Phase II Trial for Intestinal Microbiome Modulation With Antibiotics in the Neoadjuvant Treatment of Locally Advanced Rectal Cancer

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PHASE II CLINICAL STUDY OF INTESTINAL MICROBIOTA MODULATION WITH ORAL METRONIDAZOLE IN THE NEOADJUVANT TREATMENT OF LOCALLY ADVANCED ADENOCARCINOMA OF THE RECTUM.

DEPARTMENTAL PROJECT

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SUMMARY

Colorectal cancer is the second most common cancer in the world in both sexes. One third of these tumors are located in the rectum, and treatment can involve up to three modalities: radiotherapy, chemotherapy and surgery. In cases where radiotherapy and chemotherapy are indicated.

Neoadjuvant chemotherapy and radiotherapy reduces the risk of locoregional relapse in locally advanced tumors. Schemes that brought chemotherapy into the preoperative phase, called totally neoadjuvant treatment (TNT), have also shown a gain in disease-free survival. For some years now, thanks mainly to Brazilian researchers, subgroups of patients have been selected for treatment without surgery, when chemotherapy for preoperative purposes induces a complete clinical response. This strategy is now known as "Watch and Wait" (WW). Even higher organ preservation rates have been reported with a long-course chemotherapy and radiotherapy regimen, followed TNT with FOLFOX. These treatment regimens have reached a plateau in clinical response rates, which has led researchers around the world to look for strategies that can increase response rates. Intestinal microbiota studies carried out by our group, well as results from other international groups, have shown that overpopulation of some genders of anaerobic bacteria is associated with worse response to treatment. To date, no study has attempted to intervene in the intestinal microbiota in order to increase the complete response rate in rectal cancer. Our proposal aims to modulate the intestinal microbiota by means of a phase 2 clinical trial, the intervention for which is the use of metronidazole.

INTRODUCTION

The treatment of carcinoma of the rectum involves different modalities. The main goal of preoperative treatment is to achieve maximum clinical and pathological response. Previous studies have shown that some genera of anaerobic bacteria are associated with poor response and open up prospects for increasing response rates to neoadjuvant treatment by modulating the intestinal microbiota at the start of treatment. Our proposal is based on the use of the antimicrobial metronidazole at the start of radiotherapy, with the aim of reducing the anaerobic population in the rectal lumen and inducing higher response rates.

Achieving a complete clinical response to radiochemotherapy allows for a non-operative therapeutic alternative, also known as Wacht and Wait (WW). This strategy is now adopted worldwide and was developed by the Brazilian surgeon, Prof. Angelita Habr-Gama. (Habr-Gama, 2004; Van der Valk, 2018). Current cytotoxic treatment regimens seem to have reached a clinical response plateau (Garcia-Aguilar ;2022; Jimenez-Rodriguez; 2021). Our proposal involves a simple, low-cost intervention with great potential to increase complete response rates to treatment and avoid high morbidity surgeries in a greater number of patients with rectal cancer.

The multidisciplinary Colorectal Cancer Group team at the A.C. Camargo Cancer Center has a strong track record in this line of research. Both clinical and translational research, more than 10 scientific articles have been published in the last 5 years in indexed journals investigating molecular factors that predict response to neoadjuvant treatment in rectal cancer. The group has also conducted a randomized clinical trial in the treatment of rectal cancer. More recently, the group has published highly relevant data on intestinal microbiota and response to treatment.

Colorectal cancer is already a public health problem in Brazil's major metropolitan regions.

Particularly in the case of rectal cancer, treatment often causes significant social and work-related consequences.

Based on previous research conducted and published by our group, we now propose a simple, low-cost and effective clinical intervention, very low risk of adverse events, which has the potential to change the current results in the treatment of rectal cancer.

THEORETICAL FRAMEWORK

Diagnosis and Staging of Rectal Cancer:

The diagnosis of rectal cancer is made with a biopsy of the lesion and anatomopathological confirmation, usually via colonoscopy, rectosigmoidostomy or anuscopy. The most common histological type is adenocarcinoma, followed by squamous cell carcinoma and other rarer neoplasms such as gastrointestinal stromal tumors (GISTs), neuroendocrine tumors and melanoma (INCA 2020).

After diagnosis, the disease is staged using imaging tests. The recommended imaging tests are CT scans of the chest and upper abdomen and MRI of the pelvis. In selected cases, PET scans (PET-CT) are added. The tumor marker carcinoembryonic antigen (CEA) is routinely requested before treatment begins, as it is a useful marker in the patient's oncological follow-up (Valadão, 2022).

As already established in the scientific community, the international TNM-AJCC classification is used.

Treatment of Rectal Cancer:

The treatment of rectal cancer is complex and involves a multidisciplinary approach.

Patients with low and middle rectal cancer, clinical stages II and III, receive neoadjuvant treatment with chemotherapy and radiotherapy, followed by surgery. The earliest cases, clinical stage I, go for surgery, while the most advanced patients, with clinical stage IV, are referred for systemic treatment with chemotherapy (Kasi, 2020).

Neoadjuvance for rectal cancer has the role of reducing the recurrence rate, increasing the disease-free survival rate (Kasi, 2020).

It is important to note that tumors of the upper rectum, above the peritoneal reflection, follow the treatment protocol for colon tumors, which begins with surgical treatment in the non-metastatic scenario (Benson, 2018).

Surgical treatment of rectal cancer:

Surgery for rectal cancer is a major challenge for surgeons, as it is located in the pelvis, in an anatomical region that is difficult to access and where the anorectal sphincter muscles, responsible for human fecal continence are located.

The male anatomy of the pelvic region, where the rectum is located, is extremely delicate; it is narrower than the female anatomy and contains the prostate. These

peculiarities make the surgical approach difficult. The female pelvis, on the other hand, is less narrow and, together with other attributes, facilitates surgical treatment.

Along with radiotherapy, the standardization of the surgical technique for rectal cancer, with total excision of the mesorectum, has improved the surgical outcome of these patients, reducing local recurrence and improving the disease-free survival rate (Martling, 2002).

Oncological surgery for rectal cancer, rectosigmoidectomy, is well standardized, with the crucial points being ligation of the inferior mesenteric artery at its origin, total excision of the mesorectum and a tumor safety margin. In most cases, these patients are left with a temporary protective ileostomy, due to the risk of fistula. After an endoscopic examination confirming that the anastomosis has healed properly, the ileostomy can be closed. Some patients, due to technical or clinical difficulties, undergo Hartmann's surgery, rectosigmoidectomy with terminal colostomy.

In cases of low rectum, in which the sphincter muscles of the anal canal are compromised, Abdominoperineal amputation (Miles surgery) is indicated, a surgical technique that results in a definitive colostomy.

In early cases, there is the possibility of transanal and endoscopic resection. Another possibility is total excision of the transanal mesorectum combined with an abdominal approach. It is not uncommon for patients to be diagnosed with locally advanced or metastatic lesions, which cause obstructive symptoms. In these situations, derivative ostomies are indicated; another option is endoscopic prostheses. When the tumor invades adjacent organs to the rectum, such as the bladder, uterus and prostate, in selected cases, pelvic exenterations are indicated, an oncological surgery that involves resection of the organs compromised by the neoplasm in a monobloc. When it involves resection of the bladder or prostate, urinary diversions such as wet colostomy or bricker may be necessary.

Undeniably, there have been exciting technological advances in recent decades when it comes to surgical treatment for rectal cancer. With a considerable increase in minimally invasive surgery for rectal cancer, such as laparoscopy and robotics. These new technologies seem to reduce hospitalization time and postoperative pain, without compromising the oncological outcome (Crippa, 2021).

Currently, a multidisciplinary perioperative approach is advocated in the surgical treatment of rectal cancer, in an attempt to minimize preoperative complications. A proper nutritional assessment is essential, as these patients are often indicated for protein and other nutrient supplementation in the preoperative period.

Surgical treatment of rectal cancer can lead to various complications for patients, such as fistulas, infection, bleeding, paralytic ileus, thromboembolic events,

anastomosis stenosis and anterior rectal resection syndrome are the most common (Andreoni, 2007).

“Wacht and wait" strategy

The English term "*Watch and Wait" (WW)* allows a non-operative alternative as a therapeutic strategy for patients with rectal cancer.

The literature shows that around 30%-40% can have a complete response after neoadjuvant therapy and avoid surgery. After completing neoadjuvant therapy, the patient is re-staged by MRI and clinical assessment (rectal touch + rectoscopy) between eight and 12 weeks after treatment. In cases where complete clinical response (cCR) found, the option of WW is proposed and discussed with the patient, and the risks/benefits of this approach are presented. (Garcia-Aguilar J, 2022)

METHODOLOGY

Study design:
Single-center, phase 2, single-arm clinical study compared to historical control.

Intervention:
The intervention consists of administering metronidazole orally at a daily dose of 1,500 mg, in three doses of 500 mg every 8 hours, during the first seven days of neoadjuvant radiotherapy.

The researchers will arrange for the metronidazole to be dispensed with the support of the group of research nurses.

Adherence to the use of metronidazole will be assessed through a record in the patient's diary (Appendix 1), as well as questioning during medical visits and recorded in the medical records.

Adverse events associated with the use of metronidazole are uncommon and include gastrointestinal disorders. Very rare reactions (occur in less than 0.01% of patients using this medicine) and include disorders of the immune system, nervous system, hepatobiliary system and skin tissue. All adverse effects will be carefully evaluated by the study team.

Patients can stop taking the medication for any of the following reasons: disease progression, death, adverse event(s) leading to permanent discontinuation of QTRT, medication intolerance, withdrawal of consent by the patient (or legal representative) or the need for new systemic cancer therapy or surgery.

Objective

Main objective

Determine the effectiveness of the use of antimicrobial therapy at the beginning of neoadjuvant treatment in patients with locally advanced adenocarcinoma of the rectum.

Secondary objectives:

- Evaluate changes in the profile of intestinal bacteria through intestinal analysis (RNA-16S analysis) in fecal samples before and after treatment.
- Measure the organ preservation rate.
- Verify the rate of pathological complete response among the operated patients.
- Monitor toxicity to neoadjuvant treatment.
- Calculate 3-year locoregional recurrence-free survival.

Outcomes

Main outcome:

Complete clinical response to neoadjuvant treatment, defined by the absence of residual disease detectable by the following methods: clinical rectal examination; rectal endoscopic examination (flexible rectoscopy); magnetic resonance imaging (MRI).

Secondary outcomes:

- Evaluate changes in the profile of intestinal bacteria through intestinal analysis (RNA-16S analysis) in fecal samples before and after treatment.
- Organ preservation rate.
- Complete pathological response rate among operated patients.
- Toxicity of neoadjuvant treatment.
- Locoregional recurrence-free survival at 3 years.

Study population:

Inclusion criteria:

- Patients over the age of 18 with adenocarcinoma of middle and lower rectum, classified as locally advanced or whose tumor location requires rectal amputation surgery.
- Tumors located below the peritoneal reflection, as determined by MRI, will be defined as middle and lower rectum.
- Patients who are candidates for rectal amputation are those with a tumor whose distal limit is less than 2.0 cm from the pectineal line by rectoscopy or less than 2.0 cm from the anorectal ring by touch or MRI.

- For patients who are not candidates for rectal amputation (middle rectum), patients with tumors that are candidates for radiotherapy in neoadjuvant treatment will be included, defined by clinical staging by MRI, according to the following criteria: mesorectal fascia compromised by direct extension of the primary tumor or by a compromised lymph node; cT4 tumors.

Exclusion criteria:

- Patients without performance for total neoadjuvant treatment.
- Histologies other than adenocarcinoma.
- Adenocarcinomas with microsatellite instability phenotype, by immunohistochemistry.
- Patients with a tumor of the mid-rectum, without indication for radiotherapy in the neoadjuvant treatment regimen, according to the institutional protocol: cT3N0 tumors, with free mesorectal fascia; cT1-3 N+, with free mesorectal fascia.

Neoadjuvant treatment regimen:

The standard neoadjuvant treatment regimen consists of long-course radiotherapy, with 4.500 cGy fractionated into 25 applications of 180 cGy and boost on the primary tumor up to a total dose of 54 Gy, concomitant with fluoropyrimidine (intravenous 5-fluorouracil or oral capecitabine). The current practice is to carry out all the pre-surgery neadjuvant treatment (consolidation TNT), which consists of chemo-radiotherapy followed by chemotherapy with fluoropyrimidine (intravenous 5-fluorouracil or oral capecitabine) and oxaliplatin for up to 6 cycles or starting with chemotherapy followed by chemo-radiotherapy (induction TNT).

Determining the main outcome:

The primary endpoint will be determined between 7 and 10 days after the end of total neoadjuvant consolidation chemotherapy. If, for some clinical reason, the total neoadjuvant chemotherapy regimen is induction, before radiotherapy, then the determination of clinical response will be carried out between 10 and 12 weeks after the end of radiotherapy.

For patients who are candidates for rectal amputation and achieve a complete clinical response after the end of neoadjuvant treatment, organ preservation (WW) will be indicated as the first option.

For patients with mid-rectum tumors whose surgery includes preservation of the anal sphincter, total excision surgery of the mesorectum with sphincter preservation will be offered as the first treatment option, regardless of the response pattern. In cases

of complete clinical response, the WW strategy will be offered as an alternative to patients who refuse surgery.

Concomitant medications

In general, concomitant medications that are deemed necessary for the treatment and safety of the patient are allowed, and their use must be documented in the patient's medical records.

Concomitant administration of investigational drugs is not permitted. The use of growth factors is permitted at the discretion of the investigator for patients with neutropenia, in accordance with institutional routine. The use of antibiotics during the study should be avoided, as long as it does not interfere with patient safety.

Toxicities

For the evaluation of adverse events, patients will be seen before the start of study, within 7 days of starting QTRT, according to institutional routine, and at the end of the study (in the evaluation of the primary endpoint, with a complete clinical examination and routine laboratory tests (blood count, urea, creatinine and others, if clinically indicated).

Patients with intolerable grade 2 or grade 3 toxicity will have the treatment dose reduced and/or radiotherapy interrupted, as institutional routine. All adverse effects will be graded and assessed as to whether or not they are treatment-related. Patients requiring hospitalization should be immediately identified by the Principal Investigator of the study.

As it is an approved drug and widely used commercially, we do not expect to see any serious adverse events or those leading to intolerance. However, if intolerance does occur, it will not be permitted to reduce the quantity, but we will recommend discontinuation.

Adverse events will be assessed according to the Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0. An adverse event for the purposes of this protocol is the appearance of (or worsening of pre-existing) undesirable sign(s), symptom(s) or medical condition(s) occurring after signing the Informed Consent Form (ICF), even if the event is not considered to be related to the study drug(s). The occurrence of adverse events should be obtained through direct questions to the patient or when they are voluntarily reported by the patient during or between visits or through physical examination or laboratory tests.

Whenever possible, each adverse event should be evaluated to determine:

1. Severity (mild, moderate, severe and life-threatening) or (CTCAE grade 4.0).

2. Your relationship with each study drug (suspected / not suspected).
3. Their duration (start and end dates or whether they continue into the final exam).
4. Conduct adopted (no conduct adopted; dosage of study drug adjusted/temporarily discontinued; study drug permanently discontinued as a result of said adverse event; concomitant medication administered; non-drug therapy administered; prolonged hospitalization/hospitalization).

Biological Samples - Exploratory Analysis

As part of this project, we will collect pre- and post-intervention stool samples from all the patients included in the study investigate the secondary endpoint, which consists of evaluating changes in the intestinal bacteria profile through intestinal analysis (RNA-16S analysis) in pre- and post-treatment fecal samples.

Ethical considerations

The study will be conducted in accordance with the protocol, the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines 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 undertakes to ensure that each patient in the study, or their 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 carried out. The investigator will retain one copy of each ICF signed by the patient. It will be emphasized that participation is voluntary and that the patient has the right to refuse participation or leave mid-study whenever they wish. This will not affect the patient's subsequent care. Female patients will be instructed not to become pregnant during QTRT treatment and will be instructed to use contraception if of childbearing age, as is already routinely done. There is a risk of loss confidentiality if patient data is identified, but all appropriate measures will be taken to prevent this from happening.

The protocol will be approved by the Local Ethics Committee. The study will be registered in the international clinical trial registry ClinicalTrial.gov.

Funding and Sponsorship of the Study

The patient will not incur any costs in relation to the use of metronidazole and the collection of biological samples related to the outcomes of this study. The study budget includes health insurance for expenses related to complications associated with the research. All project costs relating to the intervention, analysis and patient insurance will be funded by the CNPQ - Universal Call for Proposals, which has already been approved.

Control variables:

The following variables will be collected: age; gender; tumor location (distance from pectineal line in cm); clinical stage T; clinical stage N; clinical stage M; type of preoperative treatment; date of completion of preoperative treatment; interpretation of clinical response; date of surgery; ASA; comorbidities; surgical access route; type of surgery in relation to sphincter preservation; date of hospital discharge; postoperative complication (Clavien-Dindo scale); type of post-operative complication; response pattern; toxicities; re-operation; date of hospital discharge; re-hospitalization within 30 days; pathological stage T, pathological stage N; date of stoma closure; post-operative chemotherapy; date of last report; local recurrence; distant recurrence; date of first recurrence; date of last report or death; status at last report.

STATISTICAL ANALYSIS

The data will be collected and anonymized on the Redcap platform.

Once the data has been checked for consistency on the Redcap platform, it will be exported to SPSS software version 23 for statistical analysis. At first, exploratory analysis will be carried out, with simple frequencies of the variables. For numerical variables, the mean, median and standard deviation will be presented, and the means of quantitative variables will be compared using the Student's T-test. Association tests between qualitative variables will be carried out using the chi-square or Fisher's test.

A power of 80% and an Alpha standard error of 5% will be adopted for all analyses.

Sample size calculation

For the sample calculation, we considered the hypothesis of a 50% increase in the clinical response rate, with a change from 40% to 60% response. For this expected difference, we considered an alpha error of 5% and test power equal to 80%, with an estimated size of 100 patients.

Interim Analysis

After recruiting 50% of the estimated sample, an interim analysis will be carried out to assess safety, futility and efficacy. The O'Brien Fleming method will be used to evaluate the interim efficacy analysis.

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APPENDIX 1:

PATIENT INSTRUCTIONS

Keep this diary card with you at all times and show it to any health professional, such as a doctor, nurse or dentist that you see so that they know you are taking part in a clinical study.

Fill in this card day, recording the study drug you have used and bring it to your next study visit.

If you forget to take a dose at the usual time, you can still take it up to an hour later.

_____ hours later, but if you exceed this maximum number of hours in which you could have taken your medicine, do not use the missed dose and restart treatment with the next dose the next day, as usual.

Avoid eating or drinking _____ while taking the study drug (if indicated in the study).

Keep your study drug at room temperature (15-25°C), in a dry place and out of the reach of children.

Bring any unused study medication in its packaging to the hospital on your next visit.

Call the study team if you have any questions or concerns at the contacts described in the table below, as well as any complications.

The holder of this card is taking part in a Survey, keep it private.
In the event of an emergency, go to the Emergency Room at AC Camargo and contact the research team.
For further information and questions, please contact the research team.

Phone: (11) 2189-5000 RAMAL 2213 Monday to Friday from 8 a.m. to 6 p.m.

Card - Patient Diary RESEARCH

STUDY: _____

MEDICAL RESEARCHER: _____

TREATMENT: _____

Nº OF PARTICIPANT: _____

DO USE BY _____ TIMES A DAY, FOR _____

STARTING ON

DATE OF NEXT APPOINTMENT: ____/____/____ TIME ____:____

Observations (such as interval time before and/or after food intake):

_____
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REGISTRATION OF DRUG UNDER STUDY

Nº DAY	DATE	SCHEDULE	QUANTITY USED	NOTES (example: extra visits extra or symptoms)
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