relapsed or metastatic gastric cancer with negative HER2 expression on tissue biopsy.

2- To evaluate the response to standard treatment with combination chemotherapy with trastuzumab in relapsed or metastatic gastric cancer with positive expression of HER2 on CTC and negative on tissue biopsy.

Secondary:

1- To verify the prognostic impact of HER2 positivity in circulating tumor cells in advanced gastric tumors;

2- Evaluate HER2 expression in CTCs at the time of progression to standard first-line treatment with anti-HER2 therapy in patients who previously had HER2 positivity.

Outcomes:

Primary:

1- Frequency of HER2 expression among circulating tumor cells of patients with relapsed or metastatic gastric cancer with negative expression of HER2 in tumor tissue (primary or metastatic, preferably).

2- RECIST1.1 radiological response rate following standard treatment with combined chemotherapy with trastuzumab in relapsed or metastatic gastric cancer with positive expression of HER2 in CTC and negative in tumor tissue.

Secondary:

1- Prognostic impact measured by progression-free survival and overall survival using standard treatment with trastuzumab combination chemotherapy.

2- Frequency of HER2 immunocytochemical expression in CTCs at the time of progression to standard first-line chemotherapy and trastuzumab treatment.

Methods:

Study Design:

This is a single-phase prospective unicentric phase II study evaluating HER2 expression in circulating tumor cells and their response to standard treatment with trastuzumab combination chemotherapy in cases of positive expression in patients with relapsed or metastatic gastric cancer with expression HER2 negative in the tumor tissue.

Eligible patients who sign the Informed Consent Form (ICF) will be submitted to collection of CTCs for immunocytochemical expression (ICC) and HER2 in situ fluorescence hybridization test (FISH) at two times: in the diagnosis of relapse or disease untreated first-line palliative metastatic treatment (for eligibility assessment, see "Eligibility") and, in progression after standard treatment of platinum-based chemotherapy and fluoropyrimidine and trastuzumab. Eligible patients will be treated with standard chemotherapy (FOLFOX schedule every 2 weeks) as per institutional routine, combined with experimental use of trastuzumab at the standard dose (3) of 8 mg / kg in the first cycle D1, followed by 6 mg / kg every 2 weeks in the remaining cycles until disease progression, unacceptable toxicity or withdrawal of consent. The use of trastuzumab for HER2-positive gastric cancer is a worldwide standard and widely used in our service for patients with supplemental health insurance; Trastuzumab is not available from the Unified Health System (SUS). The standard definition of HER2 positive expression is made by immunohistochemistry (IHC) or FISH, the latter being performed only if IHQ 2+. FISH will not be performed when IHQ 0 or 1+ in tissue biopsy as the investigators will stick to the standard procedures of current care. As in this study the investigators will evaluate treatment with trastuzumab based on HER2 expression by immunocytochemistry and FISH in the CTCs of all participants, the investigators considered this intervention as experimental. Brazilian company Libbs, which produces the biosimilar trastuzumab used in AC Camargo, will donate the drug and provide insurance to cover any adverse effects associated with trastuzumab.

The following clinical data will be collected prospectively and structured in a password-protected electronic database: gender, age, gastric tumor location (esophageal-gastric transition, fundus, body or pylorus), ECOG scale performance status, sites

metastases, previous treatments, comorbidities (defined as any clinical condition requiring pharmacological therapy), concomitant chronic use drugs, body mass index, treatments received for metastatic disease (2nd and 3rd lines), response to treatment, dates of progression, further treatment and death.

Research Methodology for Circulating Tumor Cells (CTC)

A 10 ml pre-study peripheral blood sample will be collected for circulating tumor cell (CTC) screening and characterization of protein expression by immunocytochemistry. In case of results pointing to good accuracy of CTC in this neoplasia, these results can be applied as a diagnostic tool for patients with contraindication of tissue biopsy.

Isolation and Purification of CTC (blood samples):

Blood samples collected in EDTA tubes (10 mL) will be processed within 4 hours, diluted 1:10 in erythrocyte lysis buffer and filtered on the ISET system. After filtration, the membranes will be washed with PBS, dried at room temperature, protected from light and stored at -20 ° C until the time of analysis.

Immunocytochemistry in ISET Membranes:

For immunocytochemistry and protein expression analysis, membrane spots will be cut and placed in 24-well plates. Each spot will be hydrated with Tris Buffered Saline (TBS) for 10 minutes. The cells will be permeabilized with 0.2% TBS Triton X-100 for 5 min at room temperature. Following a further wash with TBS, the membranes will be incubated for 15 minutes in the dark at room temperature with a 3% hydrogen peroxide solution and washed again with TBS. Then the antibodies to be screened will be applied to the spots and incubated for one hour. For negative control, the primary antibody will be omitted. The development will be done with Dual long system HRP (DakoTM) and chromogen Diaminobenzidine 3,3 '(DAB) (DakoTM). For reading, the spots will be stained with hematoxylin for 1 minute and adhered to slides with aqueous mounting medium. CTC will

be characterized according to the following criteria: nuclear size equal to or greater than 16µm, nuclear contour irregularity, presence of visible cytoplasm, high nucleus-cytoplasm ratio (> 0.8), as described by Krebs et al. .19 When any of the described criteria is missing, the cells will be classified as atypical. Results will be given in CTC number per mL of blood.

FISH on ISET Membranes:

For FISH and HER2 mRNA expression analysis the investigators will use the ACD RNAscope® in situ Hybridization (ISH) 2.5 HD Detection Kit (Brown), previously standardized for ISET membrane spots. 5 minutes at room temperature (RT) and then with 1% formaldehyde for 5 minutes at RT Subsequent washes between steps will be performed with distilled water (2 washes) Membranes with CTCs will be incubated in ScopeHydrogen Peroxide RNA (ACD) for 10 minutes. After washing, the membranes will be incubated with cytological pepsin (ZytoVision) for 10 minutes at RT the membranes will be washed in 70%, 90% and 100% ethanol for one minute each. The membranes will be incubated with the HER2 mRNA probe in the hybridizer at 40 ° C (wet) for 2 hours From this step, the washes will be performed with the 1X Wash Buffer (ACD) (2 washes each step). Incubate the membranes with Amp 1 (ACD) for 30 minutes at 40 ° C (wet) in the hybridizer. After washing, the investigators will incubate the membranes with Amp 2 (ACD) for 15 minutes at 40 ° C (humid) in the hybridizer. Then the investigators will incubate the membranes with Amp 3 (ACD) for 30 minutes at 40 ° C (wet) in the hybridizer. After washing, the membranes will be incubated with Amp 4 (ACD) for 15 minutes at 40 ° C (wet) in the hybridizer. Washing. The investigators will incubate the membranes with Amp 5 (ACD) for 30 minutes in T.A. Wash. The membranes will be incubated with Amp 6 (ACD) for 15 minutes in T.A. Wash. The investigators will mix equal volumes of BROWN-A and BROWN-B (ACD). The membranes will be incubated with DAB in the sealed tray for 10 min at RT. The DAB will be removed and washed 2X in distilled water. The investigators will incubate in 50% hematoxylin (after dilution, lasts 1 week) for 2 min at RT. The investigators will wash 2X with distilled water. The slides will be adhered with aqueous mounting medium and coverslip. Membrane reading will be performed under a bright field microscope.

Study Population

Eligibility:

Patients will be recruited from Clinical Oncology outpatient clinics and must meet all the criteria below Inclusion and none Exclusion:

Inclusion criteria

- Age 18 or over
- Histological diagnosis of recurrent or metastatic gastric cancer
- Immunohistochemistry (IHC 0 or 1+) or FISH negative (if IHQ 2+) for HER2 on tissue biopsy, according to institutional routine
- Candidates to initiate first-line palliative treatment; Previous adjuvant treatment is allowed since its termination occurred at least 12 months ago
- ECOG performance range 0 to 2
- Informed consent form signed by patient or legal representative

Exclusion Criteria

- Patients already on or previously using anti-HER2 therapy
- Left ventricular ejection fraction (LVEF) <55% baseline, as already evaluated in the gastric cancer routine
- Pregnant or lactating women
- Patients participating in other experimental drug protocols
- Patients who received previous palliative chemotherapy
- Another synchronic neoplasia requiring systemic treatment

Treatment

The first-line standard treatment for gastric cancer is based on fluorpyrimidine and platinum-containing chemotherapy (FOLFOX). When the tumor expresses HER2 3+ on IHC or 2+ and is confirmed by FISH, trastuzumab at the standard dose of 8 mg / kg in D1 of the first cycle is added, followed by 6 mg / kg every 2 weeks for the remaining cycles until disease progression, unacceptable toxicity. (3) The investigators will use the same doses of trastuzumab, but HER2 positivity is determined by immunocytochemical expression and FISH in CTCs.

Trastuzumab is a human monoclonal IgG antibody that selectively targets HER2, a human epidermal growth factor (EGFR) receptor. Trastuzumab inhibits the growth of HER2 overexpressing tumor cells on the surface of the breast, gastric, ovarian, lung and prostate cancer cells. (27,28) Mechanisms involved include: decreased vascular epithelial growth factor (VEGF) production, antibody-dependent cell-mediated cytotoxicity, G0 / G1 cell cycle cytotoxicity, and inhibition of signaling pathways. (27)

Contraindications:

• history of hypersensitivity reaction to trastuzumab or Chinese hamster ovary cell proteins (27)

Careful:

• patients with pre-existing cardiac dysfunction or left ventricular ejection fraction (LVEF) of 55% or less (27)

• patients with pre-existing severe lung disease or extensive involvement of the lung parenchyma by tumor; Patients who experience dyspnea at rest due to comorbidities or advanced neoplasia may be at higher risk of death from infusion reaction. (27) These are ineligible for treatment with trastuzumab and often chemotherapy due to poor performance status (ECOG> 2).

ADVERSE EFFECTS:

Trastuzumab has been used by thousands of cancer patients for at least 2 decades. Its first indication was in HER2-positive breast cancer and later in gastric cancer. It is currently being used in other solid tumors with tumor expression of HER2, such as bile duct and colon tumors. It is a safe drug with few risks but requires attention for its cardiotoxic effect. Severe trastuzumab-induced cardiotoxicity is described in less than 1% of breast cancer patients. Already in patients with stomach tumors, the incidence is anecdotal, most likely because the time of use is shorter (approximately 8 to 10 months). Signs and symptoms include: dyspnea, cough, paroxysmal nocturnal dyspnea, peripheral edema, congestive heart failure (CHF), or reduced LVEF by 10% or more. (27) Trastuzumab cardiac dysfunction is cumulative dose related and is reported to be highly reversible. LVEF returns to baseline during treatment at 1.5 months after trastuzumab; however, some cases have resulted in disabling heart failure, thrombosis and stroke and / or death. (27.30)

Risk factors for trastuzumab-related cardiotoxicity include:

• advanced age (> 65 years)

• a lower left ventricular ejection fraction (LVEF)

• higher body mass index (> 25) on screening

There is some uncertainty as to whether smoking, diabetes, hypothyroidism, or hyperlipidemia are associated with cardiotoxicity following drug use. Prior or concurrent irradiation does not increase cardiac events, but may increase the incidence of leukopenia. (27) However, there are inadequate long-term data to correlate cardiac dysfunction with trastuzumab with simultaneous or prior radiation. (27) Trastuzumab disrupts the HER2 signaling pathway in the heart, which maintains normal cardiac cell growth, repair and survival. This results in changes in cardiac contractility, but does not cause myocardial cell death. (31)

Toxicity management:

Suggested management recommended for trastuzumab-related cardiotoxicity includes drug retention for approximately 3 weeks if LVEF falls 10 to 15 points below baseline and / or below 50%. Trastuzumab may be resumed when LVEF improves. If LVEF does not improve within approximately 3 weeks, discontinuation of the drug is considered. (27.31)

LVEF should be evaluated before starting trastuzumab treatment, repeated every 3 months during treatment, and then every 6 months after the end of treatment, up to 24 months from the last dose of trastuzumab.

Statistical Analysis Plan

Statistical plan

Descriptive statistics will be used to report the results of categorical and continuous variables, and respective dispersion measures. Time-to-event variables will be reported in Kaplan Meyer medians and curves.

Sample size calculation

To calculate the sample size, the investigators considered HER2 positive expression H0 in tumors with negative expression in tissue biopsy of 60%, based on the results produced by our group. (13) Two-tailed type I error margin of 10% was considered. With these calculations, the sample number will be 85 patients so the investigators can find 50 cases of HER2 positive expression in CTCs.

Recruitment and Study Duration

At AC Camargo Cancer Center, 100 new patients with gastric adenocarcinoma are treated annually. Considering that 50% correspond to the intestinal type and of these, 80% are HER2 negative on tissue biopsy, the investigators estimate to recruit the study

population within a maximum of 2 years and to complete the study within 5 years.

Schedule

TABLE 1. Complete procedural summary of the study after signing the consent form

Procedure	Screening	baseline evaluation *	D1C2 D1C3 D1C4	D1C5	D1C6 D1C7 D1C8...	PD
Eligibility Assessment						
Meets eligibility criteria	x	x				
Demographics, medical history and previous treatment		x				
CTC Collection for Evaluation of HER2 Expression**	x					x
Trastuzumab Dispensing			X	x		
Clinical Safety Assessment						

Physical exam		x	x	x	x	x
Vital signs, body weight and ECOG		x	x	x	x	x
Signs and symptoms assessment		x	x	x	x	x
Review of medications in use		x	x	x	x	x
Adverse Event Assessment			x	x	x	x
Laboratory safety assessment						
HMG, Ur, Cr, Na , K	x		X	X		
Ecocardiogram***	x			X		
Image assessment						
CT of lung, abdomen and pelvis or MRI****	x			X		x

* within 48h of D1C1

**at least 7 days before D1C1

*** Ecocardiogram: the first within 10 days of D1C1 and should be requested every 3 months during treatment

**** Within 4 weeks of D1C1 and every 4 cycles (± 5 days)

Funding

This is an academic and initiative study of the main investigator (Rachel P Riechelmann). There will be no cost to the patient in relation to the research procedures, such as blood collection for CTC evaluation and trastuzumab drug. The cost of conducting the test will be through a research grant provided by the brazilian company Libbs. In the case of CTC-positive HER2 patients and therefore with indication for trastuzumab combined with chemotherapy, Libbs will donate commercially available biosimilar trastuzumab in the

brazilian market and standard AC Camargo for treatment until disease progression, unacceptable toxicity or withdrawal of consent. The company will only have access to the final result of the work and will not participate in the study design, or in any phase of its execution, as well as not have access to the individual data of patients included in this project. Research grant money donated by Libbs includes funding for insurance to cover potential damages associated with research and coverage for treatment of adverse events associated with trastuzumab. The patient's paying source pays for routine treatment: chemotherapy, blood tests and imaging, as is already done in the treatment routine of these patients. Echocardiography is warranted as a routine test for HER2-positive gastric cancer patients because the trastuzumab package insert in Brazil describes that this medication is indicated for first-line advanced HER2-positive gastric cancer without specifying by which diagnostic test the HER2 result was obtained.

Ethical Considerations

The study will be conducted in accordance with the International Harmonization Conference protocol of Good Clinical Practice guidelines (ICH GCP), and applicable local laws and regulatory requirements. The Informed Consent Form (ICF) must comply with ICH GCP guidelines, local regulatory regulations, and legal requirements.

The investigator should ensure that each study patient, or legal representative, is fully informed about the nature and objectives of the study and possible risks associated with participation. The investigator will obtain written consent from each patient before any specific study activity is performed. The investigator will retain one copy of each consent form signed by the patient. It will be emphasized that participation is voluntary and that the patient has the right to refuse participation or leave in the middle of the study at any time. This will not impair subsequent patient care. There is a risk of loss of confidentiality if patient data is identified, however, all reasonable measures will be taken to prevent this from occurring, such as: database without participant identifying information, password protected database and only by study investigators.

This is an academic and initiative study of the principal investigator (Rachel P Riechelmann). The Libbs company that will have access only to the final result of the work and will not participate in the study design or in any phase of its accomplishment, as well as not have access to the individual data of the patients included in this project.

The protocol was approved by the Local Ethics Committee.

References:

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90.
2. World Health Organization. International Agency for Research on Cancer. GLOBOCAN 2018: stomach cancer fact sheet. 2018.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.
4. Estatísticas de câncer. INCA, Jun/2019. Disponível em https://www.inca.gov.br/numeros-de-cancer
5. Van Cutsem E, Bang YJ, Feng-Yi F, Xu JM, Lee KW, et al. HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. Gastric Cancer. 2015;18(3):476-84.
6. Lauren P. The two histological main types of gastric carcinoma: Diffuse and so-called Intestinal-type carcinoma. An attempt at histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31-49.

7. Vauhkonen M, Vauhkonen H, Sipponen P. Pathology and molecular biology of gastric cancer. Best Pract Res Clin Gastroenterol. 2006;20(4):651-74.
8. Polkowski W, van Sandick JW, Offerhaus GJ, ten Kate FJ, Mulder J, et al. Prognostic value of Laurén classification and c-erbB-2 oncogene overexpression in adenocarcinoma of the esophagus and gastroesophageal junction. Ann Surg Oncol. 1999;6(3):290-7.
9. Fitzgerald RC, Hardwick R, Huntsman D, Carneiro F, Guilford P, Blair V, et al; International Gastric Cancer Linkage Consortium. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet. 2010;47(7):436-44.
10. Lindor NM, McMaster ML, Lindor CJ, Greene MH; National Cancer Institute, Division of Cancer Prevention, Community Oncology and Prevention Trials Research Group. Concise handbook of familial cancer susceptibility syndromes - second edition. J Natl Cancer Inst Monogr. 2008;(38):1-93.
11. Haidle JL and Howe JR. Juvenile Polyposis Syndrome. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019.
12. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res. 2006;12(10):3209-15.
13. Anaya DA, Chang GJ and Rodriguez-Bigas MA. Extracolonic manifestations of hereditary colorectal cancer syndromes. Clin Colon Rectal Surg. 2008;21(4):263-72.
14. Setia N, Clark JW, Duda DG, Hong TS, Kwak EL et al. Familial Gastric Cancers. Oncologist. 2015;20(12):1365-77.
15. Ruiz-Saenz A, Moasser MM. Targeting HER2 by Combination Therapies. J Clin Oncol. 2018;36(8):808-811.
16. Boku N. HER2-Positive Gastric Cancer. Gastric Cancer. 2014;17(1):1-12.
17. Begnami MD, Fukuda E, Fregnani JH, Nonogaki S, Montagnini AL et al. Prognostic implications of altered human epidermal growth factor receptors (HERs) in gastric carcinomas: HER2 and HER3 are predictors of poor outcome. J Clin Oncol. 2011;29(22):3030-6.
18. Satoh T, Xu RH, Chung HC, Sun GP, Doi T, et al. Lapatinib plus paclitaxel versus

paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in Asian populations: TyTAN--a randomized, phase III study. J Clin Oncol. 2014;32(19):2039-49.

19. Thuss-Patience PC, Shah MA, Ohtsu A, Van Cutsem E, Ajani JA, et al. Trastuzumab emtansine versus taxane use for previously treated HER2-positive locally advanced or metastatic gastric or gastro-oesophageal junction adenocarcinoma (GATSBY): an international randomised, open-label, adaptive, phase 2/3 study. Lancet Oncol. 2017;18(5):640-653.
20. Pietrantonio F, Caporale M, Morano F, Scartozzi M, Gloghini A, et al. HER2 loss in HER2-positive gastric or gastroesophageal cancer after trastuzumab therapy: Implication for further clinical research. Int J Cancer. 2016;139(12):2859-2864.
21. Niikura N, Tomotaki A, Miyata H, et al. Changes in tumor expression of HER2 and hormone receptors status after neoadjuvant chemotherapy in 21 755 patients from the Japanese breast cancer registry. Ann Oncol. 2016; 27: 480– 7.
22. Abrahao-Machado LF and Scapulatempo-Neto C. HER2 testing in gastric cancer: An update. World J Gastroenterol. 2016;22(19):4619–25.
23. Strien L, Leidenius M, von Smitten K, et al. Concordance between HER-2 and steroid hormone receptor expression between primary breast cancer, sentinel node metastases, and isolated tumor cells. Pathol Res Pract 2010;206:253– 8.
24. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997– 4013.
25. Park SR, Park YS, Ryu MH, Ryoo BY, Woo CG, et al. Extra-gain of HER2-positive cases through HER2 reassessment in primary and metastatic sites in advanced gastric cancer with initially HER2-negative primary tumours: Results of GASTric cancer HER2 reassessment study 1 (GASTHER1). Eur J Cancer. 2016;53:42-50.
26. Abdallah EA, Braun AC, Flores BCTCP, Senda L, Chinen LTD, et al. The Potential Clinical Implications of Circulating Tumor Cells and Circulating Tumor Microemboli in Gastric Cancer. Oncologist 2019;24:e854-63
27. Hoffmann-La Roche Limited. HERCEPTIN® product monograph. Mississauga,

Ontario; 6 January 2012.

28. Voorzanger-Rousselet N, Garnero P. Biochemical markers in oncology. part I: molecular basis. part II: clinical uses. Cancer Treat Rev 2007;233:230-283.
29. Hoffman-La Roche Limited. HERCEPTIN® product monograph. Mississauga, Ontario; 15 July 2014
30. AHFS Drug Information® (database on the Internet). Trastuzumab. Lexi-Comp Inc., 15 March 2012.
31. Mackey JR, Clemons M, Cote MA, et al. Cardiac management during adjuvant trastuzumab therapy: recommendations of the canadian trastuzumab working group. Curr Oncol 2008;15(1):24-35