Mayo Clinic Cancer Center

Phase II trial of Rifaximin in patients with early stage HER2 positive breast cancer with gastrointestinal toxicities related to pertuzumab-based therapy

Study Chairs: [redacted]

Study Cochairs: [redacted]

Statistician: [redacted]

√Study contributor(s) not responsible for patient care

Drug Availability
Drug Company supplied
Salix Pharmaceuticals: Rifaximin (IND #14692)

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Protocol Resources

Questions:	Contact Name:
Patient eligibility*, test schedule, treatment delays/interruptions/adjustments, dose modifications, adverse events, forms completion and submission	[redacted]
Forms completion and submission	[redacted]
Protocol document, consent form, regulatory issues	[redacted]
Non-paraffin biospecimens	[redacted]
Serious Adverse Event Reporting	[redacted]

*No waivers of eligibility allowed

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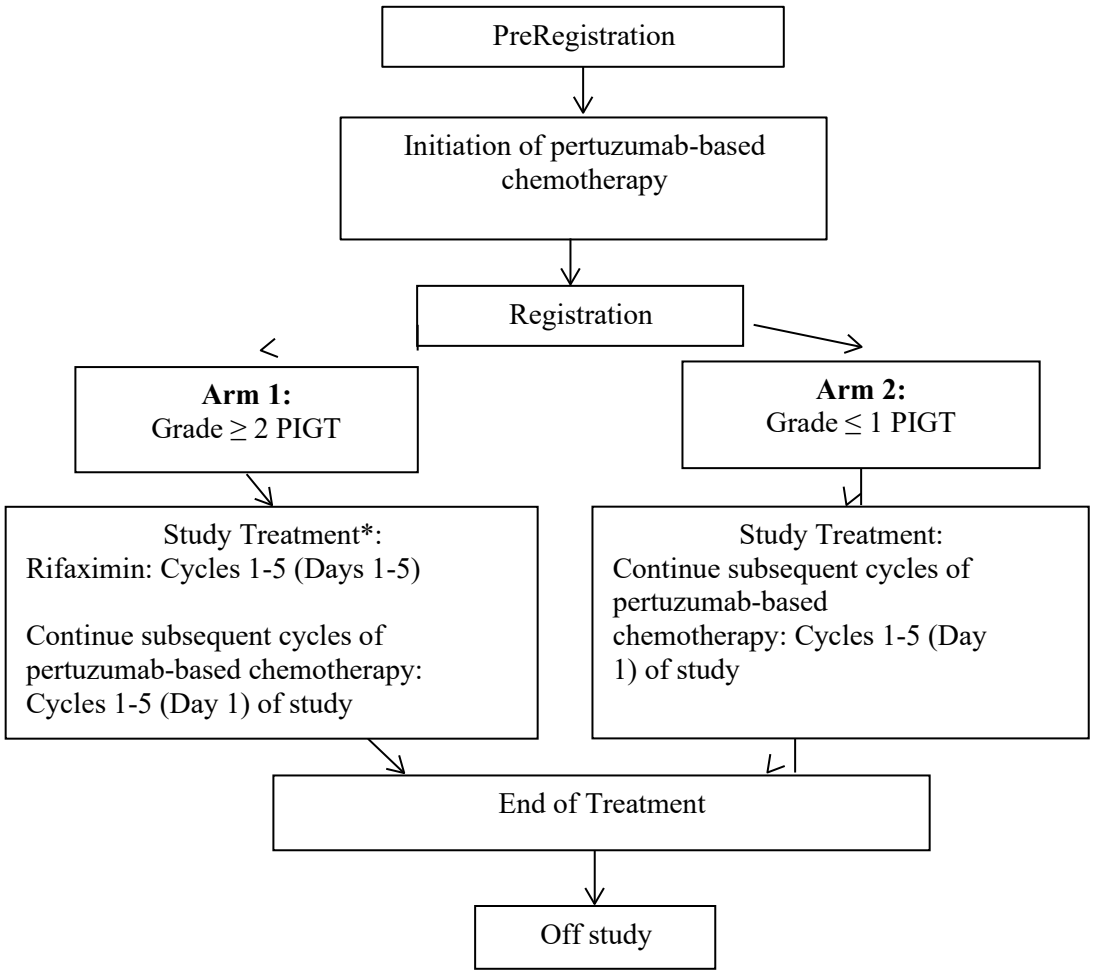
Schema

Sample size: 34 patients
Patient population: Stage I-III HER2 overexpressed breast cancer patients who will receive adjuvant and neoadjuvant pertuzumab-based chemotherapy.



Treatment Cycle = 21 days (± 7 day window)

Rifaximin treatment will be for 5 days concurrently with subsequent cycles of pertuzumab-based chemotherapy in patients with Grade ≥ 2 PIGT

End of Treatment = 21 days after the last dose of pertuzumab-based chemotherapy
*Patients that develop ≥ grade 2 PIGT ONLY according to NCI CTCAE v5.0 after initiation of pertuzumab-based chemotherapy will be eligible to receive rifaximin for 5 days on subsequent cycles of pertuzumab-based chemotherapy. Rifaximin will be given as 550 mg orally twice daily for 5 days starting on day 1 of the pertuzumab cycle. This will be repeated every 3 weeks on subsequent

Pertuzumab induced gastrointestinal toxicities (PIGT) including abdominal distension, abdominal pain, diarrhea, dyspepsia, stomach pain, and typhlitis, according to NCI CTCAE v5.0.

Generic name: Rifaximin Brand name(s): Xifaxan Mayo Abbreviation: Availability: Mayo Clinic Pharmacy

1.0 Background

1.1 HER2 positive breast cancer

Breast cancer is one of the most common malignancies among women worldwide. Human epidermal growth factor receptor 2 (HER2)-positive breast cancer is a unique subset of breast cancer, which accounts for approximately 15-30% of breast cancers (Owens MA, 2004).

Patients with HER2 positive breast cancer in addition to traditional chemotherapy are also treated with HER2 targeted therapies such as pertuzumab and trastuzumab. HER2 targeted therapies have revolutionized the treatment outcome for patients with HER2 positive breast cancer. However, gastrointestinal toxicities are common adverse events of pertuzumab in particular. The gastrointestinal toxicities can be severe enough that the immediate consequences are to reduce, delay or interrupt the planned treatment which could potentially affect the oncologic outcome.

1.2 Pertuzumab induced gastrointestinal toxicities

Pertuzumab-induced gastrointestinal toxicities (PIGT) are common side effects from pertuzumab, which has been reported in up to 70% of patients receiving pertuzumab (Swain et al, 2017). The gastrointestinal toxicities appear to be intensified when pertuzumab is given in combination with chemotherapy.

In the pivotal phase III CLEOPATRA trial, which evaluated pertuzumab in combination with trastuzumab and docetaxel in patients with metastatic HER2-positive breast cancer, all grade diarrhea was reported in 66.8% of patients receiving pertuzumab compared to 46.3% of patients receiving docetaxel and trastuzumab with placebo (Baselga J et al., 2012). Among patients with early stage HER2-positive breast cancer, all grade episodes of diarrhea were reported in the range of 43-72.4% in patients receiving pertuzumab combination.

The incidence of all grade diarrheas was highest in the TCH+P regimen in the TRYPHAENA trial with 72.4% of patients receiving this regimen had any grade diarrhea and 11.8% of those patients had ≥ grade 3 diarrhea (Schneeweiss A et al., 2013).

In the NEOSPHERE trial the incidence of all grade diarrheas reported in the THP arm was 51% (Gianni et al. 2012).

1.3 Etiology of PIGT

PIGT is likely a result of a combination of different etiologies. There are several potential mechanisms for diarrhea associated with epidermal growth factor receptor (EGFR)/HER2 signaling blockade, including dysregulation of ion channels in intestinal epithelial cells, which leads to excess chloride secretion; altered gut motility; changes in intestinal microbiome, including small intestinal bacterial overgrowth and altered nutrient metabolism.

1.31 Hydrogen Breath Test

Hydrogen breath test is a non-invasive diagnostic tool to evaluate small intestine bacterial overgrowth and carbohydrate malabsorption, such as lactose, fructose,

and sorbitol malabsorption. The test is performed after a short period of fasting of about 8-12 hours. Bacteria in the bowel generally produce hydrogen gas on fermentation of carbohydrates. In bowel, bacteria can only do this when dietary carbohydrates are not absorbed in small intestine and stay as undigested material as it travels along the digestive tract into large intestine. Though some of the hydrogen gas produced by the bacteria is expelled as flatus or in making other molecules such as sulphides, acetate and short chain fatty acids but most of the gas is absorbed across the lining of the large intestine into blood stream. The gas is then transported to lungs via the blood stream and from blood it is exchanged into the airways of lungs and breathed out. The only source of hydrogen gas in the breath can be from bacterial fermentation in the bowel. The same applies to the gas called methane which is exhaled by some people and not all. The bacteria in their large bowel make methane from the hydrogen. The amount of hydrogen and methane gases breathed out from the lungs can be easily measured by taking a breath sample, and measuring by a breath-testing machine. (Rezaie et al., 2017)

1.4 Treatment approaches for PIGT

Pharmacologic approaches to treat PIGT are not specific and mainly focus on symptomatic relief.

For the treatment of diarrhea and other PIGT, the standard of care is to use antidiarrheal agents such as loperamide, diphenoxylate/atropine, etc., however even with the use of these agents, the gastrointestinal toxicities can persist. No other drugs are approved for the treatment of pertuzumab induced gastrointestinal toxicities.

A recent case report demonstrated the safe and effective use of rifaximin in the treatment of pertuzumab induced gastrointestinal toxicities (Soyano AE et al., 2017). In this case, rifaximin was thought to reduce the small intestinal bacterial overgrowth contributing to diarrhea.

New research on this area is needed as these side effects can be disabling and negatively affect quality of life and survivorship of patients. New treatment approaches are needed to help to safely decrease the incidence and severity of gastrointestinal toxicities without having to reduce, delay or interrupt the chemotherapy schedule as that also may negatively affect the oncologic outcome.

1.5 Rifaximin

Rifaximin is a poorly absorbed bactericidal, which is a derivative of rifamycin. Rifaximin irreversibly inhibits bacterial protein synthesis by binding to the beta-subunit of the bacterial DNA-dependent RNA polymerase (RpoB) (Hartmann et al., 1967). Previous study demonstrated that rifaximin has minimal systemic absorption with < 0.4% bioavailability (Descombe et al., 1994). Due to poor absorption, previous clinical studies showed no significant drug-drug interaction with other medications (Pentikis et al., 2007; Trapnell et al., 2007). Rifaximin has been studied using multiple dosing and frequency. The 550 mg twice daily dosing is based on several clinical trials for traveler's diarrhea[1,2], irritable bowel syndrome[3], and hepatic encephalopathy[4]. The 5-day duration was based on previous clinical trials of rifaximin for the treatment of travelers' diarrhea (TD) which showed that 5-day course of rifaximin is safe and resulted in a trend for shorter duration of diarrhea compared to TMP-SMX (160/800 mg twice a day)[5]. Given the fact that

patients with PIGT do not have an established infection, this dosing schedule is likely to be adequate to prevent small intestinal bacterial overgrowth which may contribute to PIGT.

2.0 Goals

2.1 Primary Goal

To evaluate the reduction rate of grade ≥ 2 abdominal toxicities, including abdominal distension, abdominal pain, diarrhea, dyspepsia, stomach pain, and typhlitis according to the National Cancer Institute Common Terminology for Adverse Events version 5.0 (NCI CTCAE v5.0) with the use of rifaximin in stage II-III HER-2 positive breast cancer patients with pertuzumab induced gastrointestinal toxicities.

2.2 Secondary Goals

2.21 Evaluate dose reductions, dose delays and discontinuation of treatment with pertuzumab due to gastrointestinal side effects.

2.22 Evaluate and measure the change in the Bristol stool scale before and after rifaximin treatment.

2.23 Evaluate and measure the change in the 4-point Likert scale patient questionnaire before and after rifaximin treatment.

2.3 Correlative Study Goals

2.31 Evaluate changes in the fecal microbiome, hydrogen breath test, and permeability test before and after rifaximin.

2.32 Evaluate changes in the fecal microbiome, hydrogen breath test, and permeability test before and after pertuzumab-based chemotherapy.

2.33 Evaluate the difference in the fecal microbiome, hydrogen breath test, and permeability test among patients with or without PIGT.

3.0 Patient Eligibility

3.1 Pre-registration – Inclusion Criteria

3.11 Age ≥18 years

3.12 Histological confirmation of HER2 positive breast cancer stage I-III per AJCC staging 8th edition.

3.13 Provide written informed consent.

3.14 Breast cancer patients who will be receiving pertuzumab-based chemotherapy with either TCHP (docetaxel, carboplatin, trastuzumab, and pertuzumab) or docetaxel/paclitaxel, trastuzumab, and pertuzumab.

3.15 ECOG Performance Status (PS) 0, 1, or 2 (Appendix I).

3.16 The following laboratory values obtained ≤30 days prior to pre-registration:

- Hemoglobin ≥ 10.0 g/dL
- Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L
- Platelet count ≥ 100 x 10^9/L
- Total Bilirubin ≤ 1.5 x ULN (institutional upper limit of normal)
- AST (SGOT)/ALT (SGPT) ≤ 2.5 x ULN
- Serum or plasma creatinine ≤ 1.5 x ULN
- Calculated creatinine clearance ≥45 ml/min using the Cockcroft-Gault formula below:

Cockcroft-Gault Equation:

Creatinine clearance for males = $\frac{(140 - \text{age})(\text{weight in kg})}{(72)(\text{serum creatinine in mg/dL})}$

Creatinine clearance for females = $\frac{(140 - \text{age})(\text{weight in kg})(0.85)}{(72)(\text{serum creatinine in mg/dL})}$

3.17 Negative serum pregnancy test done ≤30 days prior to pre-registration, for person of childbearing potential only.

3.18 Willingness to return to enrolling institution for follow-up (during the Active Monitoring Phase of the study).

3.19a Willingness to provide mandatory stool specimen for correlative research (see section 14.0)

3.19b Ability to complete questionnaire(s) by themselves or with assistance.

3.2 Pre-registration - Exclusion criteria

3.21 History of myocardial infarction ≤6 months prior to pre-registration, or congestive heart failure requiring use of ongoing maintenance therapy for life-threatening ventricular arrhythmias.

3.22 Failure to recover from acute, reversible effects of prior therapy regardless of interval since last treatment.
EXCEPTION: Grade 1 peripheral (sensory) neuropathy that has been stable for at least 3 months since completion of prior treatment.

3.23 Uncontrolled intercurrent non-cardiac illness including, but not limited to:

- Ongoing or active infection
- Psychiatric illness/social situations
- Dyspnea at rest due to complications of advanced malignancy or other disease that requires continuous oxygen therapy
- Any other conditions that would limit compliance with study requirements

3.24 Immunocompromised patients and patients known to be HIV positive and currently receiving antiretroviral therapy.
NOTE: Patients known to be HIV positive, but without clinical evidence of an immunocompromised state, are eligible for this trial.

3.25 Receiving any other investigational agent which would be considered as a treatment for the primary neoplasm. (adjust to protocol if applicable)

3.26 Any of the following because this study involves an agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown

- Pregnant women
- Nursing women
- Women of childbearing potential who are unwilling to employ adequate contraception

3.27 Current Colostomy or ileostomy

3.28 History of inflammatory bowel disease

3.29a History of irritable bowel syndrome

3.29b History of arteriovenous malformations

3.29c History of gastrointestinal bleeds

3.29d Previous surgical resection of the small bowel or colon

3.29e Previous allergy to rifaximin or its derivatives

3.3 Registration – Inclusion Criteria

3.31 Received pertuzumab based regimens in the adjuvant or neoadjuvant setting.

3.32 The following laboratory values obtained ≤ 14 days prior to registration:

− Hemoglobin ≥ 8.0 g/dL

− Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L

− Platelet count ≥ 100 x 10^9/L

− Total Bilirubin ≤ 1.5 x ULN (institutional upper limit of normal)

− AST (SGOT)/ALT (SGPT) ≤ 2.5 x ULN

− Serum or plasma creatinine ≤ 1.5 x ULN

− Calculated creatinine clearance ≥45 ml/min using the Cockcroft-Gault formula below:

Cockcroft-Gault Equation:

Creatinine clearance for males = $\frac{(140 - \text{age})(\text{weight in kg})}{(72)(\text{serum creatinine in mg/dL})}$

Creatinine clearance for females = $\frac{(140 - \text{age})(\text{weight in kg})(0.85)}{(72)(\text{serum creatinine in mg/dL})}$

4.0 Test Schedule

4.1 Test schedule for early stage HER2 positive breast cancer with and without gastrointestinal toxicities*

Tests and procedures	Preregistration***	7-14 days after initiation of pertuzumab-based chemotherapy	Registration (21 days after initiation of pertuzumab-based chemotherapy)	Cycle 1, Day 1[10]	Cycle 1, Day 7	Cycle 2-5, Day 1	End of Treatment (21 Days after last dose of pertuzumab-based
Window			+7 days/-3 days	+3 days/-3 days	+ 7 days	± 7 days	+ 7 days
History, ECOG (PS),physical exam[1]	X		X	X		X	X
Height	X						
CBC with 5 part differential, total bilirubin, direct bilirubin (if total bilirubin is elevated), AST (SGOT), ALT (SGPT), serum, or plasma creatinine[11]	X		X[11]	X		X	X
Chemistry group[2, 11]			X[11]	X		X	X
Pregnancy test, serum[3]	X						
PIGT grading assessment			X	X		X	X
Adverse event assessment				X		X	X
4-Point Likert Scale (Appendix IV)[4]	X			X		X	X
Bristol stool scale and stool diary (Appendix V)[5]	X			X		X	X
(Optional) Hydrogen breath test[6, R]	X	X		X	X		X

Tests and procedures	Preregistration***	7-14 days after initiation of pertuzumab-based chemotherapy	Registration (21 days after initiation of pertuzumab-based chemotherapy)	Cycle 1, Day 1[10]	Cycle 1, Day 7	Cycle 2-5, Day 1	End of Treatment (21 Days after last dose of pertuzumab-based
Research stool collection (see section 14.0) [7, R]	X	X		X	X		X
(Optional) Research urine collection (See section 14.0)[8, R]	X	X		X	X		X
Patient Medication Diary[9]						X	
Concomitant Medications	X	X[12]	X[12]	X		X	X

Cycle = 21 days (± 7 day window)

*Pertuzumab induced gastrointestinal toxicities (PIGT) include abdominal distension, abdominal pain, diarrhea, dyspepsia, stomach pain, and typhlitis, according to NCI CTCAE v5.0.

**21 days after the last dose of pertuzumab-based chemotherapy. In patients who receive pertuzumab-based chemotherapy in the adjuvant and neoadjuvant setting, this follow up should be prior to surgery in patients who will proceed with surgery.

***Test/Procedures completed ≤30 days prior to pre-registration are not to be repeated.

1. History and physical exam including weight and performance status.
2. Alkaline phosphatase, BUN, albumin, total protein, uric acid, bicarbonate, calcium, chloride, glucose, LDH, magnesium, phosphorus, potassium, sodium
3. For Women of childbearing potential only. Must be ≤30 days prior to registration.
4. The 4-point Likert scale questionnaire (Appendix IV) will be completed by the patient at preregistration, prior to Initiation of pertuzumab-based chemotherapy, and on Day 1 of Cycles 1-5 of Rifaximin treatment, and at the end of treatment. Questionnaire will be completed on Day 1 of each subsequent cycle.
5. Bristol stool scale and stool diary (Appendix V) will be completed by the patient on days 1-14 of each pertuzumab- based chemotherapy cycle. Study staff will provide the patient with 14 pages of stool diaries. The diaries will be collected on the 1st day of each subsequent cycle and at the end of treatment.
6. OPTIONAL: Hydrogen breath test (See Lactulose Breath Test For Bacterial Overgrowth, Appendix VII) will be collected and submitted at preregistration, 7-14 days after initiation of pertuzumab-based chemotherapy, Cycle 1, Day 1, Cycle 1 (day 7-14), and at the end of treatment.

The hydrogen breath test and the urine permeability test must be ≥48 hours apart. Hydrogen breath test is an optional test and will be performed at physician discretion.

7. Stool kit will be given to patient prior to collection. Stool will be collected and submitted, At preregistration, 7-14 days after pertuzumab based treatment, Cycle 1, Day 1, Cycle 1 (day 7-14), and at the end of treatment.
8. OPTIONAL: Research Urine for the permeability test will be collected preregistration, 7-14 days after initiation of pertuzumab-based chemotherapy, Cycle 1, Day 1, Cycle 1 (day 7-14), and at the end of treatment. The hydrogen breath test (optional) and the urine permeability test (optional) must be ≥48 hours apart.
9. (ARM 1 only) The patient medication diary must begin the day the patient starts taking the medication and must be completed per protocol and returned to the treating institution OR compliance must be documented in the medical record by any member of the care team. (See Appendix II)
10. If test/procedure was completed prior to C1D1, do not repeat. Exam and labs must be completed prior to study treatment.
11. Laboratory values obtained ≤ 14 days prior to registration.
12. If concomitant medications are not recorded during preregistration, they may be collected 7-14 days after initiation of pertuzumab-based therapy OR at registration (21 days after initiation of pertuzumab-based chemotherapy).

R Research funded (see Section 19.0)

4.2 Survival Follow-up

This study does not follow patient survival. Once patients complete study intervention, they are off study.

5.0 Grouping Factor:

PIGT Grade: ≤ 1 VS. ≥ 2.

6.0 Registration Procedures

6.1 Pre-Registration (Step 0)

6.11 Registering a patient

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration application. The registration application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 5:00 p.m. Central Time (Monday through Friday).

The instructions for the registration application are available on the MCCC web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and an MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office [redacted] If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.12 Verification of information

Prior to accepting the pre-registration, the registration application will verify the following:

- IRB approval at the registering institution
- Patient pre-registration eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information.

6.13 Pre-registration tests/procedures

Pre-registration tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.14 Correlative Research

6.141 Mandatory - A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.19a, 14.0)

6.142 Optional – An optional research component is part of this study, there will be an option to select if the patient is to be registered onto this component (see Section 14.0)

- Patient has/has not given permission to give his/her urine sample for research testing.

6.15 Kits - Stool collection kits and urine containers are available on site.

6.2 Registration (Step 1):

6.211 Registering a patient

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration application. The registration application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the registration application are available on the MCCC web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and an MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration application can be confirmed in any of the following ways:

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- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.3 Verification of materials

Prior to accepting the registration, registration application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.4 Documentation of IRB approval

Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office [redacted] If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.5 Treatment on protocol

Treatment on this protocol must commence at Mayo Clinic Florida under the supervision of a medical oncologist.

6.6 Treatment start

Rifaximin treatment cannot begin prior to registration and must begin ≤3 days after registration.

6.7 Baseline symptoms

All required baseline symptoms (see Section 10.6) must be documented and graded.

6.8 Study drug

Study drug is available on site.

6.9b Study Conduct

The clinical trial will be conducted in compliance with regulations (21 CFR 312, 50, and 56), guidelines for Good Clinical Practice (ICH Guidance E6), and in accordance with general ethical principles outlined in the Declaration of Helsinki; informed consent will be obtained from all participating patients; the protocol and any amendments will be subject to approval by the designated IRB prior to implementation, in accordance with 21 CFR 56.103(a); and subject records will be stored in a secure location and subject confidentiality will be maintained. The investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

7.0 Protocol Treatment

7.1 Treatment Schedule

7.11 Treatment medication table

Agent	Starting Dose	Route	Administration
Rifaximin	550 mg	PO	Twice daily (Cycles 1-5,

Cycle Length = 21 days

Treatment Window = ± 7 days

Patients that develop ≥ grade 2 PIGT ONLY according to NCI CTCAE v5.0 after initiation of pertuzumab-based chemotherapy and prior to registration will be eligible to receive rifaximin for 5 days on subsequent cycles of pertuzumab-based chemotherapy. Rifaximin will be given as 550 mg orally twice daily for 5 days starting on day 1 of the pertuzumab cycle. This will be repeated every 3 weeks on subsequent pertuzumab cycles.

7.2 Return to consenting institution

For this protocol, the patient must return to the consenting institution for evaluation at least every 21±7 days during treatment and once 21 Days after last dose of pertuzumab-based chemotherapy.

7.3 Treatment by local medical doctor (LMD) is not allowed.

8.0 Dosage Modification Based on Adverse Events

If the patient develops any clinically significant adverse event attributed to rifaximin use, strictly follow the modifications in this table for the first three cycles, until individual treatment tolerance can be ascertained. Thereafter, these modifications should be regarded as guidelines to produce mild-to-moderate, but not debilitating, side effects. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Reductions or increases apply to treatment given in the preceding cycle and are based on adverse events observed since the prior dose. The patient should continue to be followed according to protocol criteria; this includes completing questionnaires.

8.1 Dose Levels (Based on Adverse Events in Tables 8.*2* and 8.3)

Dose Level	***Rifaximin***
0*	550 mg orally twice daily x 5 days
-1	550 mg orally daily x 5 days
-2	550 mg orally x 3 days
-3	Discontinue

*Dose level 0 refers to the starting dose.

If dose reduction goes below -2 level then rifaximin should be discontinued.

For patients who do not tolerate the 550 mg BID x 5 days dosing schedule, dose adjustment to 550 orally daily x 5 days is permitted in order to allow the patient to continue on study drug. The following guidelines need to be applied and these changes must be recorded on the on the specific section of the patient record.

Missed/skipped doses will not be made up (i.e. the patient should not double their dose if the previous dose was missed). When the adverse event that resulted in a dose reduction improves to and remains stable at Grade 1 or less for a minimum of 14 days, the dose can be re-escalated at the investigators discretion provided there are no other concomitant adverse events.

Dose reduction/interruption/discontinuation decisions should be based on the CTCAE grade of the adverse event (AE) and the guidelines provided below. In general, doses should not be reduced or interrupted for Grade 1 events, but treatment to control symptoms should be provided as appropriate. All AEs should be followed weekly or as clinically appropriate until stabilization or resolution.

A patient with a Grade 4 AE may resume treatment at the lower dose level if the AE recovers to grade ≤1 within 21 days of interrupting drug and, if in the opinion of the Investigator and Medical Monitor, the event is not life-threatening, and the patient can be managed and monitored for recurrence of AE. Dose interruptions of more than 21 days are not allowed unless approved by the Investigator.

Please refer to the table below for dose adjustment recommendations for rifaximin induced adverse events.

8.2 Dose Modifications Based on Interval Adverse Events

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	Grade	ACTION FOR RIFAXIMIN
Nervous system disorders	Dizziness	Grade 1	Maintain same dose of Rifaximin
		Grade 2	Omit rifaximin for current cycle. Reassess for next cycle. If dizziness worsens or does not improve, omit rifaximin until resolved to Grade ≤1. If AE does not resolve ≤ 3 weeks, resume rifaximin at 1 reduced dose level.
		Grade 3	Omit rifaximin for current cycle. Reassess for next cycle. If dizziness worsens or does not improve, hold rifaximin until resolved to Grade ≤1. If AE does not resolve ≤ 3 weeks, resume rifaximin at 2 reduced dose level.
Skin and subcutaneous tissue disorders	Rash maculo-papular	Grade 1	Maintain same dose of Rifaximin
		Grade 2	First occurrence: - Maintain dose of Rifaximin and monitor rash closely Second Occurrence: - Omit rifaximin for current cycle and monitor rash - Reassess for next cycle. If rash worsens or does not improve, Omit rifaximin until resolved to Grade ≤1. If AE does not resolve ≤ 3 weeks, resume rifaximin at 1 reduced dose level
		Grade 3	Omit rifaximin for current cycle and monitor rash Reassess for next cycle. If rash worsens or does not improve, Omit rifaximin until resolved to Grade ≤1. If AE does not resolve ≤ 3 weeks, resume rifaximin at2 reduced dose level
Skin and subcutaneous tissue disorders	Pruritus	Grade 1	First occurrence: - Maintain dose of Rifaximin and monitor closely Second Occurrence: - Omit rifaximin for current cycle and monitor - Reassess for next cycle. If rash worsens or does not improve, hold rifaximin until resolved. If AE does not resolve ≤ 3 weeks, resume rifaximin at 1 reduced dose level
		Grade 2	Omit rifaximin for current cycle and monitor pruritus Reassess for next cycle. If rash worsens or does not improve, omit rifaximin until resolved to Grade ≤1. If AE does not resolve ≤ 3 weeks, resume rifaximin at 2 reduced dose level
		Grade 3	Permanently discontinue rifaximin
Investigations	Serum Alanine	Grade 1	Maintain dose of Rifaximin and monitor closely

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	Grade	ACTION FOR RIFAXIMIN
	Aminotransferase (ALT)	Grade 2	Omit rifaximin for current cycle and monitor Reassess for next cycle. If ALT worsens or does not improve, hold rifaximin until resolved to Grade ≤1 . If AE does not resolve ≤ 3 weeks, resume rifaximin at 1 reduced dose level
		Grade 3	Omit rifaximin for current cycle and monitor. Reassess for next cycle. If ALT worsens or does not improve, omit rifaximin until resolved to Grade ≤1. If AE does not resolve ≤ 3 weeks, resume rifaximin at 2 reduced dose level
		Grade 4	Permanently discontinue rifaximin

** Use the following to describe actions in the Action column:

- **Hold/Delay** = The current dose(s) of all drugs during a cycle is delayed. The patient does make up the delayed dose(s) when the patient meets the protocol criteria to restart drugs.
- **Discontinue** = The specified drug(s) are totally stopped.
- Omit= The current dose(s) for the specified drug(s) during a cycle is skipped. The patient does not make up the omitted dose(s) at a later time.

NOTE: If patient experiences an adverse event and rifaximin was omitted for 2 consecutive cycles, discontinue rifaximin.

NOTE: If the patient experiences a significant adverse event requiring a dose reduction at the start of the next cycle, then the dose will remain lowered for that entire subsequent cycle. If that cycle is completed with no further adverse events >Grade 2, then the dose may be increased, at the investigator's discretion, one level at a time, in the following cycles.

NOTE: Adverse events requiring a dose-reduction step for any or all drugs beyond the two dose-reduction steps (levels –1 and –2) will be at the discretion of the treating physician, if the decision is made for the patient to be kept on study. These dose reductions must be clearly recorded in reported clinical

8.3 Warnings and Precautions

8.31 Clostridium difficile-associated diarrhea (CDAD)
CDAD has been reported with use of nearly all antibacterial agents, including rifaximin. Therefore, CDAD must be considered in patients who present with diarrhea. If CDAD is confirmed, rifaximin should be discontinued. Appropriate antibiotic treatment for CDAD, fluid and electrolyte management, protein supplementation, and surgical evaluation should be instituted as clinically indicated.

8.32 Development of drug resistant bacteria
Due to potential increased risk of drug-resistant bacteria, rifaximin should be discontinued in patients who are confirmed to have multi-drug resistant bacterial infection that is felt to be related to rifaximin at the discretion of the attending physician.

9.0 Ancillary Treatment/Supportive Care

9.1 Full supportive care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. Other treatment as necessary for the control of chemotherapy related symptoms is allowed. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.2 Blood products and growth factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the Journal of Clinical Oncology, Volume 33, No 28 (October 1), 2015: pp. 3199-3212 (WBC growth factors) AND Journal of Clinical Oncology, Volume 28, No 33 (November 20), 2010: pp. 4955-5010 (darbepoetin/epoetin).

9.3 Antiemetics

Antiemetics may be used at the discretion of the attending physician.

9.4 Diarrhea

The use of loperamide and/or atropine/diphenoxylate is allowed. In the event of Grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals.

10.0 Adverse Event (AE) Monitoring and Reporting

The site principal investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting [redacted]	Mayo Sites – attach to MCCC Electronic SAE Reporting Form
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: [redacted]	Will automatically be sent to [redacted]

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

Unanticipated Adverse Device Event (UADE)
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version5.0 can be downloaded from the CTEP web site:

[redacted]

a. Identify the grade and severity of the event using the CTCAE version 5.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.
An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.
NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.
Probable - The AE *is likely related* to the agent(s)/procedure.
Possible - The AE *may be related* to the agent(s)/procedure.
Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 AEs Experienced Utilizing Investigational Agents and Commercial Agent(s) on the SAME (Combination) Arm

NOTE: When a commercial agent(s) is (are) used on the same treatment arm as the investigational agent/intervention (also, investigational drug, biologic, cellular product, or other investigational therapy under an IND), the **entire combination (arm) is then considered an investigational intervention for reporting.**

- An AE that occurs on a combination study must be assessed in accordance with the guidelines for **investigational** agents/interventions.
- An AE that occurs prior to administration of the investigational agent/intervention must be assessed as specified in the protocol. In general, only Grade 4 and 5 AEs that are unexpected with at least possible attribution to the commercial agent require an expedited report, unless hospitalization is required. Refer to Section 10.4 for specific AE reporting requirements or exceptions.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

- An increased incidence of an expected adverse event (AE) is based on the patients treated for this study at their site. A list of known/expected AEs is reported in the package insert or the literature, including AEs resulting from a drug overdose.
- Commercial agent expedited reports must be submitted to the FDA via MedWatch 3500A for Health Professionals (complete all three pages of the form).

[redacted]

Instructions for completing the MedWatch 3500A:

[redacted]

10.4 Expedited Reporting Requirements for IND/IDE Agents

10.41 Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the sponsor within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization	7 Calendar Days	24-Hour 3 Calendar Days
Not resulting in Hospitalization	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

10.42 General reporting instructions

The Mayo IND Coordinator will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Use Mayo Expedited Event Report form [redacted] for investigational agents or commercial/investigational agents on the same arm.

For commercial agents:

[redacted]

To Pharmaceutical Company (Salix Pharmaceuticals):
Submit all SAEs and Pregnancy Reports to Valeant Drug Safety [redacted]

10.43 Reporting of re-occurring SAEs

ALL SERIOUS adverse events that meet the criteria outlined in table10.41 MUST be immediately reported to the sponsor within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 Other Required Reporting

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the Reportable Event coversheet and appropriate documentation to [redacted] The Mayo Regulatory Affairs Office will review and process the submission to the Mayo Clinic IRB.

10.52 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Disease Progression"** under the system organ class (SOC) of the **General disorders and administration site conditions**. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND/IDE will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 Pregnancy, Fetal Death, and Death Neonatal

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form:

[redacted]

10.551 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy loss"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "Neonatal loss"** under the General disorders and administration SOC.

10.6 Required Routine Reporting

10.61 **Baseline and Adverse Events Evaluations**

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.

Grading is per CTCAE v5.0 **unless** alternate grading is indicated in the table below:

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Nervous system disorders	Dizziness	X	X
Skin and subcutaneous tissue disorders	Rash maculo-papular	X	X
Skin and subcutaneous tissue disorders	Pruritus	X	X
Investigations	Alanine aminotransferase increased	X	X
Gastrointestinal disorders	Number of Stools	X	
	Diarrhea		X

10.62 All other AEs

All AEs and SAEs will be recorded regardless of whether or not they are considered related to the study drug(s) in the subjects's medical record and on the appropriate study specific CRF form.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 Treatment Evaluation/Measurement of Effect

Measurement of effect will be determined based on whether a patient have improvement in grade 2 abdominal adverse events; details related to the primary endpoint and its assessment are found in the statistics section of this protocol.

12.0 Descriptive Factors

None.

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 A patient is deemed ineligible if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. The patient will go directly off study.

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted.
- If the patient never received treatment, on-study material must be submitted.

13.2 A patient is deemed a major violation, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go off study. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered.

13.3 A patient is deemed a cancel if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

13.4 If the patient stops rifaximin but continues pertuzumab, the patient should continue to fill out questionnaires on-study as long as the patient is on pertuzumab based chemotherapy.

13.5 At the end of the pertuzumab based chemotherapy treatment, the patient will continue to the End of treatment Visit as indicated in Section 4.0.

13.6 If the rifaximin dose is delayed, missed, or skipped, the patient should continue to fill out questionnaires.

14.0 Body Fluid Biospecimens:

14.1 Summary Table of Stool to be collected

Research	Mandatory or Optional	Specimen collected	Volume to collect per tube (# of tubes to be collected)	≤30 days prior to Preregistration	7-14 days after initiation of pertuzumab-based chemotherapy	Cycle 1, Day 1	Cycle 1, (Day 7)	End of Treatment (21 Days after last dose of pertuzumab-based chemotherapy)	Process at site? (Yes or No)	Temperature Conditions for Storage /Shipping
Fecal microbiome test	Mandatory	Stool	2 mL (2)	X	X	X	X	X	No	Cold, Ice pack
Permeability Test	Optional	Urine	5 mL (2)	X	X	X	X	X	Yes	Frozen, Dry Ice

14.2 Collection and Processing

All samples should be labeled with the study number (MC18C3), the patient's study ID, and the designated specimen timepoint as indicated in section 14.1.

14.21 Fecal Microbiome
Collect two 2ml tubes and ship with cold pack.

14.22 Permeability Test
Collect two 5 mL 13x75 mm polypropylene tubes. The samples should be shipped frozen overnight on dry ice.

14.3 Shipping and Handling

14.31 Stool kits will be used for this study. Kits will be supplied by the Biospecimen Accessioning and Processing Share Resource (BAP) in Florida.

14.311 Stool Collection kits prepared by BAP will be provided to the patients. Participating institutions may obtain kits by submitting the supply order form to [redacted]Because we are charged for all outgoing kits, a small, but sufficient, supply of the specimen collection kits should be ordered prior to patient entry. Supply Order Forms must be filled in completely and legibly for quick processing.

14.312 Each stool kit will contain 2 or more stool collection vials that contain a collection spoon. Each kit will also contain a toilet hat, gloves, ice packs, and any other material deemed necessary for sanitary collection of stool by the patient in their home. Instructions for collection will be included with each kit.

14.313 Prior to being provided to the patient, each stool kit and vial will be labeled with the protocol number and patient ID number. Information regarding the date and time of collection will be added to the containers.

14.314 Fecal specimens may be collected by the patient at any day of the week, as long as the specimen is maintained at freezing temperature until shipping. Returning of the kit can be done in two ways: (a) in person, by depositing the kit with samples maintained under a cold temperature with ice pack to Mayo Clinic Florida; (b) by shipping the kit with samples maintained under a cold temperature with the ice pack by FedEx overnight shipping **Monday – Friday ONLY** (cost covered by the study).

14.315 Shipping Specimens

Stool samples will be sent to the following address at the end of the study via batch shipment:

[redacted]

14.32 Specimen kits will be used for this study. Kits will be supplied by the Biospecimen Accessioning and Processing Share Resource (BAP) in Florida.

14.321 Urine collection containers will be provided to the patients.

14.322 Each vial will be labeled with the protocol number and patient ID number. Information regarding the date and time of collection will be added to the vial.

14.323 Urine specimens may be collected by the patient at any day of the week, as long as the specimen is maintained at freezing temperature until shipping. When samples are ready to be shipped, an email notification must be submitted. They are to be shipped frozen overnight on dry-ice (Mon – Wed only) with a sample sheet that is to be completed and electronically returned for each batch shipped.

14.324 Shipping Specimens

Urine Samples for permeability test must be shipped to:

[redacted]

14.4 Background and Methodology

14.41 Fecal microbiome characterization

Microbial DNA will be extracted from the stool samples using the MoBio PowerSoil ® DNA Isolation Kit according to manufacturer's protocol at the University of Minnesota Genomics Center. After extraction and quality assurance, amplicons will be generated from the 16S rDNA V4 region using barcoded reverse primers and sequenced using the Illumina MiSeq platform. Reads will then be aligned using a custom multiple alignment tool (Jeraldo 2014) that merges paired end reads into a single multiple alignment and obtain bacterial taxa calls to assess bacterial abundance and diversity. Alpha diversity measures will be performed to evaluate the sample bacterial diversity observed. Distance metrics will be applied to compare the microbiome diversity of the samples before and after treatment, and with and without PIGT. A generalized mixed-effects model will be fit to the taxa counts to determine the significance of differential abundances of individual and groups of taxa across the subjects.

14.42 Permeability Test

Gastrointestinal permeability is known to play a role in a number of chronic gastrointestinal disorders such as inflammatory bowel disease, celiac disease, and irritable bowel syndrome. Performing this test measures the integrity of the intestinal barrier using probe sugars to assess the absorptive ability of the intestines. We shall use the urinary excretion of mannitol, and lactulose, to measure small intestinal and colonic permeability (Camilleri et al submitted 2009). The chemical analysis is refinement of the method of Lostia et al (2008) with 2 improvements: first, we use HPLC-tandem mass spectrometry to increase assay accuracy and test sensitivity; second, we use the sugars administered in solution or a delayed- release capsule (that dissolves at the ileocolonic junction), to identify which urine samples correspond to small intestinal or colonic absorption.

15.0 Drug Information

IND number (study specific) *or* IND exempt

[redacted]

15.1 Rifaximin

15.11 **Background:** Rifaximin is a semi-synthetic derivative of rifampin and acts by binding to the beta-subunit of bacterial DNA-dependent RNA polymerase blocking one of the steps in transcription. This results in inhibition of bacterial protein synthesis and consequently inhibits the growth of bacteria.

15.12 **Formulation**: Rifaximin tablets for oral administration are film-coated and contain 200 mg or 550 mg of rifaximin.

The 200 mg tablet is a pink-colored, round, biconvex tablet with “Sx” debossed on one side and plain on the other.

The 550 mg tablet is a pink-colored, oval, biconvex tablet with “rfx” debossed on one side and plain on the other.

15.13 **Preparation and storage**: Store rifaximin tablets at 20° to 25°C (68° to 77°F); excursions permitted to 15° to 30°C (59° to 86°F).

15.14 **Administration:** Rifaximin may be taken with or without food.

15.15 **Pharmacokinetic information**:
Absorption:
In healthy subjects, the mean time to reach peak rifaximin plasma concentrations was about an hour and the mean C_{max} ranged 2.4 to 4 ng/mL after a single dose and multiple doses of rifaximin 550 mg.

Distribution: Rifaximin is moderately bound to human plasma proteins. *In vivo*, the mean protein binding ratio was 67.5% in healthy subjects and 62% in patients with hepatic impairment when rifaximin was administered.

Metabolism: In an *in vitro* study rifaximin was metabolized mainly by CYP3A4. Rifaximin accounted for 18% of radioactivity in plasma suggesting that the absorbed rifaximin undergoes extensive metabolism.

Half-life elimination: The mean half-life of rifaximin in healthy subjects at steady-state was 5.6 hours.

Excretion: In a mass balance study, after administration of 400 mg 14C-rifaximin orally to healthy volunteers, of the 96.94% total recovery, 96.62% of the administered radioactivity was recovered in feces mostly as the unchanged drug and 0.32% was recovered in urine mostly as metabolites with 0.03% as the unchanged drug.

15.16 **Potential Drug Interactions**:
An *in vitro* study suggests that rifaximin is a substrate of CYP3A4. *In vitro* rifaximin is a substrate of P-glycoprotein, OATP1A2, OATP1B1, and OATP1B3. Rifaximin is not a substrate of OATP2B1.

However, rifaximin has minimal systemic absorption with < 0.4% bioavailability. In previous clinical studies, rifaximin has shown no significant effect on drug metabolism by cytochrome P450 isoenzymes.

P-glycoprotein Inhibitors
Caution should be exercised when concomitant use of rifaximin and a P-gp inhibitor such as cyclosporine is needed. Concomitant administration of cyclosporine, an inhibitor of P-gp and OATPs significantly increased the systemic exposure of rifaximin. In patients with hepatic impairment, a potential additive effect of reduced metabolism and concomitant P-gp inhibitors may further increase the systemic exposure to rifaximin.

Warfarin
Changes in INR have been reported post marketing in patients receiving rifaximin and warfarin concomitantly. Monitor INR and prothrombin time. Dose adjustment of warfarin may be needed to maintain target INR range. See prescribing information for warfarin.

CYP3A4 Substrates
An *in vitro* study has suggested that rifaximin induces CYP3A4. However, in patients with normal liver function, rifaximin at the recommended dosing regimen is not expected to induce CYP3A4. It is unknown whether rifaximin can have a significant effect on the pharmacokinetics of concomitant CYP3A4 substrates in patients with reduced liver function who have elevated rifaximin concentrations.

15.17 **Known potential toxicities**
Common known potential toxicities, > 10%:
Cardiovascular: Peripheral edema
Central nervous system: Dizziness, fatigue
Hepatic: Ascites
Gastrointestinal: Nausea

Less common known potential toxicities, 2% - 10:
Central nervous system: Headache, depression
Dermatological: Pruritus, skin rash
Gastrointestinal: Abdominal pain, pseudomembranous colitis
Hematologic & oncologic: Anemia
Hepatic: Increased serum ALT
Neuromuscular & skeletal: Muscle spasm, arthralgia, increased creatine phosphokinase
Respiratory: Nasopharyngitis, dyspnea, epistaxis
Miscellaneous: Fever

Rare known potential toxicities: <2%: Anaphylaxis, angioedema, *Clostridium difficile* associated diarrhea, exfoliative dermatitis, flushing, hypersensitivity reaction, urticaria

15.18 **Drug procurement:** Rifaximin will be provided free of charge to patients by Salix Pharmaceuticals.

15.19 **Nursing Guidelines:**

1. Assess for patients concomitant medications. There are several drug to drug interactions with rifaximin. For patients receiving concomitant warfarin, INR and PT will need to be monitored carefully with dose adjustments of warfarin as necessary.
2. Patients may experience peripheral edema. Instruct patients to report this and manage symptomatically.
3. Dizziness has been seen with this agent. Warn patients of this possibility.
4. Monitor LFT's while patient is on therapy as increased LFT's have been seen as has ascites.
5. Rifaximin may be taken with or without food.
6. Rarely anaphylaxis (angioedema, flushing, and hypersensitivity reaction, urticarial) has been seen with this agent. Instruct patient to report any signs or symptoms of allergic reaction to the study team immediately and/or seek out emergency medical care.
7. Gastrointestinal side effects have been seen including diarrhea, abdominal pain, and nausea. Due to the possibility of c-difficle colitis, patients should be instructed to notify the team of any diarrhea and not to self-medicate.

16.0 Statistical Considerations and Methodology

16.1 Statistical Endpoints and analysis

16.11 Primary Endpoint

The primary endpoint is to evaluate the reduction rate of ≥ grade 2 abdominal toxicities including abdominal distension, abdominal pain, diarrhea, dyspepsia, stomach pain, and typhlitis based on the NCI CTCAE with the use of rifaximin in stage I-III HER-2 positive breast cancer patients with PIGT after initiation of pertuzumab-based chemotherapy.

16.12 Secondary endpoints

16.121 Dose reductions, dose delays and discontinuation of treatment with pertuzumab due to gastrointestinal side effects

16.122 Change in the Bristol stool scale before and after rifaximin treatment.

16.123 Other PIGT such as abdominal pain, bloating and flatulence as measured by a 4 point Likert scale.

16.13 Correlative Endoints

16.131 Evaluation of changes in the fecal microbiome, hydrogen breath test, and permeability test before and after rifaximin.

16.132 Evaluation of changes in the fecal microbiome, hydrogen breath test, and permeability test before and after pertuzumab-based chemotherapy.

16.133 Evaluation the difference in the fecal microbiome, hydrogen breath test, and permeability test among patients with or without PIGT.

16.2 Study Design

We plan to conduct a single center, phase II trial of rifaximin in stage II-III HER2-positive breast cancer patients with PIGT (defined as ≥ National Cancer Institute Common Terminology Criteria for Adverse Events, NCI CTCAE v5.0, grade 2 gastrointestinal toxicities) despite standard antidiarrheal agents after the initiation of pertuzumab-based neoadjuvant or adjuvant chemotherapy. A parallel arm will comprise patients without PIGT (defined as ≤ 1 NCI CTCAE v5.0 grade gastrointestinal toxicities. No formal statistical comparisons are planned for the primary and secondary endpoints; however, we will evaluate between arm differences for the correlative endpoints in a descriptive and explorative fashion.

16.21 Decision Rule: The largest success proportion where the proposed treatment regimen would be considered ineffective in this population is 10%, and the smallest success proportion that may warrant subsequent studies with the proposed regimen in this patient population is 30%. The following 2-stage design uses 7 or 18 evaluable patients to test the null hypothesis that the true success proportion in a given patient population is at most 30%. We call it a success if the patient has reduction of ⩾ grade 2 abdominal toxicities after cycle 1.

16.211 STAGE 1: Enter 7 evaluable patients into the study. If no success is observed in the first 7 evaluable patients, we may consider this regimen ineffective in this patient population and terminate this study. Otherwise, if the number of successes is at least 1, we will proceed to Stage 2.

16.212 STAGE 2: Enter an additional 11 evaluable patients into the study. If 3 or fewer successes are observed in the first 18 evaluable patients, we may consider this regimen ineffective in this patient population. If 4 or more successes are observed in the first 18 evaluable patients, we may recommend further testing of this regimen in subsequent studies in this population.

16.213 NOTE: We will not suspend accrual between stages because long-term suspension of accrual for the first-stage analysis is likely to lead to poor accrual in the second stage. We feel that safety will not be compromised due to real time adverse event monitoring. We will consider closing accrual in the event of rapid accrual or high incidence of adverse events.

16.3 Sample Size

The sample size was determined based on the primary endpoint measured in the rifaximin arm and is based on a Simon's two-stage design (Simon, 1989), which is fully described in section 16.21. A minimum of 7 and a maximum of 18 evaluable patients will be accrued onto this phase II study unless undue adverse events are encountered. We anticipate accruing an additional 2 patients to account for ineligibility, cancellation, major treatment violation, or other reasons. Given the incidence of ≥ grade 2 diarrhea is

approximately 60% with pertuzumab-based chemotherapy; we expect to enroll a total of 34 patients to achieve 20 patients receiving rifaximin, of which 18 will be evaluable. Note that the total sample size will be determined by number of patients in the rifaximin arm, once there are 18 patients deemed evaluable in the rifaximin arm , the study will close accrual.

Assuming that the number of successes is binomially distributed, the significance level is ≤10% and the probability of declaring that this regimen warrants further studies (i.e. statistical power) under various success proportions and the probability of stopping accrual after the first stage can be tabulated as a function of the true success proportion as shown in the following table.

If the true success proportion is	0.10	0.15	0.20	0.25	0.30
then the probability of declaring that the regimen warrants further studies is	0.09	0.26	0.47	0.66	0.80
and the probability of stopping at stage 1 is	0.48	0.32	0.21	0.13	0.08

16.4 Analysis Plan

The statistical analyses will be handled in a descriptive manner.

16.41 Primary endpoint:

The proportion of successes will be estimated by the number of successes divided by the total number of evaluable patients. Confidence intervals for the true success proportion will be calculated according to the approach of Duffy and Santner (1987).

16.42. Secondary Endpoints

16.421. Descriptive statistics (frequency table) and histogram will be used to summarize dose reductions, dose delays and discontinuation of treatment with pertuzumab due to gastrointestinal side effects.

16.422 Descriptive statistics (frequency table) and histogram will be used to summarize the Bristol stool scale during the study.

16.423 Descriptive statistics (frequency table) and histogram will be used to summarize other PIGT such as abdominal pain, bloating and flatulence as measured by a 4 point Likert scale during the study.

16.43 Correlative Endpoints

16.431. Descriptive statistics (mean, sd, median, iqr) and longitudinal plots (raw value, change, change in percentage) will be used to summarize the baseline levels of hydrogen/methane peak by hydrogen breath test , diversity of gut microbiome(number of species) and specific species by fecal microbiome, and levels of urine mannitol and lactulose by permeability test before and after rifaximin.

16.432 Descriptive statistics (mean, sd, median, iqr) and longitudinal plots (raw value, change, change in percentage) will be used to summarize the baseline levels of hydrogen/methane peak by hydrogen breath test, diversity of gut microbiome(number of species) and specific species by fecal microbiome, and

levels of urine mannitol and lactulose by permeability test before and after pertuzumab-based chemotherapy.

16.433 Descriptive statistics (mean, sd, median, iqr) and longitudinal plots (raw value, change, change in percentage) will be used to the fecal microbiome, hydrogen breath test, and permeability test among patients with or without PIGT. The study is not powered to detect any differences between the two arms; the main purpose is to quantify and evaluate differences descriptively between patients who develop and do not develop PIGT (between arm 1 and arm 2) to inform subsequent research.

16.5 Accrual and duration of study

This trial will be conducted at Mayo Clinic in Florida. Based on past experience of patient accrual in previous trials and the frequency of pertuzumab therapy we estimate an accrual rate of approximately 1-2 patients per months. Therefore, we expect to complete accrual within 30 months. With a follow-up time of 6 months, the expected total duration of the study is about 3 years.

16.6 Data and Safety Monitoring

The principal investigator(s) and the study statistician will review the study monthly to identify accrual, adverse event, and any endpoint problems that might be developing. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least biannually, based on reports provided by the MCCC Statistical Office.

16.7 Adverse Event Stopping Rule

16.71 The stopping rule specified below is based on the knowledge available at study development. We do note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

16.72 Accrual will be temporarily suspended to this study if at any time we observe events that the study team considers to be at least possibly related to study treatment (i.e., an adverse event with attribute specified as “possible,” “probable,” or “definite”) that satisfy the following:

16.73 If there are ≥ 2 patients who receive rifaximin develop the same grade 3 or higher adverse events based on the NCI CTCAE v5.0 or if ≥ 1 patient develops grade 4 or higher adverse events that the study team considers to be at least possibly related to rifaximin (i.e., an adverse event with attribute specified as “possible,” “probable,” or “definite”).

16.74 We note that we will review grade 4 and 5 adverse events deemed “unrelated” or “unlikely to be related” to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse event.

16.8 Gender and minority accrual considerations

This study is open to patients for all races.

Accrual Targets				
	Sex/Gender(%)			
Ethnic Category	**Females**	**Males**	**Unknown**	**Total**
Hispanic or Latino	1	0	0	1
Not Hispanic or Latino	33	0	0	33
Ethnic Category: Total of all subjects	34	0	0	34
Racial Category				
American Indian or Alaskan Native	1	0	0	1
Asian	0	0	0	0
Black or African American	4	0	0	4
Native Hawaiian or other Pacific Islander	0	0	0	0
White	29	0	0	29
Racial Category: Total of all subjects	34	0	0	34

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens:

None.

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Data submission instructions for this study can be found in the Data Submission Schedule.

18.2 Survival Follow-up

See Section 4.2.

18.3 CRF completion

This study will use Medidata Rave® for remote data capture (rdc) of all study data. Data collection for this study will be done exclusively through the Medidata Rave® clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active account and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on either the organization roster at the enrolling site.

18.4 Site responsibilities

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation for diagnosis prior to study. These documents should be submitted within 30 days of preregistration (for prior to study entry materials).

18.6 Labelling of materials

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.7 Incomplete materials

Any materials deemed incomplete by the MCCC Operations Office will be considered "not received" and will not be edited or otherwise processed until the missing information is received. A list of the missing documents will be made available to the appropriate co-sponsor/participant.

18.8 Overdue lists

A list of overdue materials and forms for study patients will be generated monthly. The listings will be sorted by location and will include the patient study registration number. The appropriate co-sponsor/participant will be responsible to obtain the overdue material.

18.9 Corrections forms

If a correction is necessary the QAS will query the site. The query will be sent to the appropriate site to make the correction and return the query and documentation of correction back to the QAS.

19.0 Budget

19.1 Costs charged to patient: routine clinical care

19.2 Tests to be research funded: Rifaximin (study drug), Stool kit and test, Hydrogen breath test, and Permeability collection and test.

19.3 Other budget concerns:

Salix Pharmaceuticals, Inc will provide Mayo Clinic with funding to support costs of running this study and will provide study drug, rifaximin, for use in this study.

20.0 References

1. Baselga J et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* (2012) **366**(2):109-19.

2. Bass NM, Mullen KD, Sanyal A, et al: Rifaximin treatment in hepatic encephalopathy. N Engl J Med 362:1071-81, 2010.

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

[redacted]

Appendix II Patient Medication Diary (Arm 1 only)

Name ______________________________ **Study ID Number ____________________**

Please complete this diary on a daily basis. Write in the amount of the dose of rifaximin that you took in the appropriate "Day" box.

Rifaximin may be taken with or without food. On the days that you do not take any study drug or miss a dose, please write in "0" for any dose that was missed. If you forget to take your daily dose, please write in "0", but remember to take your prescribed dose at the next regularly scheduled time. Rifaxmin is required to be taken on Days 1-5. If you miss Day 5 doses, do not continue to take the drug the next day.

If you experience any health/medical complaints please record this information.

Week of: ______________________

Study Drug	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***
Rifaximin 1st dose					
Rifaximin 2nd dose					

Week of: ______________________

Study Drug	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***
Rifaximin 1st dose					
Rifaximin 2nd dose					

Week of: ______________________

Study Drug	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***
Rifaximin 1st dose					
Rifaximin 2nd dose					

Week of: ______________________

Study Drug	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***
Rifaximin 1st dose					
Rifaximin 2nd dose					

Week of: ______________________

Study Drug	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***
Rifaximin 1st dose					
Rifaximin 2nd dose					

Patient signature: ______________________________________

Health or medical complaints during this time:

Other medications or supplements taken during this time:

Name of medication or supplement	How much did you take? (example: Two 500mg pills)	When did you take it (examples: Every day Or Day 19 and Day 20)

Use a separate sheet of paper if more space is needed.

My next scheduled visit is: _____

If you have any questions, please call: _____

Study Coordinator Use Only

Number of pills returned _____	Number of vials returned: _____	
Discrepancy Yes____/No____	Verified by _____	Date_____

Appendix III NCI CTCAE v5.0 for Gastrointestinal toxicities

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Diarrhea	Increase of <4 stools per day	Increase of 4-6 stools per day over baseline	Increase of ≥7 stools per day over baseline; hospitalization indicated; limiting self care ADL	Life-threatening consequences, urgent intervention indicated	Death
Abdominal pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-
Bloating	No change in bowel function or oral intake	Symptomatic; decreased oral intake; change in bowel function	-	-	-
Flatulence	Mild symptoms; intervention not indicated	Moderate; persistent; psychosocial sequelae	-	-	-

ADL: activities of daily living

Appendix IV 4 point Likert scale questionnaire

Name _____ Study ID Number _____

A. How would you rate the severity of abdominal pain experienced during the past 3 weeks?
0. None
1. Mild
2. Moderate
3. Severe

B. How would you rate the severity of abdominal bloating experienced during the past 3 weeks?
0. None
1. Mild
2. Moderate
3. Severe

C. How would you rate the severity of flatulence/gas experienced during the past 3 weeks?
0. None
1. Mild
2. Moderate
3. Severe

D. How would you rate the severity of diarrhea experienced during the past 3 weeks?
0. None
1. Mild
2. Moderate
3. Severe

E. Do you consider your symptoms have improved since starting the study drug? (Only fill if you have received the study drug)
0. Yes
1. No

See reference:
Pimentel, Mark et al.: Rifaxmin therapy for patients with irritable bowel syndrome without constipation. The New England Journal 2011; 364:22-32.

Appendix V Bristol Stool Scale and Stool Diary

Stool form	Appearance	Type
Separate hard lumps, like nuts (hard to pass). Result of slow transit		1
Sausage-shaped but lumpy		2
Like a sausage but with cracks on its surface		3
Like a sausage or snake – smooth and soft		4
Soft blobs with clear cut edges (easy to pass)		5
Fluffy pieces with ragged edges, a mushy stool		6
Watery, no solid pieces. Result of very fast transit		7

See Reference:
Lewis, SJ et al. Stool form scale as useful guide to intestinal transit time. *Scandinavian Journal of Gastronenterology*. (*1997*) 32: 920-924.

Daily Diary Date: mm dd yy Initials: Day:

If you had no bowel movements today, please check this box: ☐ No bowel movements today.

	Describe the consistency of bowel movement	Describe the ease of passage of bowel movement	Did you feel like you completely emptied your bowels?
	1 Hard lumps 2 Lumpy sausage 3 Cracked sausage 4 Smooth sausage 5 Soft lumps 6 Mushy 7 watery	1 Manual Disimpaction 2 Enema needed 3 Straining needed 4 Normal 5 Urgent wo/pain 6 Urgent w/pain 7 Incontinent	1 No 2 Yes
1 hr. min ☐ am ☐ pm			
2 hr. min ☐ am ☐ pm			
3 hr. min ☐ am ☐ pm			
4 hr. min ☐ am ☐ pm			
5 hr. min ☐ am ☐ pm			
6 hr. min ☐ am ☐ pm			
7 hr. min ☐ am ☐ pm			

See Reference:
Vazquez-Roque et al. A Controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function. *Gastroenterology (2013)* 144(5), 903-911. https://doi.org/10.1053/j.gastro.2013.01.049