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Phase IIa Trial of Encapsulated Rapamycin (eRapa) to Prevent Progression in Familial Adenomatous Polyposis Patients under Active Surveillance

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Principal Investigator: Carol Burke, MD

IND Sponsor: Emtora Biosciences

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Authorized Signatory for the Sponsor

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator (PI) will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All investigators listed on Form FDA 1572 have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Phase IIa Trial of Encapsulated Rapamycin (eRapa) to Prevent Progression in Familial Adenomatous Polyposis Patients under Active Surveillance
Study Description:	Patients with Familial Adenomatous Polyposis (FAP) who are undergoing endoscopic surveillance will be given eRapa at one of three escalating doses/schedules (0.5mg every other day, 0.5mg daily every other week, or 0.5mg daily) for 12 months (360 days) with the aim of reducing polyp burden. Patients will serve as their own controls. Patients will be assessed with surveillance endoscopy at baseline, 6 months (180 days), and 12 months (360 days) for change in polyp burden. Pharmacokinetics (PK)/pharmacodynamics (PD) and adverse events will be compared between the treatment groups. Correlation between immune markers and clinical outcomes will be explored. Once the recommended phase 2 dose (RP2D) is identified, subjects will undergo evaluation under fed and fasted states to determine the food effect on eRapa absorption.
Objectives:	Primary Objectives: 1) To study the frequency and severity of adverse events associated with low dose encapsulated rapamycin in FAP. 2) To determine the recommended phase 2 dose (RP2D) based on assessment of safety, long-term tolerability, optimal blood levels, and clinical benefit. 3) To determine the efficacy of eRapa in delaying polyp progression in patients with FAP compared to historic controls. Secondary Objectives: 1) To further examine the clinical effect of eRapa on other markers of cancer prevention. 2) To determine the effect of high fat, high calorie meals on the pharmacokinetics of eRapa. Tertiary/Exploratory Objectives: To explore correlation between immune markers influenced by mTOR inhibition and clinical outcomes.

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Endpoints:	Primary Endpoints: 1. Safety and tolerability of eRapa as determined by graded toxicity assessed throughout the trial per CTCAE v.5.0. 2. Safety/adverse events as in endpoint 1, dose delays, dose reductions, withdrawal of treatment secondary to low-grade toxicities, and serum pharmacokinetic monitoring. 3. Percentage change from baseline in the colorectal polyp burden as measured by endoscopy at 6 months (180 days). Secondary Endpoints: 1. Percentage change from baseline in the colorectal polyp burden at 12 months (360 days). 2. Change from baseline in International Society for Gastrointestinal Hereditary Tumors (InSiGHT) Stage at 6 months (180 days) and 12 months (360 days). 3. Change from baseline in Spigelman Stage Score at 6 months (180 days) and 12 months (360 days) in patients with polyps at baseline on esophagogastroduodenoscopy (EGD). 4. Percentage change from baseline in the duodenal polyp number and size at 6 months (180 days) and 12 months (360 days) in patients with polyps at baseline on EGD. 5. Pharmacokinetic parameters (AUC[0-INF], Cmax) to assess the effect of food on the bioavailability of the anticipated RP2D Exploratory Endpoints: 1. Immunologic response will be measured and will include an overall assessment of T cell phenotype and function, specifically analyzing T cell naïve/memory/effector phenotype, exhaustion markers (PD-1, LAG3, TIM3), and homing markers (CD62L, CCR7) prior to, during and after treatment with eRapa.
Study Population:	The pilot trial will enroll 30 patients with the genetic or clinical diagnosis of FAP. The clinical diagnosis includes a lifetime history of 100 or more cumulative tubular adenomas throughout the colorectum. Patients must be undergoing surveillance for known FAP and can include those with intact colons as well as those who have undergone surgical therapy. For those patients who have undergone partial or total colectomy, they must have documented residual polyps in their rectum for which they are receiving active surveillance. The food effect study will include no more than 20 patients who have successfully demonstrated tolerance of eRapa or de novo patients if at least 15 patients are not available to participate.
Phase:	IIa
Description of Sites/Facilities Enrolling Participants:	6 to 8 sites will enroll patients (depending on enrollment, additional sites will be added at the discretion of the investigative team). They will be centers with specialty expertise in FAP treatment and surveillance within the United States. Willing sites and participants who have demonstrated tolerance of eRapa will be approached to participate in the food effect portion of the trial. Additionally, de novo patients may be approached at these sites if 15 prior patients are unavailable or unwilling to participate.

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Description of Study Intervention:	Participants will be given oral encapsulated rapamycin (eRapa) at doses of either 0.5mg every other day, 0.5mg daily every other week, or 0.5mg daily. Once the R2PD is identified, participants will be given oral encapsulated rapamycin (eRapa) in both fed and fasted states.
Study Duration:	The trial is anticipated to last approximately 24 months (12 months for enrollment and 12 months for treatment and follow up). The enrollment rate is anticipated at 0.5 pts/site/month x 5 sites, therefore, 2.5 pts/month x 12 months = 30 patients. Primary and secondary endpoints are assessed at 6 months (180 days) and 12 months (360 days) after the date of the initial dose (month 0; day 0). Upon identification of the RP2D, after a 2 week washout (14 days), subjects will be randomized into a fed and fasted (2 weeks – 14 days) two period cross over, with an intervening 2 week washout (14 days) for a total of 2 months (8 weeks).
Participant Duration:	Approximately 12 months (360 days) for treatment and follow-up for the main study and, for those who choose to participate, 2 month (60 days) for the treatment and follow-up for the food effect sub-study.

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1.2 SCHEMA

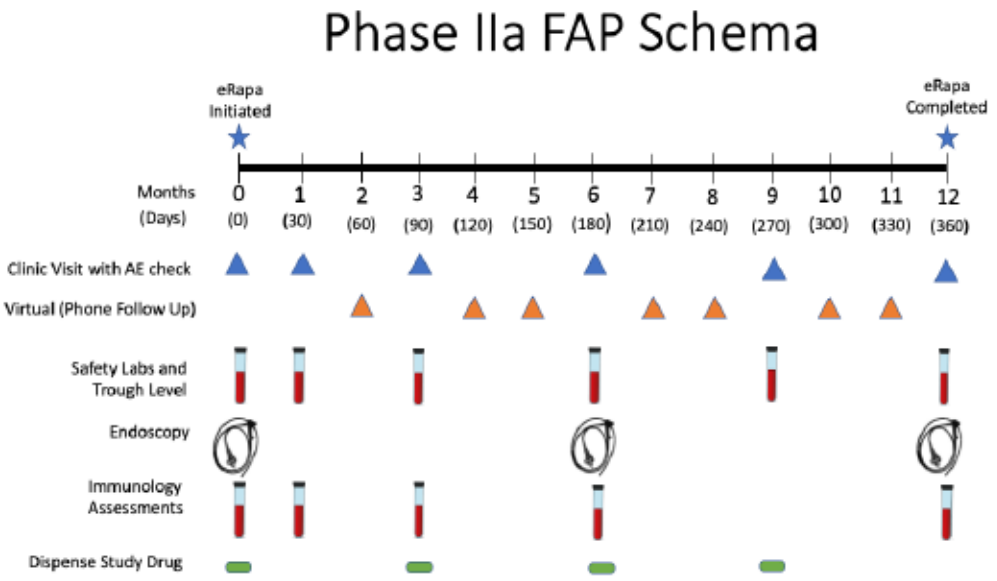
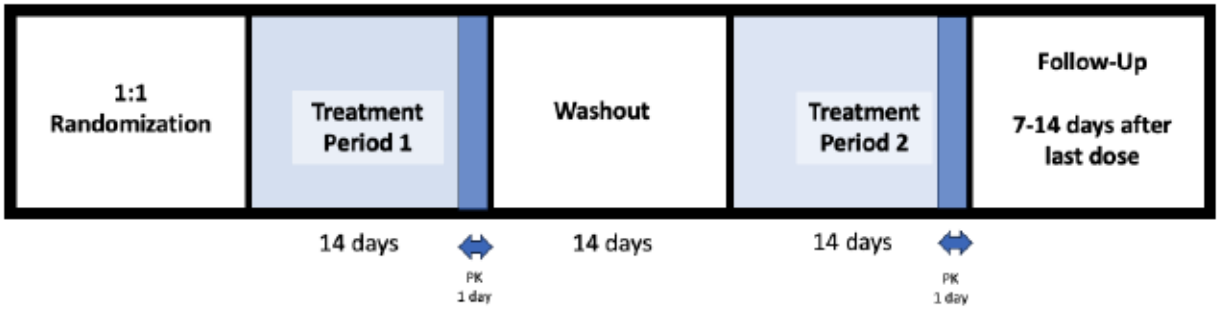


Illustration of Food Effect study design



Sequence	Treatment Period 1	Washout	Treatment Period 2
1	$eRapa_{fasted}$	Washout	$eRapa_{fed}$
2	$eRapa_{fed}$	Washout	$eRapa_{fasted}$

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1.3 SCHEDULE OF EVENTS (SOE)

Main Study SoE

Visit	Screening (In person)	Visit (In person)	Visit (In person)	Visit (Virtual)	Visit (In person)	Visit (In person/Virtual)
Procedures	Screening Month -1 (Day -30 to -1)	Enrollment and Drug Initiation Month 0 (Day 0)	Follow-Up Visits Months 1, 3, 6, 9 (Days 30, 90, 180, 270) +/- 7 days	Follow-Up Visits Months 2, 4, 5, 7, 8, 10, 11 (Days 60, 120, 150, 210, 240, 300, 330) +/- 7 days	End of Study Visit Month 12 (Day 360) +/- 7 days	End of Treatment (EoT) Visit / Off-Treatment Visits[a] (Days 30-330 as needed)
Informed consent	X					
Demographics	X					
Medical and Surgical history	X					
Concomitant medication review	X	X	X	X	X	X
Physical exam	X	X	X		X	X
Vital signs	X	X	X		X	X
Height	X	X	X		X	X
Weight	X	X	X		X	X
Performance status	X	X	X		X	X
Hematology	X	X[f]	X		X	At EoT only
Complete metabolic panel	X	X[f]	X		X	At EoT only
Lipid panel[d]	X	X[f]	X		X	At EoT only
Coagulation panel (PT/INR, PTT)	X	X[f]	X		X	At EoT only
Urinalysis	X	X[f]	X		X	At EoT only
LDH	X	X[f]	X		X	At EoT only
Pregnancy test (female of childbearing potential only)[b]	X	X	X		X	At EoT only
eRapa IP Dispensing		X	At 3, 6, & 9 months only (90, 180, & 270 days)			
PK/PD blood samples drawn at 0, 0.5, 1, 2, 4, 6, and 24 hours.[c, e]		X	At 1 month (day 30) only			
eRapa trough levels[e]			X		X	
Endoscopy w/ or w/o biopsy (+/- 14 days from visit date)	X		At 6 months (180 days)		At 12 months (360 days)	
AE review and evaluation		X	X	X	X	X
Immunology assays[e]		X	At 1, 3, & 6 months only (30, 90, & 180 days)		X	
Complete Case Report Forms (CRFs)	X	X	X	X	X	X

[a] If the patient discontinues study treatment, that visit will become the "End of Treatment" (EoT) Visit. A full history, physical, and safety lab panel will be taken as described above. "Off-Treatment Visits" will occur at the regularly scheduled monthly intervals following the EoT visit until the 12-month study limit is reached. Therefore, all patients will have 12 months of follow up, with monthly visits per the SOE. In Off-Treatment Visits, only noninvasive or safety assessments will be performed. No research blood draws will occur at these visits. If the patient discontinues study treatment prior to 6 months, endoscopy will be performed in the scheduled fashion at 6 and 12 months. If a subject discontinues treatment at a virtual visit, they should return to clinic within 14 days for the required EoT assessments as indicated above. Off-Treatment Visits will follow the same virtual and in-person schedule as noted above.

[b] Pregnancy tests can be performed using urine or serum but must be negative prior to administering study drug. Pregnancy tests are only required for females of childbearing potential.

[c] Variability in timepoints is allowed at +/- 5 minutes for the 0, 0.5, 1, 2, 4, 6 hour timepoints and at +/- 30 minutes for the 24 hours timepoint.

[d] Labs collected from fasting patients.

[e] Research lab samples to be collected on a day when the subject is scheduled to be dosed with eRapa based on the patient's assigned cohort.

[f] Safety labs can be collected on the day of dosing as long as safety lab results are reviewed by the PI and the Medical Monitor prior to the subject being dosed.

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Food Effect Sub-Study SoE

Visit	Screening (In person)	Visit (In person)	Visit (In person)	Visit (Virtual)	Visit (In person)
Procedures	Screening[e] Month -1 (Day -30 to -1)	Enrollment and Drug Initiation Week 0 (Day 0)	Follow-Up Visit Week 2 (Day 14) and Week 6 (Day 42) +/- 2 days	End of Study Visit Week 7-8 (Day 49-56)	Off Treatment/Off-Study Visit[a] (scheduled as needed)
Informed consent	X				
Demographics	X				
Randomization	X				
Medical and Surgical history	X				
Concomitant medication review	X	X	X	X	X
Adverse Event (AE) review			X	X	X
Physical exam	X	X	X		
Vital signs	X	X	X		
Height	X				
Weight	X	X	X		
Performance status	X	X	X	X	X
Hematology	X		X		X
Complete metabolic panel	X		X		X
Lipid panel[b]	X		X		X
Coagulation panel (PT/INR, PTT)	X		X		X
Urinalysis	X		X		X
LDH	X		X		X
Pregnancy test (female of childbearing potential only)[c]	X	X			
eRapa IP Dispensing		X			
Post-dose PK blood samples drawn at 0, 0.5, 1, 2, 4, 6, and 24 hours.[d]			X		
eRapa trough levels		X			
Drug/Food Diary			X		
Complete Case Report Forms (CRFs)	X	X	X	X	X

[a]If the patient discontinues study treatment, there will be an "Off Treatment/Off Study" Visit.
[b]Labs collected from fasting patients.
[c]Pregnancy tests can be performed using urine or serum but must be negative prior to administering study drug. Prenancy tests are only required for females of childbearing potential.
[d]Variability in timepoints is allowed at +/- 5 minutes for the 0, 0.5, 1, 2, 4, 6 hour timepoints and at +/- 30 minutes for the 24 hour timepoint.
[e]Screening and enrollment can occur on the same day as long as safety lab results are reviewed by the Medical Monitor prior to the subject being dosed.

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2 INTRODUCTION

2.1 STUDY RATIONALE

This is a phase IIa trial of encapsulated rapamycin (eRapa) to reduce polyp burden in patients with familial adenomatous polyposis (FAP). This new formulation of encapsulated rapamycin (sirolimus) provides a more predictable bioavailability of this drug. The encapsulated and targeted rapamycin (eRapa) can be delivered at a consistent and lower dosage, not only improving the toxicity profile but also capitalizing on the mechanism of partial and/or intermittent mTOR inhibition, making eRapa a potential immuno-oncology and chemo-preventative agent. Low dose rapamycin has been shown to prevent cancer formation, progression and/or recurrence in the majority of cancer histologies including the most prevalent: lung, breast, prostate, and colon cancers. This pilot trial will be up to a 3-arm, dose-escalation, open label trial of eRapa in FAP patients (n=30) who are under active surveillance. The eRapa treatment will continue for one year. The primary endpoint in percentage change in colorectal polyp burden at 6 months (180 days) and 12 months (360 days) will be assessed through routine endoscopy and measure the change in polyp burden from baseline. The primary endpoint of safety and tolerability will be assessed with monthly clinical exams, labs, and adverse event (AE) monitoring. Secondary endpoints will be assessed at 6 months (180 days) and 12 months (360 days). This pilot trial is patterned after the current trial assessing an anti-IL-23 mAb, guselkumab. Additionally, a subset of participants will be included in a randomized, balanced, single-schedule, two-treatment period (fed versus fasted) crossover study. Participants will receive eRapa under fed or fasted conditions in the first period, and then crossover to the other treatment (fed to fasted and vice versa) following a 2 week washout. Under this design, participants serve as their own control and will allow the assessment of the effect of food on the anticipated R2PD of eRapa

2.2 BACKGROUND

Familial Adenomatous Polyposis and Current Medical Management

FAP is caused by a mutation of the tumor suppressor gene adenomatous polyposis coli (APC) on chromosome 5. The colorectal polyp burden in FAP can range from one hundred to thousands of polyps. FAP is inherited in an autosomal dominant fashion; however, approximately 33% of cases occur as de novo mutations. FAP is distinguished from two other adenomatous polyposis syndromes, attenuated FAP which has less than 100 colonic polyps, and MUTYH-associated polyposis (MAP) which has variable polyp number and is inherited in an autosomal recessive fashion.[1]

FAP is associated with approximately 93% risk of colorectal cancer by age 40 unless colectomy is performed. The second most common cause of death in these patients is duodenal adenocarcinoma developed from polyps in the upper GI tract.[2] Patients with FAP may additionally suffer from extracolonic manifestations such as desmoid tumors, osteomas, odontomas, hepatoblastomas, thyroid tumors, and congenital hypertrophy of the retinal pigmented epithelium.[3]

Relatives of patients known to have FAP and patients clinically suspected to have FAP should undergo genetic testing. Endoscopic screening for FAP typically begins between ages 10-15 and is done annually. If significant symptoms present or endoscopy reveals an interval increase in adenoma number, high-grade dysplasia, adenomas greater than 6 mm, invasive cancer, or the colon cannot be surveyed, the patient will undergo a colonoscopy (if able and if not performed initially) followed by surgery. However, if no abnormalities are seen and the quantity of polyps is not unreasonable, the patient may continue screening and then undergo prophylactic surgical intervention around 18 years of age. Surgical options include total

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proctocolectomy with end ileostomy, total proctocolectomy with ileoanal pouch, or total colectomy with ileorectal anastomosis and continued rectal surveillance.

Although life prolonging, these surgeries are also life altering. Efforts have been made to medically manage polyp burden with limited success. Aspirin failed to exhibit a significant polyp quantity reduction.[4] Sulindac has been shown to decrease colonic polyp density and size. However, following cessation of treatment, polyps recur within 6 months. Patients have also developed colorectal adenocarcinoma while on treatment. Celecoxib was studied as an alternative to decrease gastrointestinal adverse events seen with non-selective COX inhibitors. Like sulindac, celecoxib decreased the number of polyps, but patients were given only 6 months of therapy. Furthermore, the FAP indication was withdrawn for celecoxib in 2012 because the post marketing clinical benefit study was never completed.

The compliance issues and adverse effects seen with long-term treatment of these medications as well as the small magnitude of benefit demonstrates the need for additional, superior therapies. eRapa has been assessed in multiple intestinal neoplasia (MIN) murine models. MIN is due to an APC mutation making it comparable to FAP in humans except the majority of the polyps are located in the small intestine. MIN mice were found to have delayed onset of malignancy and improved survival when fed eRapa. The extent of survival correlated to the dose of eRapa utilized and extended the treated, MIN mice lifespan past the median lifespan of wild type mice. Thus, we propose the use of eRapa as nonsurgical medical adjunct for FAP patients.

Rapamycin's Inhibition of mTOR

Rapamycin exerts its effect by acting on the mammalian target of rapamycin or mechanistic target of rapamycin (mTOR) which is an intracellular kinase that forms one of two different complexes, mTORC1 or mTORC2.[5] Both complexes contain mTOR, DEPTOR, mLST8 and the Ttil-Tel2 complex. mTORC1 exclusively is comprised of Raptor (regulatory-associated protein of mTOR) and PRAS-40. In contrast, mTORC2 is comprised of Rictor (rapamycin-insensitive companion of mTOR), mSin1, and protor.[6]

Given their different subunits, each complex exhibits different roles. mTORC1 plays a role in anabolism, aiding in the creation of nucleotides, proteins, and lipids.[7] It is involved in multiple cell processes including transcription, translation, ribosome biogenesis, regulation of the cell cycle, metabolism, and autophagy. mTORC1 is also responsive to the surrounding environment, particularly to amino acids, oxygen, and glucose.[8] mTOR is inappropriately active in 70% of malignancies and mTORC1 activation is associated with metastasis and resistance to cancer therapies. S6 ribosomal protein, a known target of mTORC1, exhibits abnormally high phosphorylation in 40% of colorectal carcinomas and adenomas.[9] While the role of mTORC2 is not fully understood, it is associated with ion transport, glucose homeostasis, proliferation, apoptosis, and regulation of actin cytoskeletons.[10,11]

mTOR inhibition in Preclinical FAP Models

FAP occurs when APC no longer can act as a tumor suppressor gene and inhibit the Wnt-β-Catenin pathway. When APC is mutated, Wnt is no longer suppressed allowing stabilization of β-Catenin. β-Catenin is then free to activate transcription factors leading to the development of malignancy. [12] APC mutation is also associated with upregulation of the mTOR pathway. Although the relationship between the Wnt and mTOR pathway is not fully elucidated, Fujishita et al. postulate that the Wnt-β-Catenin pathway increases mTOR expression, although not directly. An additional study by Fujishita et al. delved further into mTOR activation, finding c-Jun N-terminal kinase (JNK) to be a direct activator of mTOR. As more is discovered regarding the intricacies of mTOR activation, we can better understand how rapamycin will act to inhibit cancer progression.[13]

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mTOR inhibitors appear to work downstream of the Wnt-β-Catenin pathway as multiple studies have found Wnt target genes are not suppressed when treated with mTOR inhibitors.[14] Rapamycin decreases cellular proliferation but has no effect on apoptosis, indicating that upregulation of mTOR likely leads to increased proliferation causing unregulated cell growth.[15] Activated mTOR causes inhibition of elongation factor 2 kinase (eEF2K), a negative regulator of eEF2. eEF2 is then free to participate in translation elongation. Thus, when APC mutated mice with known adenomas were administered rapamycin to inhibit this pathway, resolution of clinical symptoms occurred, and survival was prolonged compared to controls.[16]

Additional preclinical trials looking at mTOR inhibition in murine models with APC mutation have also found mTOR inhibition beneficial. In a study conducted by Koehl et al., clinical markers were utilized to assess the severity of disease. Typically, with APC mutations, hematocrit and weight decline due to intestinal bleeding and inability to maintain nutritional status, respectively; in addition, splenomegaly develops. Thus, these parameters were selected for evaluation. Rapamycin treated mice had significantly higher hematocrit, weight stabilization, and splenic enlargement to a lesser degree compared to untreated APC mutant mice.[12]

Fujishita et al. determined that 50% of mice receiving high dose mTOR inhibition lived to or past 60 weeks while all placebo-controlled mice died before 30 weeks. The mTOR inhibited mice also had a significantly lower polyp burden and polyps that were present, were smaller in size compared to controls. APC mutant mice treated with mTOR inhibition also did not show a decrease in VEGF expression within polyps when compared to placebo treated controls but did show decreased microvasculature within the polyps treated with mTOR inhibition versus untreated polyps. This analysis is significant because mTOR is also known to promote angiogenesis which can facilitate tumor growth. Thus, mTOR inhibition of angiogenesis is another mechanism by which it can combat malignancy.[17]

Case Report of mTOR Inhibitors Utilized in FAP

A case report details two instances of rapamycin administered prior to surgery in pediatric FAP patients. Rapamycin was dosed at 0.05-0.1 mg/kg and blood levels were monitored with a goal range of 3-6 ng/ml. The patients were surveilled with a colonoscopy and biopsies at 6 months, 12 months, and every year thereafter. The results were promising with reduced severity of polyp dysplasia, polyp size, polyp density, and no adverse events recorded.[18]

Encapsulated Rapamycin (eRapa)

Classical mTOR inhibitors have a narrow therapeutic window and variable absorption and viability between patients, placing patients at risk for toxicities including thrombocytopenia, neutropenia, and hyperlipidemia. To limit the variability of absorption and more tightly control the blood levels of the medication, eRapa was developed. eRapa consists of submicron rapamycin particles in a methacrylic acid copolymer. This composition should prevent degradation of the active drug in the acidic gastric environment, predictably improving bioavailability and allowing for consistent drug levels after dosing. We have conducted a phase I dose escalation study of eRapa in patients with low-grade (Gleason ≤7) prostate cancer involved in an active surveillance program. For a more detailed discussion of this study, please see Section 4.3 on dosing justification.

Current and Proposed Clinical Trials

Despite the strong physiologic rationale for utilizing rapamycin in FAP, to date only one registered clinical trial has tested this medication in FAP patients. A single phase II study enrolled 5 patients following risk-reduction surgery with FAP, treated with the classical formulation of rapamycin, and

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followed these patients with serial endoscopy. While the study is complete, no data are yet available.[19]

The protocol here details our proposed phase IIa clinical trial to confirm the safety of long-term low-dose rapamycin therapy over a prolonged time course (12 months) and determine if any of the dose/schedules studied produce a measurable clinical effect on polyp burden in patients with FAP following risk reduction surgery. Patients will be treated with escalating doses of eRapa, with safety measures performed periodically throughout the study. The primary endpoints of this study will be treatment safety and percentage reduction in colorectal polyp burden as assessed on endoscopy. We hope through this study to identify a dose/schedule of rapamycin that reduces polyp burden with minimal associated toxicity.

Conclusion

FAP is a devastating disease, placing patients at risk for a variety of malignancies, but guaranteeing colorectal cancer if not intervened upon surgically. Although novel agents have been offered for medical management of the disease, none are known to be obviate or significantly delay the need for surgery. Thus, research must continue to establish an agent to better manage FAP. If eRapa is well tolerated and results in a clinical benefit in FAP patients, then patients without FAP, but at high risk for colon cancer may also gain a therapeutic benefit. This would prompt an additional clinical trial, and potentially a broadening of eRapa's indications.

2.2.1 KNOWN POTENTIAL RISKS

The potential adverse events can be drawn from other variations of rapamycin. The oncologic indications of the drug require high doses (>10 mg daily) for efficacy, resulting in significant toxicity to include serious and life-threatening events.[20] The approved dose for transplant rejection prevention is 2-5 mg daily, which is believed to be immunomodulating (not necessarily immunosuppressive)[5,21,22], and is commonly used in combination with other immunosuppressive drugs.[23-26] This dose appears to be well tolerated over extended time periods; however, blood concentration levels can vary widely (as much as 8-fold) between similarly dosed patients with sirolimus. This variability is felt to be related to the formulation and differential absorption largely related to drug degradation in the acidic conditions of the stomach. Toxicity is directly correlated to higher trough levels between doses; therefore, transplant patients routinely undergo therapeutic drug monitoring with trough level determination in order to properly dose the drug.

Rapamycin, as well as other rapalogues, exert their effect by inhibiting the mTOR pathway. This pathway is a central and critically important regulator of cellular growth and metabolism responding to a wide array of stimuli to include growth factor, hormones, nutrients, and environmental conditions. mTOR mediates profound differentiation and functional effects on major immune cell populations, prompting the use of mTOR inhibitors in anti-rejection drug regimens. Dysregulation of the mTOR pathway is closely associated with cancers and other age-related diseases, forming the basis of mTOR inhibitors as therapeutic cancer agents. These diverse uses underscore the inherent complexity in the regulation and function of the ubiquitous mTOR pathway. However, it is now understood that many of the effects and toxicities associated with mTOR inhibition are directly related to the degree of mTOR blockade. High levels of mTOR inhibition are required for a direct therapeutic impact on an established cancer while lower levels are required for immune modulation, hence, the differential dosing and toxicity as described above. Most importantly, partial and/or intermittent mTOR blockade, with minimal toxicity, has been shown to have profound beneficial effects that include prolonged lifespan and health span in multiple animal models. Two key outcomes of partial and/or intermittent mTOR inhibition are the rejuvenation of the immune system and cancer prevention.

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Below are descriptions of the risks and discomforts that patients may experience taking rapamycin. These side effects were gathered from patients taking a similar formulation of rapamycin at doses ranging from 0.5 mg daily, 5 mg weekly and 20 mg weekly. In general, it was relatively well tolerated in the lower dosed groups. There were no deaths related to this medication; there was one serious adverse event of mouth ulcers related to the medication in a patient receiving 20 mg weekly. The most common were mild mouth sores. However, the incidence of many adverse events is dose related.

Likely (>10%):
Stomatitis
Diarrhea
Abdominal pain
Nausea
Nasopharyngitis
Acne
Chest pain
Peripheral edema
Upper respiratory tract infection
Headache
Dizziness
Myalgia
Hypercholesterolemia
Hypertriglyceridemia
Hyperlipidemia
Impaired wound healing

Less Likely (1 - 10%):
Hypersensitivity (5.4%)
Indigestion (2.8%)
Fatigue (2.8%)
Blood cholesterol (low-density lipoprotein – LDL) increased (2.3%)
Tongue sores (2.3%)
Sleeplessness (1.8%)
Dry mouth (1.4%)
Abnormally low level of white blood cells (Neutropenia) (1.4%)
Mouth, gum or tooth (Oral) pain (1.4%)
Itching (1.4%)

Rare but Serious (<1%):
Rash
Laryngeal Inflammation (Sore Throat)

2.2.2 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Patients with FAP are at a high risk of developing colorectal adenocarcinoma due to a somatic APC mutation. The polyp burden is known to correlate with cancer risk. The potential benefit of eRapa in FAP patients would be reducing cancer risk as measured by reducing polyp burden. The risk of low dose eRapa would be low, as toxicity is directly correlated with high trough levels such as those historically achieved in transplant patients. eRapa is expected to be a very tolerable treatment long-term that could provide substantial benefit.

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3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
1. To study the frequency and severity of adverse events associated with low dose encapsulated rapamycin in FAP 2. To determine the RP2D based on assessment of safety, long-term tolerability, optimal blood levels, and clinical benefit. 3. To determine the efficacy of eRapa in delaying polyp progression in patients with FAP compared to historic controls.	1. Safety and tolerability of eRapa as determined by graded toxicity assessed throughout the trial per CTCAE v.5.0. 2. Safety/adverse events as in endpoint 1, dose delays, dose reductions, withdrawal of treatment secondary to low-grade toxicities, and serum pharmacokinetic monitoring 3. Percentage change from baseline in colorectal polyp burden as measured by endoscopy at 6 months (180 days)	1. As a phase IIa study, ongoing surveillance of adverse events associated with eRapa will be important, as eRapa has never been studied specifically in this population. 2. As eRapa will be given over prolonged time periods, evaluating for chronic toxicities and prolonged tolerability will be important and may impact future dosing or usage considerations. 3. Colorectal polyp burden represents a reproducible endoscopic measurement that reflects both number and size of polyp burden, which provides a more accurate assessment of any changes that might occur as a result of treatment.

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OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary		
1. To further examine the clinical effect of eRapa on other markers of cancer prevention. 2. To determine the effect of a high fat, high calorie meal on the pharmacokinetics of eRapa when administered at the anticipated RP2D.	1. Percentage change from baseline in the colorectal polyp burden at 12 months (360 days). 2. Change from baseline in International Society for Gastrointestinal Hereditary Tumors (InSiGHT) Stage at 6 months (180 days) and 12 months (360 days). 3. Change from baseline in Spigelman Stage Score at 6 months (180 days) and 12 months (360 days) in patients with polyps at baseline on EGD. 4. Percentage change from baseline in the duodenal polyp number and size at 6 months (180 days) and 12 months (360 days) in patients with polyps at baseline on EGD. 5. Observation of the exposure-response relationship under fed and fasted conditions will provide insight on the effect of food on eRapa absorption. This insight can then be used to propose dosing schedules (ie, within a certain window relative to food intake) to ensure adequate eRapa exposure in patients.	1. Following patients out to 12 months (360 days) may demonstrate durability of effect over time. 2. The InSiGHT stage is a scoring system for assessing polyp burden in patients with FAP. (Appendix A)[27] A significant reduction in polyp burden would be expected to translate to a long-term reduction in colorectal cancer risk. 3. The Spigelman score is a scoring system for assessing duodenal polyp burden in FAP, which is a clinically important component of the disease that is not affected by prophylactic colectomy. (Appendix A)[28] 4. eRapa may be able to reduce duodenal adenocarcinoma risk in patients with FAP if polyp burden is reduced. 5. AUC and C_{max} are exposure measurements used to compare the effect of food on bioavailability.
Tertiary/Exploratory		
To explore correlation between immune markers influenced by mTOR inhibition and clinical outcomes.	Immunologic response will be measured and will include an overall assessment of T cell phenotype and function, specifically analyzing T cell naïve/memory/effector phenotype, exhaustion markers (PD-1, LAG3, TIM3), and homing markers (CD62L, CCR7) prior to, during and after treatment with eRapa.	Exploring the correlation between mTOR-related immune markers and clinical outcomes may help elucidate a mechanism by which mTOR inhibition may reduce cancer risk in patients with FAP.

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4 STUDY DESIGN

4.1 OVERALL DESIGN

The primary objective of the trial is to determine the safety and efficacy of low dose eRapa in patients with FAP. This is a phase IIa trial with a primary outcome of reducing polyp burden with monitoring of adverse events. The dosing schedule is informed by preliminary data from a phase I dose escalation trial of eRapa in low-risk prostate cancer patients. Patients in this study will receive eRapa for one year while undergoing endoscopic surveillance at 6 months (180 days) and 12 months (360 days). Patients will serve as their own controls with a baseline pre-intervention endoscopy. Polyp burden will be compared via endoscopy at 6 months (180 days) and 12 months (360 days) with the baseline pre-intervention endoscopy. Multiple sites with investigators familiar with FAP will enroll patients.

After identification of the RP2D, subjects will be enrolled in a food effects two-treatment period crossover sub-study. Participants enrolled in the food effects cross-over sub-study will be randomized in a 1:1 fashion to receive eRapa under fed or fasting conditions. eRapa will be administered at the predicted RP2D, over 2 weeks, during Period 1. After Period 1, patients will undergo a 2-week washout and then crossover to Period 2, resuming administration of eRapa at the predicted RP2D, for 2 weeks. At the conclusion of the two week treatment periods one and two, particpants will undergo lab draws for PK analysis. Up to twenty patients will be enrolled in the food effects cross over study. This sample size was selected due to feasibility and given the cross-over design (ie, participants will act as their own controls).

Food Effect Schema:

Sequence	Treatment Period 1 (2 weeks)	Washout Period (2 weeks)	Treatment Period 2 (2 weeks)
1	$eRapa_{fasted}$	Washout	$eRapa_{fed}$
2	$eRapa_{fed}$	Washout	$eRapa_{fasted}$

- $eRapa_{fasted}$: RP2D of eRapa administered in the fasted state
- $eRapa_{fed}$: RP2D of eRapa administered after a high-fat, high calorie meal

Safety:
Labs (serum, urine) will be drawn pre-intervention and then at 1, 3, 6, 9, and 12 months. A formal safety review will occur after the first 3 patients enrolled in the first cohort have been on treatment for 1 month (30 days). This review will encompass all labs, exams, and interviews performed during that period. Until this safety review occurs, no additional patients in the cohort will be enrolled. A dose-limiting toxicity (DLT) is defined as any ≥Grade 3 event occurring during the DLT evaluation period that is at least possibly related to eRapa administration. To be included in this review, patients must have received > 75% of the eRapa dose, unless treatment was discontinued secondary to a DLT or a lower-grade toxicity. If there are no DLTs in the first 3 patients of the cohort, the remainder of the cohort will be enrolled (n=10 total). If there is a single DLT in the first 3 patients, then an additional 3 patients will be enrolled, and a formal safety review will be repeated at that time. If an additional DLT has occurred (i.e., 2 DLTs in the first 6 patients), then escalation will cease, and the dose immediately prior to the dose of that cohort will be determined to be the maximum tolerated dose (MTD), and all future patients as well as those currently receiving the DLT dose will be transitioned to the MTD.

If there are 2 DLTs in the first 3 patients, then escalation will cease and the dose immediately prior to the dose of that cohort will be determined to be the MTD, and all future patients as well as those currently receiving the DLT dose will be transitioned to the MTD.

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Once an entire cohort is enrolled, a second safety review will occur. Cohort enrollment is defined as time from first patient dosing through the completion of the 10th patient's Month 1 (Day 30) visit. If there are ≤3 DLTs in the entire cohort of 10 patients, then the next cohort will be initiated. If there are >3 DLTs in the cohort, then escalation will cease, and the dose immediately prior to the dose of that cohort will be determined to be the MTD, and all future patients as well as those currently receiving the DLT dose will be transitioned to the MTD.
Once safety has been shown at two assessments in the first cohort (i.e., after 3rd and 10th patient have been dosed in the cohort), the formal safety assessment after the 3rd patient in cohorts 2 and 3 will be discontinued. The safety assessment initiated after the 10th patient is dosed in each cohort will be reviewed and the DLT guidelines established will continue to be applied to this review.

In summary, the "stopping rules" for dose escalation in this study are:
- 2 DLTs in the first 3 patients of a cohort
- 1 DLT in the first 3 patients and a second DLT in the next 3 patients enrolled
- >3 DLTs in the entire cohort of 10 patients
- Significant reaction or toxicity, defined as: death, life-threatening adverse drug experience (i.e., severe anaphylactic reaction immediately after vaccine administration), inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect, or other important medical events that jeopardize the patient or require immediate medical or surgical intervention to prevent an outcome listed prior.

In the event of a fatal adverse event related to eRapa, the study will be suspended (no further enrollment at any site, cessation of therapy at all sites) until further review by the sponsor, FDA, and the IRB.

Safety will be compared to evaluate the number, frequency, duration, and relation of toxicity events to eRapa, as defined by the Common Terminology Criteria for Adverse Events (CTCAE) v 5.0; analyzed overall and compared between dosing cohorts. The IND sponsor and the overall study Principal Investigator have the right to suspend or terminate this study entirely or at a specific site at any time if the incidence or severity of AEs in this or other studies indicates a potential health hazard to patients.

Long-Term Tolerability:
This objective will assess the long-term tolerability of the medication. AEs that do not meet the criteria for a DLT (i.e., lower grade), chronic AEs, changes in drug scheduling and dosing (dose delays, dose reductions, or medication withdrawal), and the correlation of these changes or chronic events with serum drug levels (PK data) will be assessed. PIs will be instructed to track any dose delays/reductions or medication withdrawals, and these will be analyzed in the context of concurrent low-grade toxicities and PK levels from that dosing cohort at those time points.

Polyp Burden:
Colorectal polyp burden will be assessed at baseline and again at 6 months (180 days) and 12 months (360 days).

InSiGHT Stage:
InSiGHT stage will be assessed at baseline and again at 6 months (180 days) and 12 months (360 days) with endoscopy. Patients will serve as their own controls. Endoscopy findings will be scored according to the InSiGHT criteria (Appendix A).

Pharmacokinetic (PK)/Pharmacodynamic (PD) analysis:
All patients enrolled in each cohort will be asked to provide blood samples for PK/PD analysis. Single dose exposure curves will be generated by assessing the rapamycin levels in whole blood drawn at 0, 0.5,

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1, 2, 4, 6, and 24 hours after the first dose at Day 0. Additionally, multi-dose (steady-state) exposure curves will be assessed at the same time points at the end of the week after a minimum of 1 month (30 days) of dosing. In addition to dose exposure curves, the following PK parameters will be calculated: C_{max}, T_{max} C_{last}, T_{last}, K_e, half-life, and AUC. For a more detailed description of each parameter, please see Section 8.4.2. Corresponding mTOR inhibition in PBMC by assessment of phosphorylation of S6 will also be assessed at these time points. For all cohorts, drug trough levels will be drawn post-dosing at 1 month (30 days), 3 months (60 days), 6 months (180 days), 9 months (270 days), and 12 months (360 days) [all timepoints have a +/- 7 day window] with their safety labs.

For the Food Effect Sub-study, single dose exposure curves will be generated by assessing the rapamycin levels in whole blood drawn at 0, 0.5, 1, 2, 4, 6, and 24 hours after both treatment periods, at day 14 and 42. Drug trough levels will be drawn at Day 0.

Immunologic Assays:
To perform the immune assays, blood (approximately 50 mL) will be collected pre-treatment and pre-dosing at 1 month (30 days), 3 months (90 days), 6 months (180 days), and 12 months (360 days) [all timepoints have a +/- 7 day window] after initiating treatment. Immune monitoring will include an overall assessment of T cell phenotype and function, specifically analyzing T cell naïve/memory/effector phenotype, exhaustion markers (PD-1, LAG3, TIM3), and homing markers (CD62L, CCR7) prior to, during and after treatment with eRapa. Finally, as an exploratory analysis, these immunologic results will be correlated with disease progression.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

In summation from the introduction, rapamycin was initially discovered in 1972, originally as an antifungal. It was approved as an immunosuppressive in 1999 to prevent transplant rejection. Rapalogues have been developed and approved in the cancer for their ability of mTOR inhibition in breast cancer, renal cell carcinoma, and pancreatic neuroendocrine tumors. The oncologic treatment requires high doses. The immunomodulating, transplant rejection dosing is less, but usually used in combination with immunosuppressive medications. The current available formulations have variable blood concentrations requiring stringent monitoring.

FAP occurs when APC no longer can act as a tumor suppressor gene and inhibit the Wnt-β-Catenin pathway. When APC is mutated, Wnt is no longer suppressed allowing stabilization of β-Catenin. β-Catenin is then free to activate transcription factors leading to the development of malignancy.
APC mutation is also associated with upregulation of the mTOR pathway. Although the relationship between the Wnt and mTOR pathway is not fully elucidated, Fujishita et al. postulate that the Wnt-β-Catenin pathway increases mTOR expression, although not directly.[13] An additional study by Fujishita et al. delved further into mTOR activation, finding c-Jun N-terminal kinase (JNK) to be a direct activator of mTOR. As more is discovered regarding the intricacies of mTOR activation, we can better understand how rapamycin will act to inhibit cancer progression.[17]

The evaluation of food-drug interactions is required by the FDA to identify the impact on the safety and efficacy of orally administered drug products. In order to best identify how, when, and why drugs should or should not be administered with food, the food effect study will be conducted with the anticipated RP2D.

4.3 JUSTIFICATION FOR DOSE

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The goal of this trial is to provide rapamycin at a dose that simultaneously prevents disease progression and minimizes side effects. The doses chosen for dose escalation in this study are informed by a phase I dose-escalation study of eRapa patients with low-risk prostate cancer. In that study, patients were given 0.5mg weekly, 1.0mg weekly, and 0.5mg daily in a 3+3 dose escalation design. The frequency of adverse events and the PK data from that study were used to construct our current dose escalation plan and are described below.

Adverse events from this study are listed in Appendix F. There were 4 AEs in Group 1 (0.5mg weekly), 1 AE in Group 2 (1.0mg weekly), and 44 AEs in Group 3 (0.5mg daily). Of the 44 events in Group 3, 1 was a Grade 3 dose-limiting toxicity (DLT; neutropenia). Additionally, two other patients withdrew from this group following AEs that were not dose-limiting (papulopustular rash, grade 1; dizziness, grade 2). No further DLTs occurred in Group 3. From this analysis, we conclude that 0.5mg daily is tolerable but does have more toxicity than the weekly dosing cycles. Additionally, a dose of 2mg daily is used to initiate therapy with sirolimus for treatment of lymphangioleiomyomatosis for 10-20 days, with dose adjustments after that to produce serum levels of 5-15ng/mL. Daily dosing at higher doses than the highest dose rate we propose here has thus already been done and shown to have a reasonable safety profile. The weekly doses, which produce a much lower serum values (see below), were both well tolerated.

PK data from this study are shown in Appendix G. The first graph shows the PK data following a single dose of either 0.5mg or 1.0mg at the beginning of the study. Maximum blood levels were shown at 2.67h in the 1.0mg dose (C_{max}= 7.09ng/mL) and 3.33h in the 0.5mg dose (C_{max} = 1.24 ng/mL). The target range used in the case reports of FAP patients treated with rapamycin was between 3-6ng/mL, so the 1.0mg dose is slightly above this range while the 0.5mg dose is slightly below it. The second graph shows the PK data following a dose of 0.5mg in patients who had been on 0.5mg daily therapy for 3 months (Group 3). Maximum blood level in this group occurred at 1.5h and was 6 ng/mL. The trough level (time = 0h) is approximately 2 ng/mL and this is reached 2-3 days after starting the dosing regimen (graph 3). Thus, 0.5mg daily dosing is capable of producing a serum level within the 3-6 ng/mL range reported in the literature, and a sustained and constant trough level of 2-3 ng/mL. We expect this dose to be sufficient to produce a relevant clinical response. Mannick et al. evaluated patient immune response following co-administration of influenza vaccine and rapamycin at doses of 0.5mg daily, 5mg weekly, and 20mg weekly to influenza vaccine alone. Dosing with 0.5mg daily produced similar increases in antibody titer against vaccine strains as the 5mg weekly and 20mg weekly doses, and all of these were significantly elevated relative to placebo. A trend towards a positive immune impact was also seen in the eRapa prostate cancer study (higher prevalence of central memory CD8 T cells and CD3+CD56+ NK cells in the cohort that received the 0.5mg daily dose), though further immunologic study is necessary at these lower doses.

Based on the data from the eRapa in prostate cancer study as well as the data from Mannick et al., 0.5mg daily is the highest dose that we plan to test as this was reasonably well tolerated in ours and Mannick's study and produced target levels consistent with benefit seen in the FAP case reports in the literature. However, the side effect profile associated with daily dosing is not insignificant. In an attempt to limit this toxicity as much as possible while still producing a prophylactic dose, the other two groups in this study will be exposed to half this dose by altering the schedules. The first schedule is 0.5mg every other day which should provide target concentrations every other day, but limit trough levels and therefore enhance tolerability (see Appendix G; single dose curves which show drug levels go to zero at 24 hours.). The second schedule is an intermittent dosing model. The concept of intermittent dosing of rapamycin is supported in the literature. Laberge et al. demonstrated in cell culture that a single exposure of HCA2 cells to rapamycin for a period of 24h (which mirrors a single dose model as shown in the PK analysis) suppressed IL-6 secretion from the HCA2 cells by up to 80% for the 7 days immediately after treatment, which the authors noted was nearly identical to the suppression seen following continuous treatment for 7

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days. The group also noted that this was not likely to be due to intracellular retention of rapamycin, as cell-associated rapamycin had declined to 20% of the given dose by 48h and <10% of the given dose by 6 days. Therefore, the effects of a single dose appear to produce a sustained response for up to 7 days. Apelo et al. found in a mouse model that daily rapamycin treatment produced significant insulin resistance measured by a glucose tolerance test, while weekly rapamycin treatment did not produce any measurable glucose intolerance. On PK analysis, the group found that rapamycin was still detectable 3 days after a single dose and that weekly dosing still produced a significant and desired effect on immune function (greater than control, but not as profound as with daily dosing). Anisomov et al. describe results of a mouse study in which mice received either a control vehicle or rapamycin, with rapamycin doses given every other day for two weeks followed by a treatment holiday of two weeks. The group noted that the intermittent rapamycin dosing prolonged median lifespan by 10.1% and maximum lifespan by 9.3% and also decreased carcinogenesis by approximately 40%.

Cumulatively, these articles suggest that intermittent dosing can be used to produce a physiologic effect lasting up to a week while mitigating toxicity and also suggest possible dosing strategies. The intermittent dosing group in this study will be dosed at 0.5mg daily given every other week (i.e., 1 week on therapy, 1 week off therapy). Based on our previous PK data, we believe that a week of daily dosing will result in the target drug levels and then the week off will allow a reduction in any toxicities. The overall concept of intermittent dosing is supported and espoused throughout the rapamycin literature especially in the age-related studies.

In summary, the initial dose will be 0.5mg eRapa every other day, and this will be escalated to 0.5mg daily given every other week and then 0.5mg daily. Escalation will be halted if >3 DLTs occur in a single dosing cohort. If >3 DLTs occur in a single cohort, the dose below that of the >3 DLT cohort will be determined as the MTD, and all subsequent patients will receive that dose. Because dose-escalation and dose-finding is a component of this study, patients will be carefully monitored for adverse events and treatment safety will be a primary endpoint. Patients will serve as their own controls; no placebo will be given.

Long-term dosing of eRapa has not been studied, as patients in the prostate cancer trial were only treated with eRapa for 3 months and then observed for an additional 3 months after halting treatment. However, patients are maintained on much higher doses of rapamycin for years to renal transplant patients (2-5mg daily). Even accounting for differences in bioavailability, these doses are 4-10 fold higher than the highest dose we propose in our study. We therefore do not anticipate the development of any new toxicities related to prolonged dosing that have not already been identified in the eRapa study or in studies of sirolimus. However, we plan to actively monitor for dose delays/reductions, medication withdrawal, and the presence of persistent or intermittent low-grade toxicities, and analyze the long-term tolerability of each dosing regimen in the context of overall safety, appropriate serum drug levels, and overall clinical benefit.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Events (SoE*)*, Section 1.3.

The end of the study is defined as completion of the last visit or procedure shown in the main study portion of the SoE.

In the food effect portion, the end of study is defined as completion of the last procedure shown in the Food Effect SoE.

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5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form.
2. Stated willingness to comply with all study procedures and availability for the duration of the study.
3. Male or female, age at least 18 years at the time of consent.
4. Phenotypic familial adenomatous polyposis (FAP) with disease involvement of the colorectum by either genetic or clinical diagnosis: Adenomatous polyposis coli (APC) germline mutation with or without family history, or with greater than a cumulative lifetime history of (>)100 adenomas in large intestine and a family history of FAP, or FAP phenotype post colectomy for polyposis with a family history of FAP. Minimum number of polyps required for enrollment is 10.
5. Ability to safely undergo endoscopy.
6. Ability to take oral medication and be willing to adhere to the eRapa regimen.
7. For females of reproductive potential: use of highly effective contraception for at least 1 month prior to screening and agreement to use such a method during study participation and for an additional 12 weeks after the end of eRapa administration.
8. A woman must agree not to breast feed or donate eggs (ova, oocytes) during the study and for a period of 12 weeks after the last administration of study drug.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Risk-reduction surgery (colectomy or partial colectomy) within the 12 months prior to screening.
2. Use of non-steroidal anti-inflammatory drugs other than aspirin during the study. The use of 81 milligrams (mg) of aspirin a day or 650 mg of aspirin per week is allowed.
3. Treatment with other FAP-directed drug therapy (including NSAID [Nonsteroidal anti-inflammatory drug] drugs), unless completes a 4-week washout period prior to enrollment.
4. Duodenum or colon/rectum with high grade dysplasia or cancer on biopsy at screening
5. Duodenal or colorectal polyp >1 centimeters (cm) not excised at the screening evaluation
6. Pregnancy or breast-feeding.
7. Unable to provide consent or anticipated inability to attend appropriate follow-up visits.
8. Serum creatinine or measured/calculated creatinine clearance (or glomerular filtration rate [GFR]) >1.5 x ULN OR < 30 mL/min for participants with creatine levels >1.5 x institutional ULN. Bilirubin ≥ 1.5 x ULN unless conjugated bilirubin ≤ ULN; alkaline phosphatase >5x ULN; ALT/AST > 2x ULN.
9. INR or PT or aPTT > 1.5 x institutional ULN unless the patient is receiving anticoagulant therapy as long as the PT or aPTT is within therapeutic range of intended use of anticoagulants.
10. Proteinuria >1+ on urinalysis or >1g/24h on 24h urine.
11. History of interstitial lung disease or non-infectious pneumonitis.
12. Immunosuppressed state (e.g., HIV, use of chronic steroids), active, uncontrolled infection.
13. On agents known to alter rapamycin metabolism significantly. (Appendix B)
14. Concurrent involvement in other clinical trials specifically evaluating chemoprevention in FAP.
15. Patients with a colonic polyp burden too numerous to count.

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5.3 LIFESTYLE CONSIDERATIONS

Not applicable.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of polyp burden may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

The pilot trial will enroll patients with phenotypic familial adenomatous polyposis (FAP) with disease involvement of the colon and rectum by either genetic or clinical diagnosis. For patients who have undergone colectomy, they must be at least 12 months from their surgery and have documented residual polyps in their rectum for which they are receiving active surveillance. The minimum required number of polyps for all patients is 10 polyps, regardless of size.

For the sub-study, patients who have previously participated in the main study will be approached to participate in the Food Effect aspect of the trial. The patients will be dosed based on the anticipated or determined RP2D.

5.6 STUDY DISCONTINUATION

Those patients who display significant reactions (i.e., anaphylactic reaction immediately after medication administration) or serious toxicities (see next paragraph for definition of this term) will be discontinued from the study as determined by the PI and/or Sponsor. They will be followed by the study investigator until resolution of the AE.

Medications will be immediately halted if any serious adverse reactions occur to include: death, life-threatening adverse drug experience (i.e., severe anaphylactic reaction immediately after vaccine administration), inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect, or other important medical events that may not result in death, be life-threatening, or require hospitalization but which, when based upon appropriate judgment of the PI, be determined to jeopardize the patient or require medical or surgical intervention to prevent an outcome listed above. Any grade 4 adverse drug experience found to be possibly related to the experimental study treatment will result in suspension of patient enrollment to the study until additional review of the safety data by the sponsor and a determination if any changes are indicated to the protocol. Any fatal adverse event, at least possibly related to the experimental study treatment, will terminate patient accrual until additional review and approval by the IRB and FDA of any necessary changes to the protocol that may be indicated.

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Patients may withdraw from the study at any time and for any reason. A patient may be asked to withdraw from the study by the PI if they are not compliant with the timing of the medication series, observation period, or return visits to monitor for study-associated toxicities.

Additionally, if the PI determines that it is no longer safe for a patient to continue in the trial for any reason, they may be withdrawn.

If a patient is discontinued from the study for an AE, they will continue to be followed for resolution of the AE and clinical recurrences unless the patient withdraws consent for further study evaluation.

A patient may continue in the study following a Grade 1-3 AE. No dose modification is required for Grade 1 toxicities. For Grade 2-3 toxicities, PIs will be advised to hold treatment until toxicity returns to ≤ Grade 1 level. At that time, PIs will resume patients at their prior dose. If repeated AEs occur in the same patient, as above, they may be withdrawn from the study by the PI/Sponsor. These patients will continue to be followed after discontinuation of study drug to ensure resolution of all AEs. If a patient's medication is held to allow for AE resolution and the patient spends >4 weeks (28 days) off therapy, then treatment for this patient will be permanently suspended.

The reason for any premature discontinuation of a patient from the study will be recorded on the appropriate Case Report Form (CRF).

The IND sponsor and Medical Monitor have the right to suspend or terminate this study entirely or at a specific site at any time. Reasons for suspending or terminating the study may include the following:

- ☐ The incidence or severity of AEs in this or other studies indicates a potential health hazard to patients
- ☐ Patient enrollment is unsatisfactory
- ☐ Data recording are inaccurate or incomplete
- ☐ Study protocol not followed

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The eRapa (encapsulated rapamycin) drug product consists of sub-micron rapamycin particles incorporated into poly (methyl methacrylate) polymer (Eudragit® S 100). This improved formulation results in higher bioavailability and enables eRapa to consistently provide approximately 30% more drug than sirolimus (unpublished data). Improved and predictable delivery allows for consistent and sustained lower dosing, which will result in an improved toxicity profile since the latter is proven to be related to blood concentration levels.

Nomenclature
INN: Sirolimus
Chemical name (IUPAC):
(3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-
9,10,12,13,14,21,22,23,24,25,26,27,32,33,34a-hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(1S,3R,4R)-4-

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hydroxy-3-methylcyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclohentriacontine-1,15,11,28,29(4H,6H,31H)-pentone
Other nonproprietary names: sirolimus; RAPA
Manufacturer's company code: 30123
CAS registry number: 53123-88-9

6.1.2 DOSING AND ADMINISTRATION

The eRapa drug product consists of sub-micron rapamycin particles incorporated into poly (methyl methacrylate) polymer (Eudragit® L 100 / S 100). It is taken in a pill form. The dosing and bioavailability data on eRapa are all from studies in which eRapa was administered under fasted conditions. As such, patients in this study will be instructed to take eRapa 1 hour before or 2 hours after food and to avoid grapefruit juice for the duration of treatment. Patients will be instructed to take the medication at the same time of day on treatment days. Patients will be counseled to avoid fatty foods during eRapa treatment, but no specific dietary requirements are part of the study design.

Patients will receive one of three doses in a dose-escalating fashion. Cohort 1 will receive 0.5mg every other day, Cohort 2 will receive 0.5mg daily with 7 days on therapy followed by 7 days off therapy, and Cohort 3 will receive 0.5mg daily. This dosing regimen is informed by the literature and a prior phase I dose-escalation study of eRapa in prostate cancer patients, where no MTD was reached and the highest dose tested was 0.5mg daily which was reasonably well tolerated. Patients will serve as their own controls; no placebo will be given.

Patients who complete the main study will be consented to participate in the Food Effect sub-study. Participants enrolled in the food effects cross-over sub-study will be randomized in a 1:1 fashion to receive eRapa under fed or fasting conditions. Regardless of the arm, all patients will start with an overnight fast of at least 10 hours. In the fasted condition, eRapa will be consumed with no further food consumption for at least 4 hours after drug consumption. eRapa will be consumed with 240 mL (8 fluid ounces) or water. Additional water is permitted ad lib, except for the period 1 hour before and until 1 hour after administration of the eRapa. In the fed state, after an overnight fast of at least 10 hours, a high fat meal will be consumed 30 minutes (or less) prior to eRapa consumption (Appendix H). This meal should be consumed within 30 minutes. The drug should be consumed with 240 mL (8 fluid ounces) of water and additional water is allowed ad lib, except for the period 1 hour before until 1 hour after drug administration. eRapa will be administered at the predicted RP2D, over 2 weeks, during Period 1. After Period 1, patients will undergo a 2-week washout and then crossover to Period 2, resuming administration of eRapa at the predicted RP2D for 2 weeks.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The eRapa drug product is manufactured under Good Manufacturing Practice (GMP) by Southwest Research Institute (SwRI), San Antonio, TX. A complete description of the manufacturing process, including flow charts, is included in the IND. The eRapa capsules for the clinical drug supply were prepared under GMP, also by SwRI. Capsules were filled manually after blending eRapa with two excipients (talc and lactose monohydrate) needed to facilitate the filling process.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The eRapa (encapsulated rapamycin) drug product consists of sub-micron rapamycin particles

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incorporated into poly (methyl methacrylate) polymer (Eudragit® S 100). This improved formulation results in higher bioavailability and enables eRapa to consistently provide approximately 30% more drug than sirolimus (unpublished data). Improved and predictable delivery allows for consistent and sustained lower dosing, which will result in an improved toxicity profile since the latter is proven to be related to blood concentration levels.

The eRapa capsules are packaged in 31-count round HDPE bottles with a polypropylene child-resistant screw cap and foil induction seal. The drug product container also contains a ½ gram silica gel desiccant pack to provide moisture control.

6.2.3 PRODUCT STORAGE AND STABILITY

Product stability studies conducted according to International Council for Harmonisation (ICH) guidance have demonstrated that eRapa is stable for at least 12 months at freezer conditions (approximately -20°C) and for at least 6 months at refrigerator temperature (2 – 8 °C). Exposure of eRapa to ICH long-term conditions (25 °C/60% RH) and intermediate conditions (30 °C/65% RH) for 1 month (30 days) and longer resulted in significant degradation of the rapamycin active ingredient, primarily through conversion to the seco-rapamycin isomer, with attendant decrease in potency.

For long-term storage, eRapa capsules should be kept in the tightly closed original container, with the desiccant pack, in a freezer (approximately -20 °C/0 °F). For patient use, the drug product may be stored for up to 2 months (60 days) in a refrigerator (2 – 8 °C), in the tightly closed original container with the desiccant pack included. Prolonged exposure to elevated temperatures – longer than needed for the drug product to reach room temperature for dispensing – should be avoided, as these conditions may cause excessive degradation of the rapamycin active ingredient.

6.2.4 PREPARATION

The eRapa drug product is manufactured under GMP by SwRI, San Antonio, TX. A complete description of the manufacturing process, including flow charts, is included in the IND. The eRapa capsules for the clinical drug supply were prepared under GMP, also by SwRI. Capsules were filled manually after blending eRapa with two excipients (talc and lactose monohydrate) needed to facilitate the filling process.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Patients will serve as their own controls based on pre-treatment endoscopy. Polyp burden will be scored using standardized tools including polyp burden (sum of the diameter of all polyps), the InSiGHT score, and the Spigelman scoring systems (Appendix A). These assessments will be conducted by investigators who are experienced and familiar with FAP.

6.4 STUDY INTERVENTION COMPLIANCE

Drug compliance will be assessed in coordination with scheduled follow-up visits and telephone calls.

6.5 CONCOMITANT THERAPY

Use of non-steroidal anti-inflammatory drugs other than aspirin is prohibited during the study. The use of 81 mg of aspirin a day or 650 mg of aspirin per week is allowed. The rationale for this is that COX-2 inhibition is known to modulate immune response in patients with FAP but does not have sufficient

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benefit that it is considered standard treatment. In addition, it can be used as a treatment for desmoid tumors, which can be seen in patients with FAP. Other FAP-directed drug therapy is also prohibited. Patients who have been on these therapies may complete a four-week washout period prior to enrollment.

Additionally, investigators will be instructed to ensure that patients do not use either strong inhibitors or inducers of CYP3A4 and P-gp at least 14 days prior to initiation of eRapa and continuing through the duration of eRapa dosing. This limitation is informed by the drug-drug interaction section of the Rapamune label.

6.5.1 RESCUE MEDICINE

Not applicable.

6.6 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.6.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from eRapa does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

The data to be collected at the time of study intervention discontinuation will include the following:

- Serum levels of rapamycin as well as other laboratory tests including Complete Blood Count (CBC), Comprehensive Metabolic Profile (CMP) with phosphorus, and immunologic assays.
- Surveillance endoscopy will be performed at the previous scheduled interval from enrollment

6.6.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression, defined as the identification of malignancy that requires surgical intervention or any other indication for surgery related to polyps, which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the CRF. Patients who sign the informed consent form and are enrolled but do not receive the study intervention may be replaced. Patients who sign the informed consent form, and are enrolled and receive the study

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intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced.

6.6.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for three scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 7 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

7 STUDY ASSESSMENTS AND PROCEDURES

7.1 EFFICACY ASSESSMENTS

Efficacy assessments will be conducted by the investigators or other qualified personnel.

1. **Blood/serum/urine testing**: CBC, CMP, fasting lipid panel, coagulation panel, urinalysis, and LDH will be collected at screening (-30 days to day -1) and then at months 1 (30 days), 3 (90 days), 6 (180 days), 9 (270 days), and 12 (360 days) with a +/-7 day window. All of these labs will be performed at the site's local laboratory.

   Immune Assays: Blood (approximately 50 mL) will be collected at the pre-treatment visit and then at 1 month (30 days), 3 months (90 days), 6 months (180 days), and 12 months (360 days) after initiation of treatment. The immunologic blood draws will be 50 mL of blood collected as 10 mL into a BD Vacutainer Rapid Serum tube (BD, Franklin Lakes, NJ) which contain a clot activator and silicone coated interior. After receipt and centrifugation, serum will be collected, aliquoted in vials and frozen. The remaining 40 mL will be collected into four BD Vacutainer Heparin tubes (green tops) which contain an anticoagulant (sodium heparin). Once received, the heparinized blood will be pooled, diluted, and added to FICOLL HYPAQUE density gradient fluid containing tubes to allow for the separation of PBMC from the red blood cells by a single step centrifugation process. The PBMC fraction will be collected, aliquoted, and frozen for future use in immunologic assays. The immunologic assays will be performed at a central laboratory.

   Immunologic assessments will include an overall assessment of T cell phenotype and function, specifically analyzing T cell naïve/memory/effector phenotype, exhaustion markers (PD-1, LAG3, TIM3), and homing markers (CD62L, CCR7) prior to, during and after treatment with eRapa.

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De-identified patient blood samples showing only the unique study ID number will be sent from study sites via overnight delivery to our central lab facility in San Antonio, TX, where they will be stored until used for immunologic assays. At no point will laboratory personnel have access to patient identifiers. Blood will be frozen and stored under unique study numbers for up to five years for additional immunologic studies related to this protocol (for example, to repeat assays or perform new immunologic assays that do not exist at present but may become available) as needed and then destroyed. No genetic testing will be performed on this material. Study participants will not be contacted in the future for additional use of these stored blood specimens. If study participants want their blood specimens removed from storage and destroyed, they may do so by contacting the PI or research nurse at any time. Additionally, any stored blood may also be utilized to assess new generations of medications.

2. High-definition white light endoscopy will be used.

All endoscopies, both lower and upper, will be recorded using a standard video recording function, if able. Videos of each endoscopy will be submitted for central review and submitted to Cancer Insight.

At baseline, prior to video recording, all polyps ≥ 10 mm and of clinical concern will be removed. And no elements of this study will interfere with the clinical judgment of the provider in this setting. Removed polyps will be submitted for pathology per standard of care.

Local site investigators will assess polyp burden at time of endoscopy or on review of video footage as needed.

Withdrawal: During spiral clockwise withdrawal, a closed biopsy forceps, extended out approximately 2 cm from the tip of the endoscope, should be in view throughout the video recording and held in close approximation to each polyp. If polyps are larger than 5 mm, an open biopsy forceps should be held in close approximation to each polyp. Each site should document the closed and open jaw size of the forceps being utilized on the endoscopy scoring sheet.

Colonoscopy and Sigmoidoscopy: Polyp burden is calculated as the sum of polyp diameters (primary outcome). Polyp number and size will be documented. InSiGHT stage (secondary outcome) will also be calculated as follows:

Stage 0: 0-10 polyps, all less than (<)5 millimeter (mm)
Stage 1: 10-25 polyps, most < 5 mm, none greater than (>) 1 centimeter (cm)
Stage 2: 10-25 polyps, any > 1 cm, amenable to complete removal
Stage 3: > 25 polyps amenable to complete removal, or any incompletely removed sessile polyp, or any evidence of High-Grade Dysplasia (HGD), even if completely excised
Stage 4: > 25 polyps not amenable to complete removal, or any incompletely excised sessile polyp showing HGD, any invasive cancer. (Appendix A)

Intact Colons: For those with intact colons, the colon will be categorized into the following locations: cecum, ascending colon including hepatic flexure, transverse colon, descending colon including splenic flexure, and sigmoid colon. The rectum will be defined as a 20 cm segment from the anal verge. The number size of the polyps per segment of colon and rectum will be annotated. A retroflexed view of the rectum should be performed.

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Patient with total colectomy and ileorectal or ileosigmoid anastomosis:
The level of the anastomosis (cm from anal verge to anastomosis) will be annotated. The rectum will be defined as a 20 cm segment from the anal verge. The size, number and in the rectum as defined and in the sigmoid will be annotated. A retroflexed view of the rectum should be performed.

For the upper endoscopic exam:
All patients will undergo upper endoscopy at baseline. The papilla in each patient will be evaluated with a forward viewing endoscopy and if needed for visualization, cap assisted forward upper endoscopy, or with a side viewing endoscope and biopsied if grossly abnormal. Patients who have no evidence of duodenal polyposis will not receive further upper endoscopic evaluation for study purposes. Patients who have duodenal polyposis on baseline upper endoscopy will undergo endoscopy at six months and 12 months.

A tattoo will be placed 10 cm from the apex of the duodenal bulb and this defined segment will serve as the reference segment for determining size, number, and burden of polyps. Polyp number and size will be reported in this pre-defined segment of duodenum. The largest polyp within the segment will be biopsied for calculation of the Spigelman Score. This dominant polyp will be notated on the scoring form and identified on video. If this dominant polyp is incompletely excised, it will be rebiopsied on subsequent exams to maintain consistency. Spigelman classification system measures risk of developing duodenal cancer in familial adenomatous polyposis (FAP). It has been classified in following stages:

Stage 0: 0 points
Stage 1: 1-4 points
Stage 2: 5-6 points
Stage 3: 7-8 points
Stage 4: 9-12 points

The total score ranges from 0 to 12. Points are accumulated for polyps' number, size, histology, and severity of dysplasia. Stages 1-2 indicates mild disease, whereas stages 3-4 indicate severe duodenal disease. (Appendix A)

7.2 SAFETY AND OTHER ASSESSMENTS

1. **Physical examination** will be conducted by the investigator or other qualified personnel and will include height and weight, history and physical exam, and other examinations pertinent to adverse event monitoring on a quarterly basis.

2. **Fasting blood/serum/urine testing**: The CBC, CMP with phosphorus, and urinalysis will be performed at the site's local laboratory at quarterly visits for all patients.

   The safety lab draws will be 10 mL of blood collected pre-treatment and at months 1 (30 days), 3 (90 days), 6 (180 days), 9 (270 days) and 12 (360 days) with +/- 7 day window after initiation of treatment. Each safety blood draw will consist of 10 mL of blood collected in a 5mL lavender top tube (complete blood count) and a 5 mL red top (comprehensive metabolic panel). Urine will be collected as a clean-catch specimen in a sterile green-top container and be subjected to standard urinalysis to evaluate for proteinuria.

   There will be a safety review and study pause after enrollment of each cohort prior to moving on to the next cohort. The enrollment pause will go into effect after the tenth subject is dosed until the tenth patient has completed 30-day safety labs in each cohort. At this time, blood/serum/urine

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and clinical data to include all adverse events for the entire cohort will be evaluated and the decision will be made either to proceed enrolling the next cohort or to halt dose progression.

3. **Therapeutic drug monitoring:** Drug monitoring will be assessed with trough levels drawn at quarterly visits for all patients. We expect trough levels to range between 2-3 ng/mL based on results from our phase I study of eRapa in prostate cancer patients and from the Rapamune FDA label for similar doses used in treatment of lymphangioleiomyomatosis. Trough levels >20ng/mL will result in discontinuation of the drug in that patient. If trough levels 20ng/mL occur in >2 patients in the same cohort, the dose at that cohort will be discontinued and the dose from the prior cohort will become the MTD. Therapeutic drug monitoring will be performed at the central laboratory.

4. **Long-Term Tolerability:** Assessment of long-term tolerability will involve analyzing all AEs including Grade 1-2 toxicities and any changes in dosing (reduction, delay, or withdrawal) in the context of drug levels drawn for therapeutic drug monitoring, PK data obtained from all patients in each cohort (including C_{max}, T_{max}, C_{last}, T_{last}, K_e, half-life, and AUC), and clinical benefit as assessed by endoscopy.

7.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

7.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

7.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.3.3 CLASSIFICATION OF AN ADVERSE EVENT

7.3.3.1 SEVERITY OF EVENT

AEs will be graded on severity per Common Terminology Criteria for Adverse Events (CTCAE) v5.0 (Appendix D) definitions.

7.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

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Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the study drug caused the adverse event. For reporting purposes, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Factors to consider in determining if an AE should be regarded as possibly related to the use of the drug components include:

- There is a clinically plausible time sequence between onset of the AE and administration of drug components;
- There is a biologically plausible mechanism for drug components to cause or contribute to the AE;
- The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.3.3.3 EXPECTEDNESS

An unexpected AE is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current Investigator Brochure (IB); or, if an IB is not required or available, the specificity or severity of which is not consistent with the risk information described in the protocol.

Unexpected, as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the IB) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

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7.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs will be captured on the appropriate CRF. Information to be collected includes event description, date of onset, clinician's assessment of toxicity grade (severity), relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of resolution of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The investigator will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. Monthly throughout the trial, at each study visit or via phone call, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

7.3.5 ADVERSE EVENT REPORTING

With the occurrence of an AE, the first concern will be for the safety of the patient. Investigators are required to report to the medical monitor and the clinical research organization (CRO) any SAE, whether expected or unexpected within 24 hours of notification of the event. All events meeting the outlined criteria will be reported for the time period beginning any time after informed consent is obtained through the protocol-defined follow-up period. Investigators will follow reporting requirements mandated by the IRB and applicable regulations. The IND Sponsor or Sponsor's representative will report SAEs qualifying for expedited reporting to the U.S. Food and Drug Administration (FDA) per the requirements of 21 CFR Section 312.32. Refer to Appendix C for reporting algorithm.

All AEs will be recorded on the Adverse Event CRF.

7.3.6 SERIOUS ADVERSE EVENT REPORTING

Investigators will report all SAEs to the CRO and designee within 24 hours of notification of the event. Investigators will report events to the IRB of their site as required by the IRB and applicable regulations (see SAE Reporting Algorithm, Appendix C). Sponsor or Sponsor's representative will review the serious AE to determine the need for expedited reporting to the FDA. If expedited reporting is required, a MedWatch Form FDA 3500A (Appendix E) will be completed. Sponsor or Sponsor's representative will report SAEs qualifying for expedited reporting to the FDA as required by the U.S. Code of Federal Regulations.

MedWatch 3500A reporting guidelines

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In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (Section B) of the MedWatch 3500A form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Assessment of the relationship of the AE to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (e.g., D.O.B. initials, patient study number, protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted.) (The patient identifiers are important so that the new information is added to the correct initial report.)

The CRO or designee may contact the reporter for additional information, clarification, or current status of the patient for whom an AE was reported. For questions regarding SAE reporting, you may contact the CRO clinical or regulatory staff.

The study sponsor will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. Refer to Appendix C for reporting algorithm.

7.3.7 REPORTING REQUIREMENTS FOR IND SPONSOR

For sponsored IND Studies, there are reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR § 312.32. Events meeting the following criteria need to be submitted to the FDA as expedited IND Safety Reports according to the following guidance and timelines:

- 7 Calendar-Day Report: The sponsor is required to notify the FDA of any unexpected fatal or life-threatening suspected adverse reaction. An unexpected AE is one that is not already described in the IB for the drug. A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Unexpected suspected adverse reactions that are fatal or life-threatening are to be reported by the sponsor to the FDA within seven calendar days of first learning of the event.

- 15 Calendar-Day Report: The sponsor is required to notify the FDA and all participating investigators, in a written IND Safety Report, of any unexpected, serious, suspected adverse reaction within 15 days of determining an event qualifies for reporting. An unexpected AE is one that is not already described in the Investigator Brochure. A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.
    - Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32 (c)(1). All safety reports previously filed with the IND

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concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.

- Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch (Form FDA 3500A), but alternative formats are acceptable (e.g., summary letter).

7.3.8 REPORTING EVENTS TO PARTICIPANTS

Not applicable

7.3.9 EVENTS OF SPECIAL INTEREST

Not applicable

7.3.10 REPORTING OF PREGNANCY

In the event that a patient has a positive pregnancy test, she will be informed immediately by the site coordinator or investigator. She will be considered to be in violation of eligibility criteria and the study intervention will immediately be discontinued. No additional surveillance endoscopy will be performed as part of the study, unless otherwise indicated by her treating physicians.

8 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL HYPOTHESES

Primary Endpoint:

1. Safety and tolerability of eRapa as determined by graded toxicity assessed throughout the trial per CTCAE v.5.0.
2. Safety/adverse events as in endpoint 1, dose delays, dose reductions, withdrawal of treatment secondary to low-grade toxicities, and serum pharmacokinetic monitoring.
3. Percentage change from baseline in the colorectal polyp burden as measured by endoscopy at 6 months (180 days).

   Null hypothesis: there is no difference between pre-treatment and post-treatment colorectal polyp burden in patients receiving eRapa at 6 months (180 days).

   Alternative hypothesis: there is a significant decrease in post-treatment colorectal polyp burden compared to pre-treatment stage in patients receiving eRapa for 6 months (180 days).

Secondary Endpoints:

1. Percentage change from baseline in the colorectal polyp burden at 12 months (360 days).
2. Change from baseline in International Society for Gastrointestinal Hereditary Tumors (InSiGHT) Stage at 6 months (180 days) and 12 months (360 days).
3. Change from baseline in Spigelman Stage Score at 6 months (180 days) and 12 months (360 days) in patients with polyps at baseline on esophagogastroduodenoscopy (EGD).
4. Percentage change from baseline in the duodenal polyp number and size at 6 months (180 days) and 12 months (360 days) in patients with polyps at baseline on EGD.

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For all of the above:

- Null hypothesis: there is no difference between pre-treatment and post-treatment in patients receiving eRapa for the specified timeframe.
- Alternative hypothesis: there is a difference between pre-treatment and post-treatment in patients receiving eRapa for the specified timeframe.

5. Pharmacokinetic parameters (AUC[0-INF], C_{max}) to assess the effect of food on the bioavailability of the anticipated RP2D.

Exploratory Endpoints:

1. Immunologic response will be measured and will include an overall assessment of T cell phenotype and function, specifically analyzing T cell naïve/memory/effector phenotype, exhaustion markers (PD-1, LAG3, TIM3), and homing markers (CD62L, CCR7) prior to, during and after treatment with eRapa.

8.2 SAMPLE SIZE DETERMINATION

The trial is planned to enroll 30 patients, with 10 patients enrolled in each dosing cohort. This number will allow for a 20% drop out rate and still yield 24 evaluable patients. Patients will serve as their own control as the primary endpoint compares the change from baseline in polyp burden at 6 months (180 days). Assuming an effect size of 35% reduction in colorectal polyp burden compared to baseline endoscopies, a power of 80%, and alpha level 0.05, 24 patients would be needed to show a statistically significant difference between paired samples using a one-sided *t*-test.

Up to 20 patients will be enrolled in the food effect portion of the study based on practical considerations. Patients will be randomized 1:1 to one of two crossover arms to assess bioavailability.

8.3 POPULATIONS FOR ANALYSES

Patients will serve as their own controls. The control group will consist of data collected at baseline endoscopy. The treatment group will consist of data collected at 6 months (180 days) and 12 months (360 days) at surveillance endoscopy. They will be analyzed as paired samples. An analysis of the primary efficacy endpoint at 6 months (180 days) after enrollment of the 30th patient will serve as the primary analysis. A per-protocol analysis will be conducted for patients who complied with eRapa administration at least 80% of the time within the treatment period. A safety analysis will be conducted on all patients receiving at least one dose of study drug.

The food effect sub study will include up to 20 patients. Patients will serve as their own controls and will participate in each treatment periods. Data will be collected at 2 weeks and 6 weeks.

8.4 STATISTICAL ANALYSES

8.4.1 GENERAL APPROACH

Demographic characteristics of all patients will be summarized including age, sex, race, BMI, age at diagnosis, smoking history, alcohol use history, NSAID use history, PPI use, family history of colorectal cancer, family history of duodenal cancer, personal history of extraintestinal malignancy, documented APC mutation, desmoid tumor burden, results of any prior esophagogastroduodenoscopy (worst stage of

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duodenal polyposis recorded), history of known prior colorectal cancer, type and date of colectomy performed, if applicable.

Continuous variables will be summarized using the number of patients, mean, standard deviation, median, minimum, and maximum; and categorical variables will be summarized using the frequency count and the percentage of patients in each category. Differences between treatment groups will be determined using a chi-square test for categorical variables and a t-test for continuous variables.

8.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Safety will be compared to evaluate the number, frequency, duration, and relation of adverse events to eRapa, as defined by the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 (Appendix D); analyzed overall and compared between dosing cohorts. This will also be assessed based on duration of treatment.

The incidence of treatment-emergent AEs, SAEs, severe AEs, AEs related to study drug (defined as not, unlikely, possibly, probably, or definitely related to study drug), and AEs which lead to study discontinuation will be summarized by treatment group. Differences between treatment groups will be determined using Fisher's exact tests.

Time to AE onset will be estimated using the Kaplan-Meier method and treatment differences will be determined using the log-rank test. In addition, duration of any AE with >10% incidence, total number of AEs, SAEs, and related AEs will be summarized, and treatment group differences assessed using a t-test or Wilcoxon rank sum test, depending on the distribution of the data.

Long-term tolerability and the determination of the optimal long-term dose will include the AE analysis listed above as well as analysis of serum drug concentrations, dose delays, dose reductions, and medication withdrawals. The ideal dose will be determined to be that which was most readily tolerated (i.e., fewest AEs, fewest dosing schedule modifications) while still producing a physiologic effect (i.e., appropriate serum levels and reduction in polyp burden on endoscopy).

PK analyses will include all subjects who received eRapa with ≥1 sample collected at the appropriate time point. Any missing pre-dose PK samples will be reported as a concentration of "0 ng/mL." Demographic characteristics, patient history characteristics, timing of dose and blood draws, and protocol deviations will all be reported with the PK analysis. At the conclusion of the study, a subgroup analysis will be conducted examining the PK data exclusively from patients who completed the trial without any protocol violations or deviations that may have potentially affected the resulting PK data. Final PK data will be reported using this subgroup, and any patients excluded (i.e., patients with protocol deviations or violations) will be noted along with the reason for exclusion in the final PK report. Reported values will include C_{max} (maximum plasma concentration, in ng/mL), T_{max} (time of maximum plasma concentration, reported in hours), C_{last} (last detectable plasma concentration, reported in ng/mL), T_{last} (time of last detectable plasma concentration, reported in hours), K_e (elimination rate constant, reported in 1/h), half-life (time required for the drug concentration to decrease by a factor of one-half, reported in hours), AUC (area under the concentration-time curve, reported in units of h x ng/mL). PK values will be compared between cohorts using mean, standard deviation (SD), coefficient of variation as a percent (CV%), standard error (SE), and standard error of the mean (SEM). Values between groups will be compared using t-tests, with significance set at $p<0.05$.

The primary efficacy endpoint, measured as percentage change in colorectal polyp burden at 6 months (180 days), will be compared using a one-sided, paired samples *t*-test with an alpha level of 0.05.

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8.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

1) Percentage change from baseline in colorectal polyp burden at 12 months (360 days) will be compared using a one-sided paired t-test with an alpha level of 0.05.
2) Change from baseline in International Society for Gastrointestinal Hereditary Tumors (InSiGHT) Stage at 6 months (180 days) and 12 months (360 days), will be compared using a one-sided, paired samples t-test with an alpha level of 0.05
3) Change from baseline in Spigelman Stage Score at 6 months (180 days) and 12 months (360 days), will be compared using a one-sided, paired samples *t*-test with an alpha level of 0.05
4) Percentage change from baseline in the duodenal polyp number and size at 6 months (180 days) and 12 months (360 days) in patients with duodenal polyposis at baseline: measured in sum of diameters of polyps, will be compared using a one-sided, paired samples *t*-test with an alpha level of 0.05
5) For patients enrolled in the food effect portion of the study, AUC[0-INF] and C_{max} will be compared using population geometric means ratios of log-transformed data. If the 90% confidence interval of for the population geometric mean ratios is contained in the equivalence limits of 80%-125%, then an absence of a food effect is established. Additional details will be provided in the statistical analysis plan.

8.4.4 BASELINE DESCRIPTIVE STATISTICS

Not applicable

8.4.5 PLANNED INTERIM ANALYSES

Not applicable

8.4.6 SUB-GROUP ANALYSES

Given the small, planned sample size, sub-group analyses are not planned for this study.

8.4.7 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data will be listed by measure and time point.

8.4.8 EXPLORATORY ANALYSES

Immunologic responses will be determined using phenotypic and functional assays (ex vivo).

Expression percentages will be compared using a chi-square test while continuous outcomes will be compared using a paired t-test (within treatment group) and a two-sample t-test (between treatment groups) for the ex vivo response evaluated by the phenotypic and functional assays during the trial.

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Changes from pre-medication levels to each time point will be compared, and differences within treatment groups will be evaluated using a paired t-test while between treatment group differences will be evaluated using two-sample t-tests.

In a series of exploratory analyses, the different immunologic response indicators (in vivo and ex vivo) at different time points will be correlated with clinical outcome in order to assess for any predictors of outcome. Additional exploratory outcomes will compare clinical and immunological outcomes based on dosing cohort.

For the food effect portion of the study, T_{max}, t1/2. Cl/F, and Vd/F will be estimated. Summary statistics of each measure will be provided.

9 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

9.1.1 INFORMED CONSENT PROCESS

9.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

9.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved, and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

9.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided

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by the suspending or terminating party to study participants, investigator, the IND sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB, and/or Food and Drug Administration (FDA).

9.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, applicable regulations, and sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at Cancer Insight. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Cancer Insight research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at Cancer Insight.

9.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

De-identified patient blood samples showing only the unique study ID number will be sent from study sites via overnight delivery to our central lab facility in San Antonio, TX, where they will be stored until used for immunologic assays. At no point will laboratory personnel have access to patient identifiers. Blood will be frozen and stored under unique study numbers for up to five years for additional

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immunologic studies related to this protocol (for example, to repeat assays or perform new immunologic assays that do not exist at present but may become available) as needed and then destroyed. No genetic testing will be performed on this material. Study participants will not be contacted in the future for additional use of these stored blood specimens. If study participants want their blood specimens removed from storage and destroyed, they may do so by contacting the PI or research nurse at any time. Additionally, any stored blood may also be utilized to assess new generations of medications. These samples could be used to research the causes of FAP, its complications and other conditions for which individuals with FAP are at increased risk, and to improve treatment. The repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biological specimen storage may not be possible after the study is completed.

9.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Medical Monitor**
Carol Burke, MD Gastroenterologist Director of the Center for Colon Polyp and Cancer Prevention	Vance Sohn, MD Director of Medical Affairs
Cleveland Clinic	LumaBridge
Cleveland Clinic Main Campus Mail Code A30 9500 Euclid Avenue Cleveland, OH 44195	1305 East Houston St San Antonio, TX 78205
216-444-7000	253-722-3144
BurkeC1@ccf.org	vsohn@lumabridge.com

9.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Medical Monitor listed above and in accordance with the clinical monitoring plan, data monitoring plan, and quality assurance (QA) plan as described below.

9.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), ICH GCP, and applicable regulatory requirement(s).

9.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Edit checks will fire in real time during data entry flagging missing fields and/or data that is outside parameters identified by the protocol. Edit checks will be reviewed by study team and sites will be required to provide detailed justification for data

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anomalies if not a data entry error. Ongoing data review throughout the lifecycle of the study will be used to identify data trends and any potential issues with study conduct.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices [GLP], Good Manufacturing Practices [GMP]).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

9.1.9 DATA HANDLING AND RECORD KEEPING

9.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Relevant study data will be collected on each patient and entered into an Electronic Data Capture (EDC) system. The URL for the EDC system is *https://login.medrio.com*. Supported operating systems are Microsoft Windows XP, Microsoft Windows 2000, Microsoft Windows 7 and 8, Microsoft Vista, and Mac OS X. Microsoft Internet Explorer (version 9 and later), Firefox (version 27 and later), Chrome (version 30 and later), and Safari (version 9.0 and later) are acceptable web browsers.

Medrio software supports compliance with regulations such as 21 CFR Part 11, Annex 11, EU Safe Harbor, Good Clinical Practice, and HIPAA. Procedural controls include electronic audit trails of all changes to study data, electronic signatures, data encryption, and access restrictions to Protected Health Information (PHI). Access to the Medrio EDC system will only be granted to study personnel after training is conducted. User accounts are permission based which grants limited access and functionalities dependent upon permissions granted by study administrator.

All data that goes into and out of the server is encrypted using 128-bit Secure Sockets Layer (SSL) and 1024-bit RSA public keys. The servers, hosted by Medrio, are housed in a fully redundant N+1 data center with redundant power, cooling, and network connectivity. All servers reside behind Cisco firewalls and advanced intrusion detection/prevention systems. Medrio performs nightly backups to locations both on-site and off-site and maintains redundant hardware.

Data collection will begin at the signing of informed consent. All data must be submitted within 72 working hours of the visit in which data was collected. Edit checks (often called queries) will fire in real time as data is being entered to ensure quality data is provided. In addition to edit checks, manual queries will be generated by Data Managers and Monitors. Sites will have 10 working days to address all edit checks and manually created queries. Source Data Verification (SDV), Data Management Review and electronic signature by site PI will all take place prior to locking data.

9.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Council for Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed

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without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

9.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site investigator to use continuous vigilance to identify, report, and record deviations according to the study specific monitoring plan. All deviations must be addressed in study source documents and recorded on the applicable CRF within the clinical database. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

9.2 ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
APC	Adenomatous polyposis coli (gene)
AST	Aspartate Aminotransferase
AUC	Area Under the Concentration-Time Curve
AUC[0-INF]	Area Under the Concentration-Time Curve from Time Zero Extrapolated to Infinite Time
CBC	Complete Blood Count
CFR	Code of Federal Regulations
C_{last}	Last Observed Concentration
Cl/F	Apparent Clearance
C_{max}	Maximum Concentration
CMP	Clinical Monitoring Plan / Comprehensive Metabolic Panel
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
EDC	Electronic Data Capture
EGD	Esophagogastroduodenoscopy
EoT	End of Treatment
FAP	Familial Adenomatous Polyposis
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Council for Harmonisation
IND	Investigational New Drug Application
INF	Infinity
INR	International Normalized Ratio
InSIGHT	International Society for Gastrointestinal Hereditary Tumors
IRB	Institutional Review Board

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K_e	Elimination Rate Constant
LDH	Lactate Dehydrogenase
MAP	MUTYH-associated Polyposis
MTD	Maximum Tolerated Dose
mTOR	Mammalian Target of Rapamycin / Mechanistic Target of Rapamycin
NCT	National Clinical Trial
NSAID	Nonsteroidal Anti-Inflammatory Drug
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QA	Quality Assurance
QC	Quality Control
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SOE	Schedule of Events
SOP	Standard Operating Procedure
SwRI	Southwest Research Institute
t1/2	Terminal Elimination Half-Life
T_{last}	Time to Last Observed Concentration
T_{max}	Time to Maximum Concentration
ULN	Upper Limit Normal
US	United States
Vd/F	Volume of Distribution

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APPENDIX A – ENDOSCOPIC SCORING SYSTEMS

InSiGHT Polyposis Staging System-Rectum

Stage#	Polyp Description
0	0-10 polyps, all <5mm
1*	10-25 polyps most <5mm, none >1cm
2*	10-25 polyps, any >1cm, amenable to complete removal
3*	> 25 polyps amenable to complete removal, or any incompletely removed sessile polyp, or any evidence of HGD, even if completely excised
4	>25 polyps not amenable to complete removal, or any incompletely excised sessile polyp showing HGD; any invasive cancer

Clinical Intervention		Comments:
(A)	Repeat FS in 1 Year	
(B)	Ablate polyps; repeat sigmoidoscopy in 1 year	Chemo-preventive may be considered
(C)	Repeat sigmoidoscopy 6 months Polypectomy preferred	Removal of large polyps clearly necessary Chemo-preventive valuable
(D)	Repeat sigmoidoscopy 3-6 months; consider proctectomy	Large polyps must be removed; second opinion on polyp management helpful
(E)	Proctectomy / pouch revision +/- ileostomy clearly indicated within 3 months	Any decision to delay surgery must be highly individualized and based on compelling circumstances

*Presence of High-Grade Dysplasia Warrants Upstaging of Patient to Stage 4.

Patients who cannot be allotted a particular stage (e.g. patients with mix polyposis) call for an external discussion in a multidisciplinary specialty team.

Spigelman scoring system

Findings at duodenoscopy	I point	2 points	3 points
Number of adenomas	1–4	5–20	>20
Size (mm)	1–4	5–10	>10
Histology	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Notes: Staging according to score: stage 0: 0 points; stage I: 4 points; stage II: 5–6 points; stage III: 7–8 points; stage IV: 9–12 points. Data from Spigelman et al.[48]

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APPENDIX B – MEDICATIONS THAT INTERACT WITH RAPAMYCIN OR SIROLIMUS

Risk X: Avoid combination medications

Antihepaciviral Combination Products: May increase the serum concentration of Sirolimus.

BCG (Intravesical): Immunosuppressants may diminish the therapeutic effect of BCG (Intravesical).

BCG (Intravesical): Myelosuppressive Agents may diminish the therapeutic effect of BCG (Intravesical).

Conivaptan: May increase the serum concentration of CYP3A4 Substrates (High risk with Inhibitors).

Crizotinib: May increase the serum concentration of Sirolimus.

Deferiprone: Myelosuppressive Agents may enhance the neutropenic effect of Deferiprone.

Dipyrone: May enhance the adverse/toxic effect of Myelosuppressive Agents. Specifically, the risk for agranulocytosis and pancytopenia may be increased

Enzalutamide: May decrease the serum concentration of Sirolimus.

Erythromycin (Systemic): May increase the serum concentration of Sirolimus. Sirolimus may increase the serum concentration of Erythromycin (Systemic).

Fusidic Acid (Systemic): May increase the serum concentration of CYP3A4 Substrates (High risk with Inhibitors).

Grapefruit Juice: May increase the serum concentration of Sirolimus.

Idelalisib: May increase the serum concentration of CYP3A4 Substrates (High risk with Inhibitors).

MiFEPRIStone: May increase the serum concentration of Sirolimus. Management: Avoid sirolimus during and 2 weeks following mifepristone for treatment of hyperglycemia in Cushing's syndrome. The interaction magnitude could be lower with single doses used to terminate pregnancy, but neither effect has been studied clinically.

Natalizumab: Immunosuppressants may enhance the adverse/toxic effect of Natalizumab. Specifically, the risk of concurrent infection may be increased.

Pimecrolimus: May enhance the adverse/toxic effect of Immunosuppressants.

Posaconazole: May increase the serum concentration of Sirolimus.

Tacrolimus (Systemic): Sirolimus may enhance the adverse/toxic effect of Tacrolimus (Systemic). Tacrolimus (Systemic) may enhance the adverse/toxic effect of Sirolimus. Sirolimus may decrease the serum concentration of Tacrolimus (Systemic).

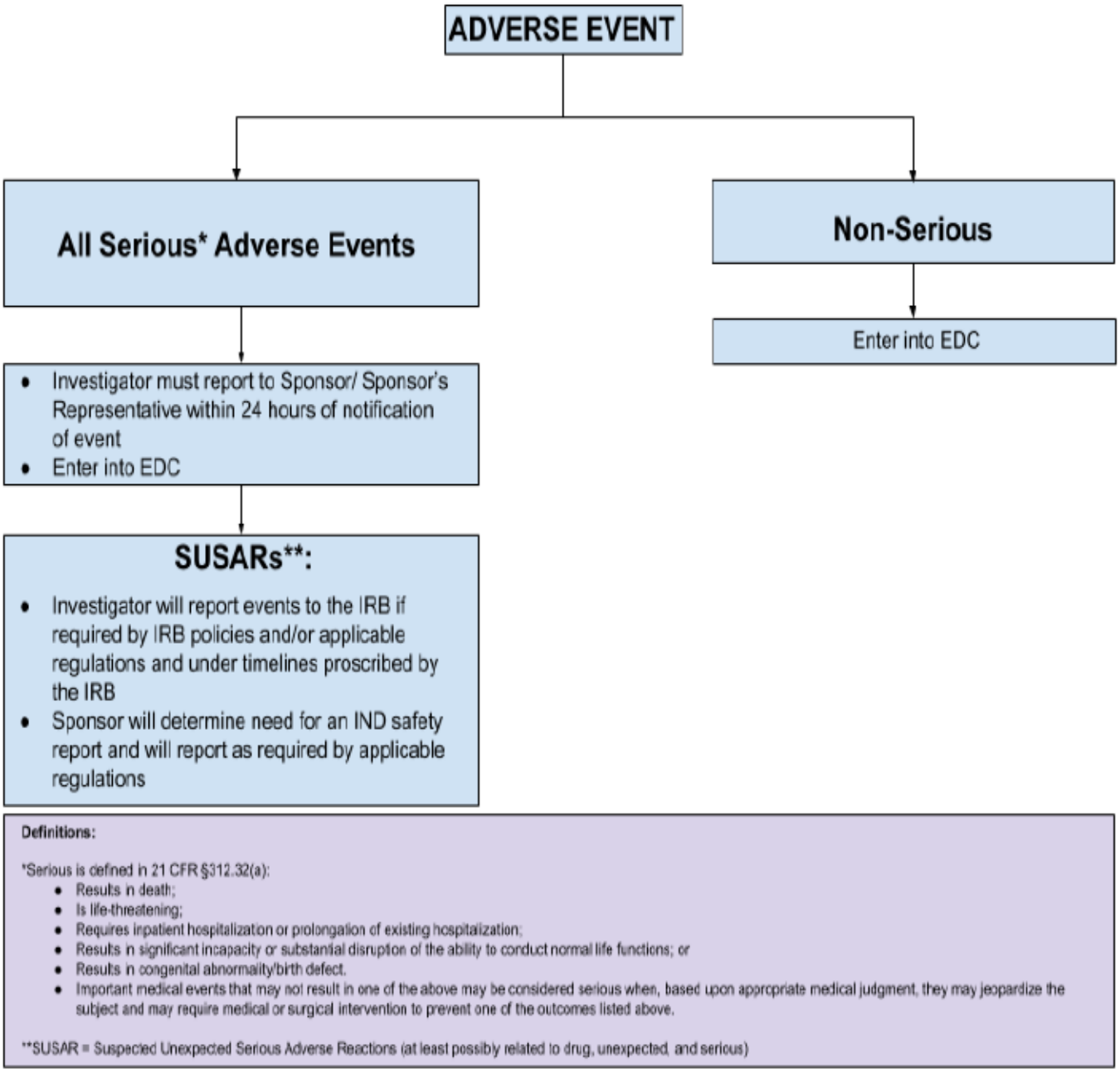
Tacrolimus (Topical): Sirolimus may enhance the adverse/toxic effect of Tacrolimus (Topical). Tacrolimus (Topical) may enhance the adverse/toxic effect of Sirolimus.

Vaccines (Live): Immunosuppressants may enhance the adverse/toxic effect of Vaccines (Live). Immunosuppressants may diminish the therapeutic effect of Vaccines (Live). Management: Avoid use of live organism vaccines with immunosuppressants; live-attenuated vaccines should not be given for at least 3 months after immunosuppressants.

Voriconazole: May increase the serum concentration of Sirolimus.

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APPENDIX C – ADVERSE EVENT REPORTING ALGORITHM



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APPENDIX D – CTCAE V 5.0

NCI Common Terminology Criteria for Adverse Events, v5.0

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

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APPENDIX E – MEDWATCH FORM FDA 3500A LINK

https://www.fda.gov/downloads/aboutfda/reportsmanualsforms/forms/ucm048334.pdf

https://www.fda.gov/downloads/aboutfda/reportsmanualsforms/forms/ucm295636.pdf

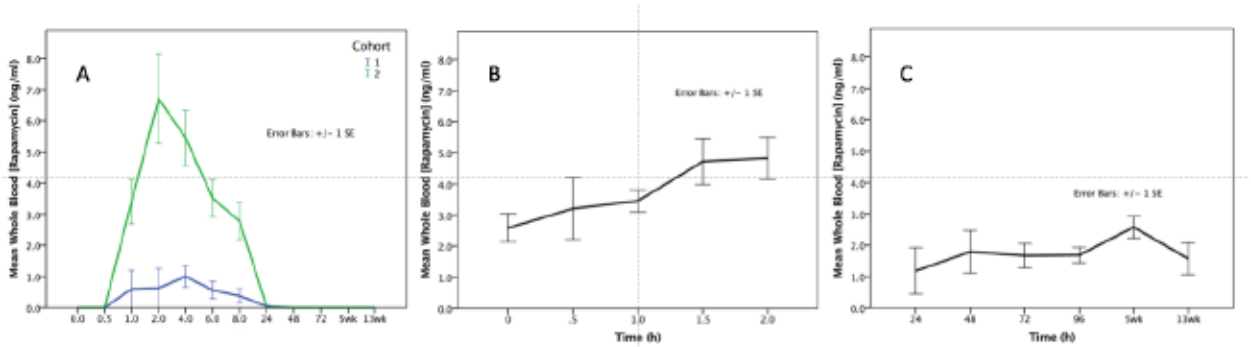
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APPENDIX F – ADVERSE EVENTS FROM PREVIOUS ERAPA STUDY

Cohort	No. Patients	No. Adverse Events, Total	No. of Patients w/ Adverse Event, Total (%)	List of Adverse Events	No. of Events	No. of Patients w/ Adverse Event (%)	Toxicity Grade	Expected (y/n)	Related to Treatment	Action Taken	Outcome	Serious (y/n)
1	3	4	3 (100)	Oral pain	1	1 (33)	1	Y	Possible	None	Recovered	N
				Maculopapular rash	1	1 (33)	1	N	Unlikely	None	Recovering	N
				Pruritus	1	1 (33)	1	Y	Unlikely	None	Recovering	N
				Headache	1	1 (33)	1	Y	Possible	None	Recovered	N
2	3	1	1 (33)	Diarrhea	1	1 (33)	1	Y	Possible	None	Recovered	N
3	9	44	7 (78)	Fatigue	5	3 (33)	1	Y	Probable	None	Recovered	N
							1	Y	Possible	None	Recovered	N
							1	Y	Possible	None	Recovered	N
				Headache	3	1 (11)	1	Y	Possible	None	Recovered	N
				Oral pain	8	2 (22)	1	Y	Probable	None	Recovered	N
							1	Y	Probable	None	Ongoing	N
				Insomnia	5	2 (22)	1	Y	Possible	None	Recovered	N
							1	Y	Possible	Drug withdrawn due to other toxicity	Recovered	N
				Diarrhea	2	2 (22)	1	Y	Possible	None	Recovered	N
							1	Y	Possible	None	Recovered	N
				Constipation	1	1 (11)	1	Y	Possible	None	Recovered	N
				Bone pain	1	1 (11)	1	N	Unlikely	None	Recovered	N
				Neutrophil count decreased	1	1 (11)	3	Y	Probable	Drug withdrawn	Recovered	N
				Lymphocte count decreased	1	1 (11)	2	Y	Probable	Drug withdrawn due to other toxicity	Recovered	N
				Papulopustular rash	1	1 (11)	1	N	Probable	Drug withdrawn	Recovered	N
				Laryngeal inflammation	2	2 (22)	2	N	Probable	Drug withdrawn due to other toxicity	Recovered	N
							1	Y	Possible	None	Ongoing	N
				Allergic reaction	1	1 (11)	2	N	Probable	Drug withdrawn due to other toxicity	Recovered	N
				Dizziness	8	2 (22)	2	N	Probable	Drug withdrawn	Recovered	N
							1	N	Unlikely	None	Recovered	N
				Nystagmus	3	1 (11)	2	N	Probable	None	Recovered	N
				Dry mouth	1	1 (11)	1	Y	Possible	None	Ongoing	N
				Pruritus	1	1 (11)	2	Y	Probable	None	Resolved	N

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APPENDIX G – PK DATA FROM PREVIOUS ERAPA STUDY



A. Cohort 1 and 2, single dose max
B. Cohort 3, multi-dose max
C. Cohort 3, multi-day trough levels

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APPENDIX H – COMPOSITION OF A HIGH FAT MEAL

Total Calories: 800-1000

Calories from Protein: 150

Calories from Carbohydrates: 250

Calories from Fat: 500-600

Percent Calories from Fat: >50

An example of a high-fat breakfast:

- Two eggs fried in butter
- Two strips of bacon
- Two pieces of toast with butter
- Four ounces of hash brown potatoes
- Eight ounces of whole milk

*50 percent of calories are derived from fat. Substitutions can be made to this meal if the content, volume, and viscosity are maintained.

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APPENDIX I – INVESTIGATOR'S AGREEMENT

Protocol title: Phase IIa Trial of Encapsulated Rapamycin (eRapa) in Familial Adenomatous Polyposis Patients under Active Surveillance

Protocol number: EMT-FAP-001

Study Drugs: Encapsulated rapamycin (eRapa)

Principal Investigator: Carol Burke, MD

Program Director: George E. Peoples, MD, FACS

Protocol Version/Date: v3.0 / 04 Dec 2023

INVESTIGATOR'S AGREEMENT / INVESTIGATOR'S SIGNATURE PAGE

I have read the protocol described above. I have fully discussed the objectives of this trial and the content of this protocol with the sponsor or the sponsor's representative. I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical review of the trial, without written authorization from the sponsor. It is, however, permissible to provide information to a patient in order to obtain consent. I agree to conduct this trial according to the protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with all applicable regulations, and guidelines as stated in the protocol and other information supplied to me. I understand that the sponsor may decide to suspend or prematurely terminate the trial at any time, for whatever reason. Such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial, I will communicate my intention immediately, in writing to the sponsor.

Signed: _____ Date: _____

Investigator's Name and Address:

_____

_____

_____