MSK PROTOCOL COVER SHEET

Randomized Trial Comparing Rifaximin and Placebo in the Treatment of Bowel Dysfunction After Anterior Resection for Rectal Cancer.

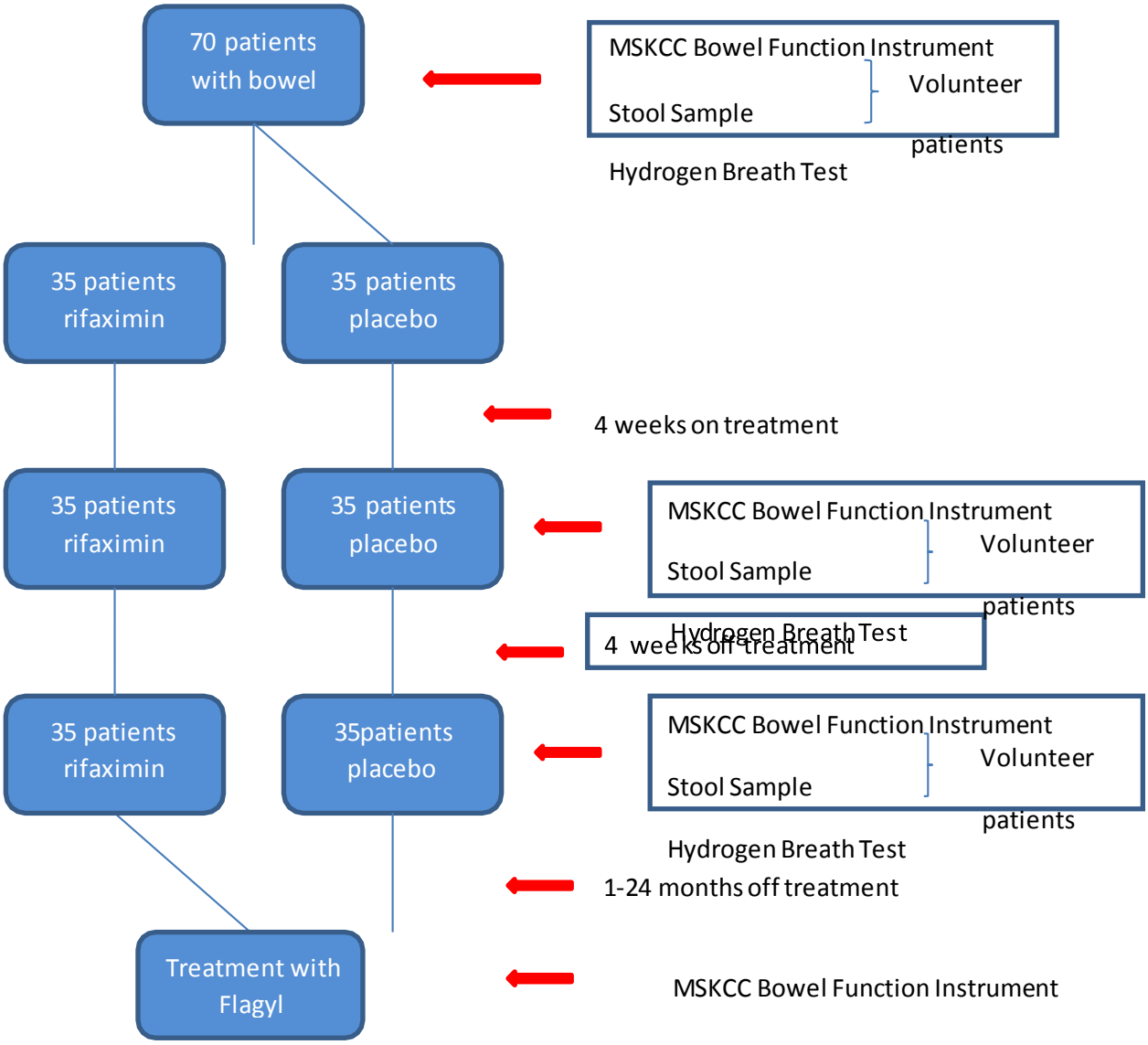
Principal Investigator/Department: Philip Paty, MD/Surgery

1.0 PROTOCOL SUMMARY AND/ORSCHEMA 3
2.0 OBJECTIVES AND SCIENTIFIC AIMS 4
3.0 BACKGROUND AND RATIONALE 5
4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION 6
4.2 Design 6
4.3 Intervention 7
5.0 THERAPEUTIC/DIAGNOSTIC AGENTS 8
6.1 CRITERIA FOR SUBJECT ELIGIBILITY 8
6.2 Subject Inclusion Criteria 8
6.3 Subject Exclusion Criteria 9
7.0 RECRUITMENT PLAN 9
8.0 PRETREATMENT EVALUATION 10
9.0 TREATMENT/INTERVENTIONPLAN 10
10.0 EVALUATION DURING TREATMENT/INTERVENTION 10
11.0 TOXICITIES/SIDE EFFECTS 12
12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT 16
13.0 CRITERIA FOR REMOVALFROM STUDY 16
14.0 BIOSTATISTICS 16
15.1 RESEARCHPARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES 18
15.2 Research Participant Registration 18
15.3 Randomization 18
16.1 DATA MANAGEMENT ISSUES 19
16.2 Quality Assurance 19
16.3 Data and Safety Monitoring 19
17.1 PROTECTION OF HUMAN SUBJECTS 20
17.2 Privacy 20
17.3 Serious Adverse Event (SAE) Reporting 20
18.0 INFORMED CONSENT PROCEDURES 21
19.0 REFERENCES 21
20.0 APPENDICES 23

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a blinded randomized control trial comparing a four week treatment with the antibiotic rifaximin versus placebo in the management of bowel dysfunction after Anterior Resection (AR) or Sphincter Preserving Surgery (SPS) for rectal cancer. Patients will be randomized into 2 groups, one group will receive rifaximin 400mg bid for 4 weeks and the other group will receive a placebo bid for 4 weeks. Prior to commencement of treatment patients bowel symptoms will be assessed using the MSKCC Bowel Function Instrument (BFI score). For those patients that agree, stool samples will be taken and stool bacterial content will be assessed. The same patients will also undertake breath testing to assess Small Intestinal Bacterial Overgrowth (SIBO). Following treatment, all patients will again be assessed using the BFI, and stool sampling and breath testing will be preformed on those patients that agreed to it. Four weeks after cessation of treatment all patients will undertake a third round of testing using the BFI and in respective patient, a third round if stool sampling and breath testing will occur. 1-24months following completion of the study, we will ask patients to participate in a follow up study using metronidazole 500mg po tid x 3 weeks on a voluntary basis. In this case we will use a phase II design and not compare with a placebo control.

The primary objective of this study is to compare the efficacy of rifaximin and placebo in the treatment of bowel dysfunction as measured by MSKCC Bowel Function Instrument in patients following AR or SPS for rectal cancer. A study schema is depicted below.



2.1 OBJECTIVES AND SCIENTIFIC AIMS

2.2 Primary objective.

- To compare the efficacy of rifaximin and placebo in the treatment of bowel dysfunction as measured by MSKCC Bowel Function Instrument (BFI score) in patients following AR or SPS for rectal cancer.

2.3 Secondary objectives

- To determine if reduction in intestinal bacterial quantity correlates with improvement in bowel symptoms (BFI score).
- To explore the bacterial composition of stool before and after antibiotic treatment.
- To identify dynamic changes in bowel function during and after antibiotic treatment using a bowel function log.

- To identify the efficacy of metronidazole in the treatment of bowel dysfunction as measured by the MSKCC BFI in patients following AR or SPS for rectal cancer for patients who have no improvement following treatment with rifaximin or placebo.

3.0 BACKGROUND AND RATIONALE

The surgical treatment of rectal cancer has become increasingly directed towards sphincter preservation where possible, with the avoidance of a permanent stoma. This has been accomplished in part by refinement of the conventional anterior resection with surgical techniques such as ultra low anterior resection (LAR), intersphincteric dissection, and coloanal anastomosis (CAA). [1, 2] The widespread use of neo-adjuvant radiotherapy and chemotherapy has also led to the down staging of low rectal tumors pre-operatively which previously would have necessitated an abdomino-perineal resection again facilitating sphincter preserving surgery (SPS).[3]

The large number of patients treated by SPS has led to a greater awareness of the bowel dysfunction following surgery or so called anterior resection syndrome (ARS).[4] Up to 90% of patients develop symptoms of bowel dysfunction following SPS for rectal cancer. The most common symptoms include incomplete evacuation, clustering of bowel motions, food affecting frequency, unformed stool, and gas incontinence; the less common but more debilitating symptoms are stool frequency and incontinence.[5] Several surgical treatment factors are known to affect bowel function. In addition, the use of adjuvant or neo-adjuvant radiotherapy to the pelvis has also been shown to exacerbate these symptoms and worsen bowel function.[6, 7] These symptoms have a significant effect on patient quality of life with 27% of patients describing their symptoms as severely affecting their lifestyle and significantly impacting on their quality of life.[5] The mechanisms responsible for these symptoms are not clearly understood but are thought to involve a combination of etiological factors, including loss of fecal reservoir (rectum), injury to pelvic nerves and sphincter injury.[8-11] A variety of novel surgical techniques have been developed with the goal of minimizing bowel dysfunction: these include creation of a neo-rectum using a colonic-J-pouch, coloplasty, end to side colorectal anastomosis, construction of artificial anal sphincters, and sacral nerve stimulation.[7, 12] These methods have been met with varying degrees of success; however, none have resulted in completely satisfactory bowel function.[13] Currently there is no standard of care for the medical management of this bowel dysfunction. Patients are generally treated individually based on symptoms

with a combination of high fibre diet, antidiarrheal agents, laxatives and/or probiotics without significant success.

Another theory explaining ARS is that denervation of the distal colon and reduction of rectal volume leads impaired / inefficient rectal evacuation and a state of rectal pseudo-obstruction. The resulting stasis of stool within the colon leads to increased numbers of colonic bacteria and subsequently overgrowth of bacteria in the large and small bowel contributing to the symptoms of bowel dysfunction.

Altered bowel function as a consequence of increased intestinal bacteria is not a new concept. Antibiotics are used in many intestinal conditions to improve bowel function. Patients with Crohns disease and small intestinal bacterial overgrowth SIBO have been shown to benefit from treatment with metronidazole and rifaximin individually, with both a reduction in SIBO and bowel symptoms.[14, 15] In active inflammatory bowel disease, antibiotic therapy also plays a role in its management. Metronidazole appears to induce significant clinical improvement in patients with pouchitis compared to placebo,[16] and long term treatment using rifaximin and metronidazole reduces the incidence of clinical flare ups of IBD again as compared to placebo.[17, 18] Similarly in irritable bowel syndrome, treatment with rifaximin has demonstrated both improvement in symptoms and a reduction in SIBO.[19] Finally in patients with chronic liver disease antibiotics again specifically both metronidazole and rifaximin have been used to reduce ammonia producing bacteria in the intestine and with that the incidence of hepatic encephalopathy and spontaneous bacterial peritonitis.[20]

Within the past two years, the PI has used metronidazole (500mg po tid for 14 days) to treat severe bowel symptoms in over 20 patients. Over 80% of these rectal cancer patients have reported marked improvement in their bowel function, including better stool formation, more complete evacuation, fever bowel motions, and less gas formation. No adverse effects were noted. However, after stopping metronidazole bowel symptoms were noted to return gradually over one to two months. More recently the PI has begun treating patients with rifaximin 400mg bid as it has fewer side effects, with similar results. This experience has led us to the theory that by treating patients with antibiotics the quantity of fecal bacteria is reduced, which in turn improves stool formation, enhances rectal evacuation, and alleviates the symptoms of bowel dysfunction.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

Consented patients will undertake the MSKCC Bowel Function Instrument, a validated questionnaire developed by the Co-PI that assesses bowel function. [5] **Appendix 2.** Patients will then be invited to undertake a hydrogen breath test and fecal

sampling to assess for bacterial content in their small intestine and stool. Following stratification of patients by the method of anastomosis (stapled or hand sewn), the patients will then be randomized into two groups. The patients will receive treatment with rifaximin or placebo depending on the arm of the trial to which they have been randomized. Patients will at this stage on a volunteer basis be asked to submit stool samples and undertake a breath test. Patients will receive the respective treatment for four weeks. Following cessation of treatment all patients will be assessed using the BFI and respective patients will have repeat stool sampling and breath testing. Patients will then have these tests repeated 4 weeks later. This corresponds to 8 weeks after the start of the trial. For those patients not undertaking stool and breath testing, they will be reminded by phone, mail or email to fill out the BFI. Before and during the trial patients will be asked to fill out a weekly stool diary to evaluate trends on bowel function. **Appendix 3.** Both the patients and the physicians will be blinded to the patient treatments. The Initial consultation along with subsequent rounds of testing with submission of stool samples and breath testing will be undertaken by one of the consenting physicians at a specifically arranged and designated "research clinic" at one of MSKCC's network centers. (Sleepy Hollow, Commack, Main Campus and 53rd St). Patients may also be seen and consented at their regular follow up colorectal clinic visits. At 1 – 24 months after completion of the phase III portion of the trial, patients will be contacted by phone and given the option of receiving metronidazole in a followup, single arm study in which all patients receive the antibiotic. Patients who wish to participate will be mailed a drug prescription for a 3 week course of metronidazole 500mgs tid as well as the BFI forms and stool diary. Pretreatment and posttreatment BFI scores will be collected and analyzed for change in bowel function as was done in the initial Phase III study.

4.3 Intervention

The antibiotic used will be rifaximin. Rifaximin acts by inhibition of bacteria synthesis of RNA. It is active against gram positive and gram negative bacteria including both aerobes and anaerobes however it is not absorbed in the gastro-intestinal system facilitating its use in enteric infections. It is licensed for the treatment of acute bacterial diarrhea and SIBO. [21] We have chosen to use rifaximin over metronidazole because it is not enterically absorbed and as such has less of a side-effect profile. Metronidazole causes a higher incidence of nausea and GI upset, has a greater amount of drug interactions and it also has a metallic taste which could effect blinding and introduce bias.

For this trial, both rifaximin and placebo will be prepared in identical capsules by the research pharmacist at MSKCC and distributed to the outpatient pharmacies. We have chosen a rifaximin dose of 400 mg bid for 4 weeks as this has been shown to be efficacious in the treatment of SIBO at this dose over 14 days. We have added a further

2 weeks to the treatment regime as from prior experience by the PI's using rifaximin in this condition, the beneficial effect tends to reduce over one week. As the BFI is validated for use over a 4 week period (ie assessing bowel function over a 4 week period), to show an effect on bowel function as assessed by the BFI, treatment is needed over a four week period. We plan to assess bowel function at 4 weeks after the completion of treatment to determine if the response to treatment is transient or whether it is a more durable effect. Following the completion of the trial patients will be offered a 3 week treatment with 500 mg of metronidazole tid. They will be assessed with the BFI at pretreatment and again at 0-1 and 4-6 weeks post treatment. Should the effect be transient, this will lead to future studies to assess if adding extra treatment with other medications such as pro-biotics may prolong the effect.

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

5.2 Rifaximin (Xifaxan®)

Rifaximin is a non-aminoglycoside semi-synthetic, nonsystemic antibiotic derived from rifamycin SV with a chemical name of 5,6,21,23,25- pentahydroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-2,7(epoxypentadeca-[1,11,13]trienimino)benzofuro[4,5-e]pyrido[1,2-á]-benzimidazole-1,15(2H)dione, 25-acetate. It comes in preparations of 200mg and 550mg tablets. It can be stored at room temperature and is FDA licensed for use in the treatment of bacterial intestinal infections.

5.3 Metronidazole (Flagyl®)

Metronidazole is an oral synthetic antiprotozoal and antimicrobial agent with a chemical name of 1-(β-hydroxy-ethyl)-2-methyl-5-nitroimidazole. It comes in oral preparations of 250mgs and 500mgs tablets. It can be stored at room temperature and is FDA licensed for the treatment of bacterial intestinal infections.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

- Patients with a history of rectal cancer treated with an anterior resection preformed at MSKCC (tumor at or below 12cm from anal verge) with restoration of bowel continuity ≥1 and ≤ 5 years. (Patients may also have had

procedures to construct neo-rectums including j-pouch, coloplasty, and end to side anastomosis).

- Patients ≥ 21 years of age.
- Presence of anterior resection symptoms by patients own assessment. These symptoms may include any of the following: incomplete evacuation, clustering of bowel motions, frequency of bowel motions, unformed stool, excessive flatus, or incontinence of flatus and/or feces.

6.3 Subject Exclusion Criteria

- Local recurrence of rectal cancer.
- Antibiotic treatment within the last 4 weeks for any condition.
- Pregnancy or breast feeding.

7.0 RECRUITMENT PLAN

Patients who have had an anterior resection for rectal cancer at MSKCC will be identified from a prospective database maintained by the Colorectal Surgery Service. These patients will be contacted by a letter inviting them to participate in a randomized study designed to evaluate the beneficial effects of rifaximin and placebo on bowel function. **Appendix 1.** We will ask patients to participate in the trial based on their own personal perception of their symptoms. Patients will be asked in the letter to contact Dr. Philip Paty (PI) or Dr. James Smith (Research Fellow) by email, telephone, or letter. Patients who have not responded to the letter after one month will be contacted by phone and invited to participate. **Appendix 4.** Every attempt will be made to recruit women and minorities to participate in this study. Participation is voluntary. Patients who are interested in participating will be scheduled for a research clinic visit with Dr. Philip Paty or another of the consenting professionals. Consenting patients will be registered and randomized at the initial clinic visit. We hope to recruit 70 patients, 35 in each arm in an attempt to have at least 30 patients per arm for analysis allowing for patient dropout.

Patients will also be asked to volunteer to submit stool samples and breath tests. Those who agree will be tested for stool bacteria and SIBO at the relevant time points along with the MSKCC BFI. For those patients who do not agree to volunteer to supply stool samples and breath test, they will be tested using the MSKCC BFI alone at the relevant time periods. All patients will fill out the stool log/diary. We hope to recruit half of the patients in each arm to participate in the extra testing. We are not testing all patients with stool sampling and breath tests as we feel it will hamper accrual into the trial. Patients will be reimbursed with a $100 gift card for each outpatient visit to cover transport costs for participating in this trial. Following completion of the phase III portion of the trial, patients will be contacted by phone and offered to participate in a follow up single arm study to assess the efficacy of metronidazole in the treatment of bowel dysfunction.

Patients already enrolled on version A(0) or A(1) will also be asked via phone to participate in the follow up study. If they wish to participate, they will be reconsented. Consent forms will be mailed to them with a self addressed return envelope.

8.1 PRETREATMENT EVALUATION

- Routine clinic evaluation at MSKCC ≤ 12 months of beginning the trial.
- Serum beta-HcG levels for pre-menopausal women (last menses within one year) ≤ 2 weeks.

9.0 TREATMENT/INTERVENTION PLAN

The planned treatment during this trial will be the administration of the antibiotic rifaximin 400mg bid for 4 weeks or a placebo bid for 4 weeks to patients with bowel dysfunction. Both rifaximin and placebo will be prepared in identical capsules by the research pharmacist at MSKCC and distributed to the outpatient pharmacies. Following registration to the trial patients will be given a prescription for the trial, which they will be able to fill out at one of the MSKCC satellite center outpatient pharmacies. Patients will be instructed to take then treatment at the same time in the morning and the evening. Any missed doses will be recorded in the stool diary/log. Once treatment has been completed patients will be asked to mail back the treatment container along with any un-used medication.

Once the trial has been completed and patients have been unblinded, patients may elect to be treated with Rifaximin if they so wish.

10.1 EVALUATION DURING TREATMENT/INTERVENTION

Pre-treatment intervention & evaluation all undertaken in an outpatient clinic (day 0)

- MSKCC BFI (for all patients)
- Breath test (for patient volunteers)
- Stool Sampling (for patient volunteers)

Treatment with 400 mg rifaximin bid x 4 weeks or placebo bid x 4 weeks.

Post treatment evaluation at an outpatient clinic, for those patients who volunteered for extra testing. (days 26 - 35 and days 53 – 63)

- MSKCC BFI
- Breath test
- Stool Sampling

Post treatment evaluation for patients not volunteering for extra tests which can be undertaken in the patients home during the following periods. (days 26 - 35 and 53 – 63)

- MSKCC BFI

All patients will be required to fill out a weekly stool log/diary for the duration of the trial. There are questions in the log/diary to assess patient compliance also.

One to twenty-four months following completion of the phase III trial patients will be offered a 3 week course of metronidazole. They will then be assessed again using the BFI at pretreatment and at 0-1 and 4-6 weeks post-treatment. (21-30 days and 49 – 63 days following starting metronidazole)

MSKCC BFI

The MSKCC BFI is a validated instrument in the assessment of bowel function following rectal cancer surgery over a 4 week period.[5] It consists of 18 items to which patients respond using a 5-point Likert scale, ranging from Always to Never. There is a summary score, and three subscales with good internal consistency (Frequency (α=0.75), Dietary (α=0.78), Urgency (α=0.79)) and test-retest reliability (total score =0.84, Frequency=0.74, Dietary=0.62, Urgency=0.87). The instrument has demonstrated discriminant and construct validity. It is written at a grade 8 reading level, and takes 5-10 minutes to complete. The BFI if filled out at home can be emailed or mailed back to the investigator where they will be stored on a secure database and a locked drawer. Should there be any incomplete or ambiguous answers a follow up phone call will be made by one of the consenting physicians to clarify the answer. However in a prior trial by the Co-PI (Larissa Temple 06-151), of the 125 patients who answered the BFI on multiple occasions less then 5 items per question were missing or filled out inadequately.

Breath Test

Methane and hydrogen breath testing is currently the best non-invasive way of assessing small bowel bacterial overgrowth.[22] The test takes 1 hour and can be taken in the outpatient clinic. The patient must fast at least 12 hours prior to starting the test. The patient takes a baseline breath and exhales into a container. He/she then ingests a quantity of lactulose. If there is overgrowth of bacteria in the small intestine the sugar is not absorbed but is instead fermented by the bacteria in the gastrointestinal tract producing hydrogen and methane. The human body does not endogenously produce either of these gasses. These gasses can then be detected in the breaths of patients indicating bacterial overgrowth in the small bowel. Subsequent breaths are then taken at varying intervals up to 90 minutes from the initial ingestion of the sugar and are assessed for methane and hydrogen levels. The samples will be analyzed at Metabolic Solutions Incorporated®, a commercial laboratory.

Stool Sampling

Stool samples will be taken during the outpatient visits. Patients can submit a sample by defecating into a container or a sample can be taken by gentle rectal examination. The samples will be collected in the outpatient setting and frozen at -80 degrees Celsius. DNA will be extracted using commercial kits (Qiagen®). Bacterial culture has proven to be unreliable as a quantitive measure of fecal content as only a tiny minority of fecal bacteria will survive in culture. As such we plan to quantify fecal bacteria using real time quantitive PCR for the gene for 16s ribosomal RNA as previously described.[23] This gene is present in all bacteria and has both conserved and variable domains. For quantification of total fecal bacteria, PCR primers amplifying the conserved domain will be used and the PCR product will be measured using the Syber Green method.

To evaluate the spectrum of fecal bacteria, a PCR plus 454 pyrosequencing technique optimized by one of the Co-Investigators (Eric Pamer) will be used. The conserved and variable domains of the gene 16S ribosomal RNA will be amplified as one PCR product using previously described primers. From this product, the variable domain will be analyzed using 454 pyrosequencing technique. Microbial complexity will be determined and comparisons between samples will be performed using UniFrac and Principal Coordinates Analysis. Approximately 120 species of bacteria will be represented in the various Pyrosequencing primers. The read out of the assay will give a positive fluorescent signal for each bacterial species present in the feces.

Expertise in both quantifying total bacterial load and identifying fecal colonization patterns will be provided by Dr Eric Pamer and his laboratory.[24]

Stool Diary/Log

The stool diary/log will allow the investigators to follow trends in bowel function and to assess how long the antibiotic effect lasts. Patients will be asked to fill the diary out once per week (+/- 2 days) on paper or by email before and through the duration of the trial. The questions asked will address range of stools per day, stool formation, flatus, incontinence, ability to completely evacuate. Patient compliance will also be assessed in the log. It will not take longer than 3 minutes to fill out. Once the log is competed it can be posted or emailed back to the investigator where it will be stored on a secure database and a locked drawer.

11.1 TOXICITIES/SIDE EFFECTS

11.2 Rifaximin

The safety of Rifaximin for the treatment of infectious diarrhea was assessed in 2 placebo controlled trials. Discontinuation of the drug due to adverse reactions occurred in < 0.4% of patients. Those adverse reactions were taste loss, diarrhea, weight loss anorexia, nausea and nasal passage irritation.

Side effects include: [25]

>10%:

- Cardiovascular: Peripheral edema (15%)
- Central nervous system: Dizziness (13%), fatigue (12%)
- Hepatic: Ascites (11%)
- Gastrointestinal:

Nausea (14%) 2% to 10%:

- Cardiovascular: Chest pain (>2% to 5%), hypotension (>2% to 5%)
- Central nervous system: Headache (10%), depression (7%), fever (6%), amnesia (>2% to 5%), attention disturbance (>2% to 5%), confusion (>2% to 5%), hypoesthesia (>2% to 5%), pain (>2% to 5%), tremor
- Dermatological: Pruritus (9%), rash (5%), cellulitis (>2% to 5%)
- Endocrine and metabolism: Hyper-/hypoglycemia (>2% to 5%), hyperkalemia (>2% to 5%), hyponatremia (>2% to 5%)
- Gastrointestinal: Abdominal pain (6% to 9%), anorexia (>2% to 5%), dehydration (>2% to 5%), esophageal varices (>2% to 5%), weight gain (>2% to 5%), xerostomia (>2% to5%)
- Hematologic: Anemia (8%)
- Neuromuscular & skeletal: Muscle spasms (9%), arthralgia (6%), myalgia (>2% to 5%)
- Respiratory: Nasopharyngitis (7%), dyspnea (6%), epistaxis(>2% to 5%), pneumonia (>2% to 5%), rhinitis (>2% to 5%), upper respiratory tract infection (>2% to 5%)
- Miscellaneous: Influenza-like illness (>2% to 5%)

<2%, postmarketing, and/or case reports (limited to important or life-threatening):

Abnormal dreams, allergic dermatitis, anaphylaxis, angioneurotic edema (including tongue and facial edema with dysphagia), CDAD, dysuria, exfoliative dermatitis, flushing, hematuria, hypersensitivity reactions, insomnia, lymphocytosis, monocytosis, motion sickness, neutropenia, polyuria, proteinuria, sunburn, tinnitus, urticaria

Drug interactions: [25]

Although in vitro studies have suggested that rifaximin induces CYP3A4, no differences in midazolam (also induces CYP3A4) concentrations were found after administration of midazolam alone and midazolam and rifaximin together. Rifaximin had also been shown to have no effect on the oral contraceptive pill. No drug interactions have been reported to date.

11.3 Metronidazole

The two most significant adverse reactions reported when taking metronidazole are convulsive seizures and peripheral neuropathy; these occur in <0.5% of patients and are nearly always associated with prolonged administration. The most commonly experienced side effect is nausea (12% of patients).

Other side effects include: (Taken from Lexi-Drugs online)

- Cardiovascular: Flattening of the T-wave, flushing, syncope
- Central nervous system: Aseptic meningitis, ataxia, confusion, coordination impaired, depression, dizziness, encephalopathy, fever, headache, insomnia, irritability, seizure, vertigo
- Dermatologic: Erythematous rash, pruritus, Stevens-Johnsonsyndrome, urticaria
- Endocrine & metabolic: Disulfiram-like reaction, dysmenorrhea
- Gastrointestinal: Nausea (~12%), anorexia, abdominal cramping, constipation, diarrhea, epigastric distress, furry tongue, glossitis, pancreatitis (rare), proctitis, stomatitis, unusual/metallic taste, vomiting, xerostomia
- Genitourinary: Cystitis, darkened urine (rare), dyspareunia, dysuria, incontinence, libido decreased, pelvic pressure, polyuria, vaginal dryness, vaginitis
- Hematologic: Neutropenia (reversible), thrombocytopenia (reversible, rare)
- Local: Thrombophlebitis
- Neuromuscular & skeletal: Dysarthria, peripheral neuropathy, weakness
- Ocular: Optic neuropathy
- Respiratory: Nasal congestion, pharyngitis, rhinitis, sinusitis, pharyngitis
- Miscellaneous: Flu-like syndrome, joint pains resembling serum sickness, moniliasis.

Drug interactions include: (Taken from Lexi-Drugs online)

- Alcohol (Ethyl): Metronidazole may enhance the adverse/toxic effect of Alcohol (Ethyl). A disulfiram-like reaction may occur.
- BCG: Antibiotics may diminish the therapeutic effect of BCG.
- Busulfan: Metronidazole may increase the serum concentration of Busulfan.
- Calcineurin Inhibitors: Metronidazole may decrease the metabolism of Calcineurin Inhibitors.
- Colchicine: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Colchicine. Management: Reduce colchicine dose as directed when using with a moderate CYP3A4 inhibitor, and increase monitoring for colchicine-related toxicity. Use extra caution in patients with impaired renal and/or hepatic function.

- CYP3A4 Substrates: CYP3A4 Inhibitors (Moderate) may decrease the metabolism of CYP3A4 Substrates.
- Disulfiram: Metronidazole may enhance the adverse/toxic effect of Disulfiram.
- Eplerenone: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Eplerenone. Management: A lower starting dose of eplerenone (25 mg once daily for adults) is recommended in patients with hypertension who are also taking drugs that are moderate inhibitors of CYP3A4.
- Everolimus: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Everolimus. Management: Use of this combination is not recommended without close everolimus concentration monitoring/dose adjustments. For renal cell carcinoma, an initial everolimus dose reduction to 2.5 mg/day (adult dose) is recommended.
- Fentanyl: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Fentanyl. Management: Monitor patients extra closely for several days following initiation of the combination, and fentanyl dosage reductions should be made as appropriate.
- Halofantrine: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Halofantrine.
- Mebendazole: May enhance the adverse/toxic effect of Metronidazole. Particularly the risk for Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis may be increased.
- Mycophenolate: Metronidazole may decrease the serum concentration of Mycophenolate. Specifically, metronidazole may decrease concentrations of the active metabolite of mycophenolate.
- Phenobarbital: May decrease the serum concentration of Metronidazole.
- Phenytoin: Metronidazole may increase the serum concentration of Phenytoin. Phenytoin may decrease the serum concentration of Metronidazole.
- Pimecrolimus: CYP3A4 Inhibitors (Moderate) may decrease the metabolism of Pimecrolimus.
- Ranolazine: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Ranolazine. Management: Limit the ranolazine adult dose to a maximum of 500 mg twice daily in patients concurrently receiving moderate CYP3A4 inhibitors (e.g., diltiazem, verapamil, erythromycin, etc.).
- Salmeterol: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Salmeterol.
- Saxagliptin: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Saxagliptin.
- Tipranavir: Metronidazole may enhance the adverse/toxic effect of Tipranavir.
- Tolvaptan: CYP3A4 Inhibitors (Moderate) may increase the serum concentration of Tolvaptan.

- Typhoid Vaccine: Antibiotics may diminish the therapeutic effect of Typhoid Vaccine. Only the live attenuated Ty21a strain is affected. Management: Vaccination with live attenuated typhoid vaccine (Ty21a) should be avoided in patients being treated with systemic antibacterial agents. Use of this vaccine should be postponed until at least 24 hours after cessation of antibacterial agents.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The ultimate response to treatment with antibiotics for ARS will be with the MSKCC bowel function instrument. This is an 18 point questionnaire which takes about 10 minutes to complete. It can be completed either in the outpatient setting or at home. This instrument has been validated in the assessment of bowel function following rectal cancer surgery over a 4 week period.[5] The BFI score will be calculated by the consenting physicians.

The purpose of the correlative studies is to assess if symptoms of ARS, indicated by a reduced MSKCC bowel function instrument score are associated with increased bacterial content in the gastro-intestinal tract or with a specific pattern of bacterial colonization. To this end bacterial content in the small intestine will be assessed using the breath test and bacterial content and colonization patterns will be assessed from fecal stool samples.

13.1 CRITERIA FOR REMOVAL FROM STUDY

A patient has the right to withdraw from the study at any time without prejudice to his/her future medical care by his/her physician at the institution

Criteria for removal for the study include:

- Patient discretion
- Significant noncompliance by the patient (<75% of drug dose taken)
- Development of an grade 3 or 4 adverse reaction to rifaximin
- Recurrence of their rectal cancer
- Should a treating physician deem it necessary in the best interest of the patient

14.0 BIOSTATISTICS

This is a blinded randomized control trial to compare the efficacy of a four week treatment with the rifaximin versus placebo in the management of Anterior Resection Syndrome (ARS) following Sphincter Preserving Surgery (SPS) for low rectal cancers. Patients will be randomized into 2 groups, one to receive rifaximin 400mg bid for 4 weeks and one group to receive a placebo bid for 4 weeks.

The primary endpoint is the change in the MSKCC bowel function index from baseline to 4 weeks after the start of therapy. At 4 weeks the patients will have been on therapy for 4 consecutive weeks. The MSKCC bowel function index has been validated to examine 4 week changes in the bowel function. [5] It consists of 18 items to which patients respond using a 5-point Likert scale, ranging from Always to Never. There is a summary score, and three subscales with good internal consistency (Frequency (α=0.75), Dietary (α=0.78), Urgency (α=0.79)) and test-retest reliability (total score =0.84, Frequency=0.74, Dietary=0.62, Urgency=0.87).Each subscale is scored by summing up the responses to the items in the subscale. If there are missing items within a subscale, the mean of the completed subscale items will be substituted for the item when >50 percent of the items in the subscale is completed. A global score will be calculated by adding the subscale scores. An additional aggregate score (total score) will also be calculated with the subscales and the single items. A subscale will be considered missing if more than one-half of the items are missing. The global score will be considered missing if any of the subscales or single items were missing. The average total score (frequency, urgency, dietary, plus four single items) among the 129 MSKCC rectal patients following SPS used to develop the bowel function index was 63.7 with standard deviation of 11.6 and range of 35 – 83. Larger BFI scores indicate better function. In this study the main comparison of interest is to examine whether the change in global-BFI from baseline to 4 weeks after four weeks of rifaximin is different from the change from baseline to 4 weeks of placebo. The analysis will be a done using an ANCOVA model, with baseline BFI and treatment indicator as covariates and 4 week BFI as the dependent variable.

Based on clinical observation, we expect the difference in BFI between the two treatments to be fairly large. We would like to accrue 30 patients in each arm stratified by surgical procedure (hand sewn versus stapled anastomosis) for randomization to ensure the two arms are balanced, as surgical procedure is known to correlate with BFI.

Power calculations are based on the global score on the BFI. Based on Dr Temple's work when developing the BFI score in 129 MSKCC rectal cancer patients, the lowest mean BFI score was found among the hand sewn patients. The mean BFI score for these patients was 42 with a standard deviation of 8and 51.6 with a standard deviation of 9 among patients with stapled anastomosis. In a current prospective study of 225 patients which is still maturing, we have found that at 6 months, the BFI discriminates between many clinical parameters with one of the most significant predictors being hand sewn vs stapled anastomosis (a surrogate for tumor level) (Mean 43.3, std 7 (n=36) vs Mean 49.4, std 8 (n=81). Based on these data, we anticipate that a difference of 6 points on the BFI is clinically and statistically meaningful.

For our power calculation, we set the type I error at 5%. We assume that the placebo arm will have no change in BFI from baseline to 4 weeks after the start of therapy. We also assume that the baseline for both treatment groups will be on a similar

magnitude. In line with the above average scores and standard deviations in Dr Temple's past and ongoing studies, we assume that the standard deviation of the differences to be 8 and the average BFI score at baseline to be 44. Using a two-sided, two sample t-test, we will have 80% power to detect a difference of differences from 0 in the placebo arm to 5.8 in the rifaximin arm with 30 patients in each arm. We expect to accrue the 60 patients for this study in 1 year, with a plan to recruit 35 in each arm to account for dropout.

In secondary analyses, we will examine the change in BFI scores adjusted for possible cofounders, such as peri-op radiotherapy. We will also examine bowel function data from the weekly logs in order to learn in what time frame (onset, duration) the effects of rifaximin and placebo occur. The data will be evaluated by graphical display without rigorous statistical testing. The weekly logs will also record drug compliance. Bacterial quantity and content in stool along with presence of SBIO will also be assessed in secondary analysis. These are both exploratory analyses.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.3 Randomization

This is a double blind randomized comparison of the antibiotic rifaximin and placebo in the treatment of bowel dysfunction following rectal resection for cancer. After eligibility is established and immediately after consent is obtained, patients will be registered in the Protocol Participant Registration (PPR) system and randomized using the Clinical Research Database (CRDB), by calling the MSKCC PPR Office at 646-735-8000 between the hours of 8:30 am and 5:30 pm, Monday - Friday. Randomization will

be accomplished by the method of random permuted block, and patients will be stratified by the variable of type of anastomosis (stapled or hand sewn). Since this is a double blind study, the patients' treatment assignments can be viewed in the CRDB only by the hospital pharmacists who are dispensing the study drugs.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study at MSKCC. The RSA will be responsible for project compliance, data collection, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected from this study will be entered into a secure MSKCC database in regulation with HIPAA guidelines. Hard copies of the MSKCC bowel function instrument questionnaire will be kept in a locked drawer.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration of data. Routine data quality reports will be generated to asses missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and SafetyMonitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring*

Board (DSMB) for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board

17.1 PROTECTION OF HUMAN SUBJECTS

Patient wellbeing will always take precedence in this trial however there will be minimal patient risk during this trial. Adverse drug reactions will be the most significant risk to patient health; however rifaximin carries minimal side effects. Should patients develop grade 3 or 4 toxicity to the antibiotic treatment, their treatment will be stopped and they will be removed from the study. The patient will be responsible for all costs related to treatments and complications of treatment except the antibiotic treatment, the placebo, breath testing and stool sampling which will be provided by MSKCC. Participation in this clinical trial is voluntary and at any stage patients may opt out to pursue any alternative treatment they wish. Once the trial is unblinded patients who received placebo during the trial may be treated with rifaximin if they so wish.

17.2 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.3 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)

- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

19.0 REFERENCES

1. Rullier, E., et al., *Sphincter-saving resection for all rectal carcinomas: the end of the 2-cm distal rule.* Ann Surg, 2005. **241**(3): p. 465-9.

2. Schiessel, R., et al., *Technique and long-term results of intersphincteric resection for low rectal cancer.* Dis Colon Rectum, 2005. **48**(10): p. 1858-65; discussion 1865-7.
3. Ceelen, W.P., Y. Van Nieuwenhove, and K. Fierens, *Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer.* Cochrane Database Syst Rev, 2009(1): p. CD006041.
4. Karanjia, N.D., D.J. Schache, and R.J. Heald, *Function of the distal rectum after low anterior resection for carcinoma.* Br J Surg, 1992. **79**(2): p. 114-6.
5. Temple, L.K., et al., *The development of a validated instrument to evaluate bowel function after sphincter-preserving surgery for rectal cancer.* Dis Colon Rectum, 2005. **48**(7): p. 1353-65.
6. Lundby, L., et al., *Long-term anorectal dysfunction after postoperative radiotherapy for rectal cancer.* Dis Colon Rectum, 2005. **48**(7): p. 1343-9; discussion 1349-52; author reply 1352.
7. Emmertsen, K.J. and S. Laurberg, *Bowel dysfunction after treatment for rectal cancer.* Acta Oncol, 2008. **47**(6): p. 994-1003.
8. Lee, W.Y., et al., *Surgical autonomic denervation results in altered colonic motility: an explanation for low anterior resection syndrome?* Surgery, 2008. **143**(6): p. 778-83.
9. Sun, W.M., et al., *Anorectal function after restorative proctocolectomy and low anterior resection with coloanal anastomosis.* Br J Surg, 1994. **81**(2): p. 280-4.
10. Horgan, P.G., et al., *Effect of anterior resection on anal sphincter function.* Br J Surg, 1989. **76**(8): p. 783-6.
11. Lewis, W.G., et al., *Why do some patients experience poor functional results after anterior resection of the rectum for carcinoma?* Dis Colon Rectum, 1995. **38**(3): p. 259-63.
12. Brown, S.R. and F. Seow-Choen, *Preservation of rectal function after low anterior resection with formation of a neorectum.* Semin Surg Oncol, 2000. **19**(4): p. 376-85.
13. Ho, Y.H., F. Seow-Choen, and M. Tan, *Colonic J-pouch function at six months versus straight coloanal anastomosis at two years: randomized controlled trial.* World J Surg, 2001. **25**(7): p. 876-81.
14. Castiglione, F., et al., *Antibiotic treatment of small bowel bacterial overgrowth in patients with Crohn's disease.* Aliment Pharmacol Ther, 2003. **18**(11-12): p. 1107-12.
15. Biancone, L., et al., *Effect of rifaximin on intestinal bacterial overgrowth in Crohn's disease as assessed by the H2-Glucose Breath Test.* Curr Med Res Opin, 2000. **16**(1): p. 14-20.
16. Madden, M.V., A.S. McIntyre, and R.J. Nicholls, *Double-blind crossover trial of metronidazole versus placebo in chronic unremitting pouchitis.* Dig Dis Sci, 1994. **39**(6): p. 1193-6.
17. Rutgeerts, P., et al., *Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection.* Gastroenterology, 1995. **108**(6): p. 1617-21.
18. Gionchetti, P., et al., *Rifaximin in patients with moderate or severe ulcerative colitis refractory to steroid-treatment: a double-blind, placebo-controlled trial.* Dig Dis Sci, 1999. **44**(6): p. 1220-1.
19. Pimentel, M., *Review of rifaximin as treatment for SIBO and IBS.* Expert Opin Investig Drugs, 2009. **18**(3): p. 349-58.
20. Phongsamran, P.V., et al., *Pharmacotherapy for hepatic encephalopathy.* Drugs, 2010. **70**(9): p. 1131-48.

21. Garey, K.W., et al., *Rifamycin antibiotics for treatment of Clostridium difficile-associated diarrhea.* Ann Pharmacother, 2008. **42**(6): p. 827-35.
22. Bures, J., et al., *Small intestinal bacterial overgrowth syndrome.* World J Gastroenterol, 2010. **16**(24): p. 2978-90.
23. Turnbaugh, P.J., et al., *A core gut microbiome in obese and lean twins.* Nature, 2009. **457**(7228): p. 480-4.
24. Ubeda, C., et al., *Intestinal domination by Vancomycin-resistant Enterococcus precedes bloodstream invasion in humans.* Journal of Clinical Investigation. *In press.*
25. http://online.lexi.com/crlsql/servlet/crlonline.

20.0 APPENDICES