RESEARCH PROTOCOL

‘Sirolimus for the treatment of severe intestinal polyposis in patients with familial adenomatous polyposis (FAP); a pilot study’

Correspondence to:
Prof. dr. E. Dekker
Department of gastroenterology and hepatology, C2
Academic Medical Centre
Meibergdreef 9
1105 AZ Amsterdam

PROTOCOL TITLE 'Sirolimus for the treatment of severe intestinal polyposis in patients with familial adenomatous polyposis (FAP), a pilot study'

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Coordinating investigator/project leader	Prof. dr. E. Dekker Department of gastroenterology and hepatology, C2 Academic Medical Centre Meibergdreef 9 1105 AZ Amsterdam
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	Prof. dr. E. Dekker Department of gastroenterology and hepatology, C2 Academic Medical Centre Meibergdreef 9 1105 AZ Amsterdam
Sponsor (in Dutch: verrichter/opdrachtgever)	Department of gastroenterology and hepatology, C2 Academic medical centre Meibergdreef 9 1105 AZ Amsterdam
Subsidising party	Pfizer BV Rivium Westlaan 142 2909 LD Capelle a/d IJssel
Independent expert (s)	Prof. dr. P. Fockens Department of gastroenterology and hepatology, C2 Academic Medical Centre Meibergdreef 9

	1105 AZ Amsterdam
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PROTOCOL SIGNATURE SHEET

Name	**Signature**	**Date**
Head of Department: Prof. dr. P. Fockens		
[Coordinating Investigator/Project leader/Principal Investigator]: Prof. dr. E. Dekker		

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CRC	Colorectal cancer
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EKG	Electrocardiography
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
FAP	Familial adenomatous polyposis
GCP	Good Clinical Practice
HRQoL	Health related quality of life
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IPAA	Ileo-anal pouch anastomosis
IRA	Ileo-rectal anastomosis
LGI	Lower gastrointestinal
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.

SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper limit of normal
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Due to the presence of numerous colorectal polyps, nearly all patients with familial adenomatous polyposis (FAP) develop colorectal cancer (CRC) at an average age of 45 years, if left untreated. Therefore, a prophylactic colectomy is recommended. After surgery, adenomas are likely to reappear in the pouch or rectum. Recently, studies in APC-deficient mice have shown that the mTOR inhibitor sirolimus can cause intestinal tumour cells to undergo growth arrest and differentiation and could even lead to regression of polyps. In current practice, sirolimus is used as an immunomodulator for patients after renal transplantation. Sirolimus has never been investigated in patients with FAP. We hypothesize that sirolimus could lead to regression of intestinal polyps in patients with FAP.

Objective: The aim of our study is to investigate the effect of sirolimus on the progression of intestinal adenomas in patients with FAP and to assess the safety of this treatment.

Study design: A prospective phase II pilot study with a follow-up of 6 months.

Study population: Five patients with FAP will be selected and invited for study participation. They need to be 18 years or older, have a genetically confirmed APC mutation with a classical FAP phenotype and a subtotal colectomy with an ileo-rectal anastomosis (IRA) or a total colectomy with an ileo-anal pouch anastomosis (IPAA) with severe polyposis.

Intervention: All patients will receive sirolimus for the duration of the study, with a trough level target range of 5-8 ng/ml.

Main study parameters/endpoints: The main study parameters are the effect of sirolimus on the size of 5 marked polyps and safety of this treatment. Safety outcomes will be assessed by summary analysis of adverse events, clinical laboratory abnormalities and regular physical examination. Additional parameters are the effect on the number of polyps, global polyp burden, histopathology and patient-reported quality of life. Cell proliferation and immunohistochemistry of mTOR targets in healthy intestinal mucosa and adenomatous tissue will be assessed.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: At baseline and at three monthly visits a medical history will be taken and physical examinations will be performed, as well as laboratory tests and HRQoL questionnaires. Trough level testing of sirolimus will be measured at day 7 after start of the study drug and weekly until the therapeutic range has been achieved, after which the next trough level will be measured at 3 and 6 months follow-up. Finally, monthly telephone check-ups will be carried out. LGI endoscopies will be done at baseline and at 6 months. For this study, we include patients with severe rectal or pouch polyposis as they are expected to have an indication for invasive surgery on a short-term base and no other less invasive alternative therapy is available.

1. INTRODUCTION AND RATIONALE

Familial adenomatous polyposis (FAP) is a syndrome caused by mutations in the adenomatous polyposis coli (APC) tumor suppressor gene. It follows an autosomal dominant pattern of inheritance and up to 25% of all cases are due to *de novo* mutations (1). This syndrome is characterized by the presence of numerous colorectal adenomas, usually more than hundred, from a young age (2). If left untreated, colorectal cancer (CRC) develops in nearly 100% of all patients with a mean age at diagnosis of 45 years (3). Therefore, international guidelines recommend a prophylactic colectomy for these patients (4-7). After surgery, surveillance sigmoidoscopies are needed as adenomas are likely to reappear in the pouch or rectum (8, 9). In addition to colorectal adenomas, patients with FAP usually develop duodenal adenomas as well as extraintestinal manifestations, such as desmoid tumors and fundic gland polyps (10-12).

In recent years several drug therapies have been investigated that aimed to decrease the number and size of polyps in patients with FAP, with none of them providing sufficient evidence of success (13). However, recently studies in APC-deficient mice demonstrated that mTOR inhibitor sirolimus can cause intestinal tumour cells to undergo growth arrest and differentiation (14). Signalling through the mTOR pathway is required for epithelial cell proliferation and tumour growth (15, 16). It was shown that mTOR complex 1 (mTORC1) activity is required for the proliferation of APC-deficient enterocytes. This complex mediates inhibition of eEF2 kinase, which is needed for the proliferation of APC-deficient cells. Sirolimus targets eEF2 resulting in growth arrest and differentiation of tumour cells. The effect of sirolimus on colonic adenoma regression was confirmed in other mice studies, with one of them showing beneficial effects in the small bowel (17-22). Sirolimus seems to inhibit polyp formation and could even lead to regression of polyps (21, 23). Moreover, it seems to increase survival and time to progression to dysplasia (20, 21).

Sirolimus has been widely investigated in human studies. It received FDA approval in 1999 as immunosuppressive agent for the treatment of patients with renal transplantations. Data on toxicity, contra-indications, interactions and dosing advice are available and based on data collected in patients with renal transplantations (24). Some of the most frequently (>10%) occurring side-effects are thrombocytopenia and anemia, hypertension, electrolyte disorders, urinary tract infections, hypercholesterolemia, hyperglycemia and abdominal pain. Less frequent, but more severe side-effects are sepsis, deep venous thrombosis and skin cancer (Table 1).

This drug has not yet been evaluated in patients with FAP. In this pilot study (phase II), the possible polyp reducing effect of sirolimus will be investigated in a small number of patients with FAP, who suffer from severe intestinal polyposis. Patients who are expected to undergo rectal or pouch-related surgery on a short- term base (to avoid the development of cancer), will be selected and invited for study participation. Both rectal and pouch surgery are invasive and associated with a significant morbidity and mortality and neither does it prevent the development of adenomas in the remaining intestinal tissue as previously described. Therefore these patients could potentially benefit from a new and less invasive FAP related drug therapy to postpone, or eventually even prevent, surgery.

The aim of our study is to investigate the effect of sirolimus on the progression of intestinal adenomas in patients with FAP and to assess the safety of this treatment.

2. OBJECTIVES

Primary objective:

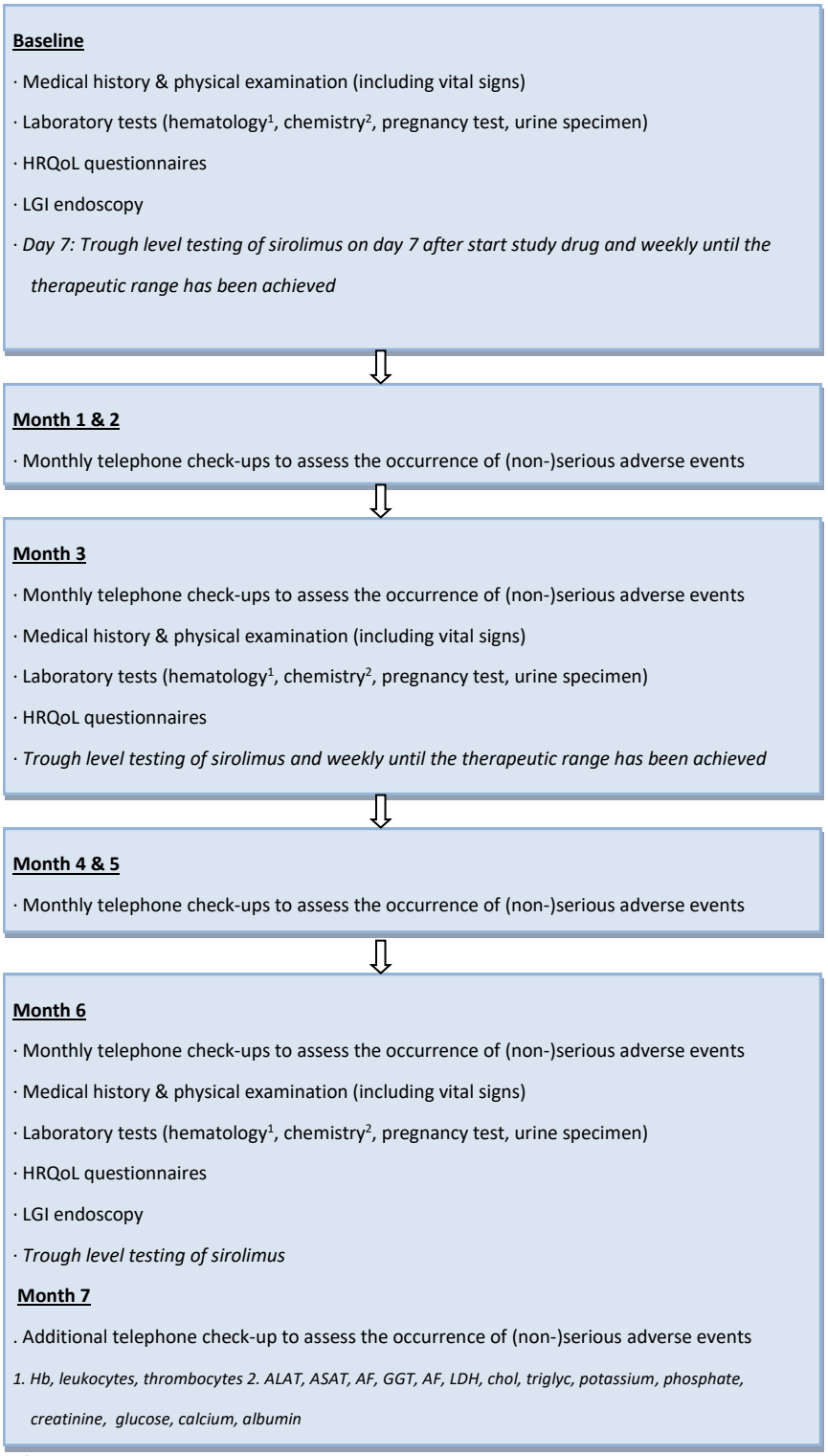
- To evaluate the effect of sirolimus on the size of 5 marked polyps in 5 patients with FAP and a large intestinal polyp burden
- To assess safety outcomes by summary analysis of adverse events, clinical laboratory abnormalities and regular physical examination.

Secondary Objectives:

- To evaluate the effect on number of polyps
- To evaluate the effect on global polyp burden (see definition in chapter 7.2)
- To evaluate the effect on histology (tubular, tubulovillous or villous histology and degree of dysplasia)
- To evaluate patient reported quality of life using health related quality of life (HRQoL) questionnaires (EORTC QLQ-C30, SF-36, CR29 and EQ-5D-5L)
- To measure the rate of cell proliferation in healthy intestinal mucosa and adenomatous tissue
- To assess immunohistochemistry of mTOR targets (such as eEF2 kinase, phospho-S6) in healthy intestinal mucosa and adenomatous tissue

3. STUDY DESIGN

This is a prospective phase II pilot study that will take place at the Academic Medical Center (AMC). Five patients with FAP will be invited for study participation. The total study duration will be 6 months. At baseline and at 6 months follow-up all included patients will undergo a lower gastrointestinal (LGI) endoscopy. Safety outcomes will be assessed by laboratory tests, physical examinations and telephone check-ups. Sirolimus trough levels will be measured regularly and if needed dosing adjustments will be made.



4. STUDY POPULATION

4.1 Population (base)

Five patients with FAP will be selected and invited for study participation. These patients must have severe rectal or pouch polyposis for which surgery is expected within a few years. These patients are selected from our large cohort of patients with FAP (n≈200). Selection will not be based on sex and age.

4.2 Inclusion criteria

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

- ≥ 18 years

-A genetically confirmed APC mutation

-Classical FAP phenotype (100-1000 colorectal adenomatous polyps)

-Subtotal colectomy with ileorectal anastomosis (IRA) or total colectomy with ileo-anal pouch anastomosis (IPAA)

-Severe rectal or pouch polyposis, defined as having >25 polyps amenable to complete removal (InSiGHT 2011 Staging System score of 3, see below)

-Fertile patients must use effective contraception during study treatment and until 12 weeks after study treatment

InSiGHT 2011 Staging System (InSiGHT Meeting 2011, San Antonio, TX)

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

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

<u>General exclusion criteria:</u>

-Inability to give informed consent

-Participation in another interventional clinical trial

Medical history:

-Subjects who are pregnant or breast-feeding, proved with a negative pregnancy test if female of child-bearing potential

-Prior pelvic irradiation

-Invasive malignancy in the past 5 years

-Subjects who are HIV positive

-Subjects with severe systemic infections, current or within 2 weeks prior to study start

-Subjects with known severe restrictive or obstructive pulmonary disorders

-Known sucrase insufficiency, isomaltase insufficiency, fructose intolerance, glucose malabsorption, galactose malabsorption, galactose intolerance or Lapp-lactase deficiency

-History of pulmonary embolism or deep venous thrombosis

-Major surgery less than or equal to 2 weeks prior to enrollment or any planned surgery within treatment period

-Active post-operative complication, e.g. infection, delayed wound healing

Co-medication:

-History of hypersensitivity to sirolimus or to drugs of similar chemical classes

-Regular NSAID use (defined as more than twice a week for 4 consecutive weeks) within 3 months prior to baseline

-Use of other FAP directed drug therapies (accepted if discontinued 3 months prior to start of the study)

-Subjects requiring systemic anticoagulation

-Co-medication that could interact with sirolimus

Lab results:

-Abnormal laboratory results (assessed within 14 days prior to start of study drug):

-Significant abnormalities in hepatic function

-ALAT, ASAT, GGT, AF, LDH: > 1.5 times ULN

-Significant hematologic abnormalities

-Hb: <7.0 mmol/L

-Thrombocytes: <100 10E9/L

-Leukocytes: <4.0 10E9/L

-Increased fasting serum cholesterol or triglyceride (whether or not on lipid-lowering therapy)

-Serum cholesterol: >7.8 mmol/L

-Serum triglycerides: >4.5 mmol/L

-Increased glucose (venous, fasting): >6.4 mmol/L

-Electrolyte abnormalities

-Total serum calcium (corrected for albumin): <2.0 mmol/L

-Potassium: <3.0 mmol/L

-Phosphate: <1.3 mmol/L

-Calculated glomerular filtration rate (GFR) less than 40 mL/min/1.73m2 using the simplified Modification of Diet in Renal Disease (MDRD) formula

-Urine specimen:

-Spot urine protein to creatinine ratio (UPr/Cr) greater than or equal to 0.5

4.4 Sample size calculation

As this is a pilot study that has not been performed in humans previously, no sample size calculation is being done. We believe a number of 5 patients is sufficient for the explorative purpose of our study.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Participants will be given sirolimus tablets. The starting dose is 2 mg once daily which will be given in 1mg tablets. On day 7 the first trough level is measured (using the LC-MS/MS method) and if not within the target range of 5-8ng/ml, dosing adjustments are made. In case of dosing adjustments, the next trough level is measured seven days later and this is repeated weekly until the target range is achieved. In case trough levels are within the target range, the next trough level measurement is at month 3, after which dosing adjustments are made if necessary, and at month 6. The maximum daily dose is 40mg. No placebo is given.

We estimate that a total number of 2000 tablets of 1mg are needed: 2 tablets a day for 6 months for 5 patients resulting in approximately 1800 tablets, plus 200 additional tablets in case of loss or if dosing adjustments are needed.

5.2 Use of co-intervention

Female participants who are sexually active must use adequate contraception as confirmed by three monthly pregnancy tests and male patients need to use adequate contraception as well. Adequate contraception needs to be continued until 12 weeks after therapy. Furthermore, any new concurrent medication that is started during the study needs to be reported. If known to have an interaction with sirolimus, an additional trough level measurement needs to be done. Patients will visit the AMC at baseline, month 3 and month 6 for the interventions as mentioned in chapter 1 ‘Study design’ (physical examinations, laboratory tests, pregnancy test, urine specimen , questionnaires) and weekly for sirolimus trough level testing until the target range has been achieved. Moreover, monthly telephone check-ups will be performed.

Endoscopies that take place at baseline and at 6 months follow-up are not considered co-interventions, as these are performed in a frequency that is consistent with our practice guidelines. Tattoos that are placed during these procedures are considered additional interventions and biopsies that are done can partly be considered as additional interventions, as some of these would have been done in routine care: 5 biopsies of normal mucosa are additional, 5 biopsies of adenomatous tissue are part of routine care.

5.3 Escape medication

In case of pain due to any cause, the use of paracetamol is recommended instead of NSAIDS.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product

The investigational product is sirolimus. Please see the SPC text of sirolimus for further information.

6.2 Summary of findings from non-clinical studies

Please see the SPC text of sirolimus. The text is based on an indication (immunotherapy in patients who received a renal transplantation) that is different than the indication we will investigate. This drug has not yet been tested in patients with FAP. Our hypothesis of the polyp reducing effect of sirolimus is based on mouse model studies. As described in the introduction, recently it was shown that the mTOR inhibitor sirolimus can cause tumour cells to undergo growth arrest and differentiation in APC-deficient mice (14). Signalling through the mTOR pathway is necessary for epithelial cell proliferation and tumour growth (15, 16). It was shown that mTOR complex 1 (mTORC1) activity is required for the proliferation of APC-deficient enterocytes. This complex mediates inhibition of eEF2 kinase, which is needed for the proliferation of APC-deficient cells. Sirolimus targets eEF2 resulting in growth arrest and differentiation of tumour cells. The effect of sirolimus on colonic adenoma regression was confirmed in other mice studies, with one of them showing beneficial effects in the small bowel (17-22). Sirolimus seems to inhibit polyp formation and could even lead to regression of polyps (21, 23). Moreover, it seems to increase survival and time to progression to dysplasia (20, 21).

6.3 Summary of findings from clinical studies

Please see the SPC text of sirolimus. This drug has not yet been tested in patients with FAP. Therefore findings from clinical human studies investigating the effect of sirolimus for our hypothesized indication cannot be reported. Since February 2014 an American center has been recruiting 15 participants for a pilot study aiming to investigate if mTor pathway activation decreases in patients with surgically-resectable desmoid tumors which are removed following pre-operative treatment with sirolimus. That study is being conducted in children and young adults (up to 29 years), which includes patients with FAP. Results are expected in 2016 (25).

6.4 Summary of known and potential risks and benefits

Please see the SPC text of sirolimus. Some of the most frequently (>10%) occurring side-effects are thrombocytopenia and anemia, hypertension, electrolyte disorders, urinary tract infections,

hypercholesterolemia, hyperglycemia and abdominal pain. Some of the less frequent, but more severe side-effects are sepsis, deep venous thrombosis and skin cancer and self-limiting oral stomatitis. All known side-effects can be found in the Table 1. The incidence of several of these side-effects can increase by an increasing trough level of sirolimus. By applying a relatively low dosing range and frequent trough level testing, we believe these risks have been minimized in our study.

Side-effects of sirolimus as reported in the SPC text apply to patients with a renal transplantation. Most of these side-effects were reported in patients who used sirolimus in combination with other immunosuppressive drugs. We exclude patients who use other immunosuppressive therapy. Moreover, compared to patients with FAP, patients with a renal transplantation more frequently have (severe) comorbidities and additionally, sirolimus will be given for a short amount of time (6 months). Therefore, side-effects of sirolimus might be less frequently found in our participants.

We will use tattoos with SPOT^{TM} for marking the polyp sites, which has been used for many years in colonoscopy practice (26). No side-effects of SPOT have been described in literature. Furthermore, colonic biopsies are accompanied by a minimal risk of perforation, bleeding and infection. These complications have been described in some case reports after hot biopsy but mostly in patients with pre-existent ulcerative colitis or infectious colitis. Bleeding after hot biopsy has been reported to occur in up to 0.26% and perforation in up to 0.01% after hot biopsy. During our study only cold biopsies will be obtained. Only case reports have reported on complications after cold biopsy (27, 28). These potential complications are cited in the written patient information.

As described in chapter 6.2, a significant polyp reducing effect of sirolimus is to be expected. The patients that will be included, are likely to undergo a subtotal colectomy with ileoanal pouch anastomosis or an end ileostomy on a short term base due to the severity of polyposis. These invasive types of surgery are accompanied with a significant risk of comorbidities (29). Even after surgery, patients will develop polyps in the remaining intestinal tissue and therefore surveillance sigmoidoscopies are needed to prevent the development of cancer (5, 30-32). There is currently no alternative treatment available for these patients. Therefore, a medical treatment to postpone or even prevent surgery could be beneficial. If a polyp reducing effect is shown, this therapy could possibly also be beneficial in postponing a preventive colectomy in patients with FAP who have their colon in situ and in postponing a duodenectomy in patients with severe duodenal polyposis.

Table 1. Side-effects of sirolimus (SPC)

System/organ	Very frequent (≥1/10)	Frequent (≥1/100 to <1/10)	Less frequent (≥1/1000 to <1/100)	Seldom (≥1/10.000 to <
Infections	Pneumonia Fungal infection Viral infection Bacterial infection Herpes simplex infection Urinary tract infection	Sepsis Pyelonephritis CMV infection Herpes zoster infection	Clostridium difficile colitis Mycobacterial infection (incuding tuberculosis) EBV viral infection	
Neoplasms, benign, malignant and non-specified (including cysts and polyps)		Skin cancer	Lymphoma/ post-transplantation lymphoproliferative conditions	
Blood and lymphatic disorders	Thrombocytopenia Anemia Leukopenia	Hemolytic-uremic syndrome Neutropenia	Pancytopenia Thrombotic thrombocytopenic purpura	
Hepatic and biliary disorders			Hepatic failure	
Immune system disorders		Hypersensitivity disorders such as anaphylactic/anaphylactoid reactions, angio-edema		
Feeding and metabolic disorders	Hypokalemia Hypophosphatemia Hyperlipidemia (such as Hypercholesterolemia) Hyperglycemia Hypertriglyceridemia Diabetes mellitus			
Nervous system disorders*	Headache			
Cardiac disorders	Tachycardia	Pericard effusion		
Vascular disorders	Lymphocele Hypertension	Venous thrombosis (such as deep venous thrombosis)	Lymphedema	
Respiratory, chest and mediastinal disorders		Pulmonary embolism Pneumonitis Pleural effusion Epistaxis	Pulmonary hemorrhage	Alveolar protei
Gastrointestinal disorders	Abdominal pain Diarrhea Constipation Nausea	Pancreatitis Stomatitis Ascites		
Cutaneous and subcutaneous disorders	Rash Acne		Expholiative dermatitis	Hypersensitive
Musculoskelatal and connective tissue disorders	Arthralgia	Osteonecrosis		
Renal and urinary system disorders	Proteinuria		Nephrotic syndrome Focal segmental glomerulosclerosis	

System/organ	Very frequent (≥1/10)	Frequent (≥1/100 to <1/10)	Less frequent (≥1/1000 to <1/100)	Seldom (≥1/10.000 to <
Reproductive system disorders and breast disorders	Menstruation disorders (including amenorrhea, menorrhagia	Ovarial cysts		
General disrders and disorders at administration site	Edema Peripheral edema Pyrexia Pain Disturbed wound healing	Disturbed wound healing Edema		
Laboratory testing disorders	Increased LDH Increased creatinin Increased liver function tests (such as increased ASAT and ALAT)			

* Side-effect of unknown frequency: posterior reversible encephalopathy syndrome

6.5 Description and justification of route of administration and dosage

In patients with a renal transplantation, the starting dose is 6 mg orally and then 2 mg once daily. After a week a first trough level assessment is done. The aim in these patients is to obtain a trough level of 12-20 ng/ml in full blood (24). The current practice in the AMC is a target dose of 5-8 ng/ml for patients with a renal transplantation as this results in less adverse events compared to a trough level of 12-20 ng/ml but still results in a clinical effect. As safety and efficacy of this drug has not yet been assessed in patients with FAP, the target range in this study is therefore set at 5-8 ng/ml as recommended by an AMC renal transplantation expert. The starting dose is 2 mg once daily. On day 7 the first trough level is measured and if not within the target range, dosing adjustments are made. In case of dosing adjustments, the next trough level is measured seven days later and this is repeated weekly until the target range is achieved. In case trough levels are within the target range, the next trough level measurement is at month 3, after which dosing adjustments are made if needed, and at month 6. The maximum daily dose is 40 mg.

6.6 Dosages, dosage modifications and method of administration

See chapter 6.5

6.7 Preparation and labelling of Investigational Medicinal Product

Sirolimus will be provided free of charge by Pfizer. Labelling of sirolimus will be done by the AMC Hospital Pharmacy according to GCP guidelines (GCP and GMP-annex 13). The registered product Sirolimus will be used, meaning that the accessory label text of the producer will be used. This will be added with another label that indicates: name of the study, name of the principal investigator, name of the sponsor (AMC, Meibergdreef 9, 1105 AZ Amsterdam, tel 020-5669111),

"uitsluitend ten behoeve van klinisch onderzoek", "Buiten bereik van kinderen bewaren", "Gebruiken volgens voorschrift studieprotocol", patient number, patient name.

6.8 Drug accountability

As described in chapter 6.7, sirolimus will be delivered at the AMC Hospital Pharmacy by Pfizer. Sirolimus will be stored and dispensed on a patient named basis by the AMC Hospital Pharmacy and drug accountability of receipt, dispensed, returned and destroyed medication will be kept conform GCP guidelines.

7. METHODS

7.1 Study parameters/endpoints

7.1.1. Main study parameter/endpoint

- Size of 5 marked polyps in 5 patients with FAP and a large intestinal polyp burden
- Safety outcomes reported by summary analysis of adverse events , clinical laboratory abnormalities and regular physical examination

7.1.2. Secondary study parameters/endpoints (if applicable)

- Number of polyps
- Global polyp burden (see definition in chapter 7.2)
- Histology (tubular, tubulovillous or villous histology and degree of dysplasia)
- Patient reported quality of life using HRQoL questionnaires
- Rate of cell proliferation in healthy intestinal mucosa and adenomatous tissue
- Immunohistochemistry of mTOR targets (such as eEF2 kinase, phospho-S6) in healthy intestinal mucosa and adenomatous tissue

7.2 Study procedures

Baseline and follow-up measurements and monitoring

-Medical history and physical examination (including vital signs) at baseline and at three monthly visits

-Lab testing at baseline and at three monthly visits: pregnancy test, urine specimen, hemoglobin, thrombocytes, cholesterol, triglycerides, glucose, potassium, phosphate, calcium, LDH, ALAT, ASAT, GGT, AF, leukocytes, creatinine, albumin.

-Three monthly HRQoL questionnaires

-Trough level testing of sirolimus (using the LC-MS/MS method) on day 7 after start study drug and weekly until the therapeutic range has been achieved, after which the next trough level will be measured at three months

-Monthly telephone check-ups to assess the occurrence of (non-)serious adverse events

-LGI endoscopies at baseline and at six months. Endoscopic procedures will be performed after bowel preparation with sedation according to the standard of care by experienced endoscopists.

Baseline LGI endoscopy

A video record will be made of the endoscopic examination, which will be conducted in a spiral fashion. Disease stage will be assessed according to the IPSS stage. Polyps >10mm and other relevant polyps can be managed at the discretion of the endoscopist. In all patients, 5 polyps between 3-10 mm will be photographed (with an open biopsy forceps visible), measured (using the open biopsy forceps), and marked using tattoo dye (using SPOT^{TM} 1 cm from the polyp at 9 o'clock, without NaCL and a maximum of 0.5cc SPOT^{TM}), photographed with ink marking, and 5 biopsies of normal mucosa and one biopsy of 5 other adenomatous polyps (other than those marked for the primary outcome measure) will be taken in order to evaluate histology and to measure the rate of cell proliferation and immunohistochemistry. The 5 marked polyps need to have an endoscopic adenomatous appearance, as sirolimus is expected to cause regression of adenomas (and not hyperplastic lesions) (14, 17). There is no evidence concerning the minimum or maximum size of adenomas that could be influenced by sirolimus. We chose not to include adenomas >10mm as we would usually resect them due to an increased risk of developing cancer (33). We chose a minimum size of 3mm as it is our experience that these patients tend to have many small (1-2mm) polyps and by choosing to mark these small polyps, it might be difficult to recognize the marked polyp amongst the adjacent small polyps.

Follow-up LGI endoscopy:

A video record will be made of the endoscopic examination, which will be conducted in a spiral fashion. Disease stage will be assessed according to the IPSS stage. The follow-up LGI endoscopy will be conducted in a similar fashion to the baseline examination. All marked polyps will be photographed and measured. Five biopsies of normal mucosa and biopsies of 5 adenomatous polyps (other than those marked for the primary outcome measure) will be taken in order to evaluate histology and to measure the rate of cell proliferation and immunohistochemistry.

At the end of the study, histology of adenomatous polyps will be reviewed by an expert GE pathologist.

The above endoscopies are part of routine care. However, extra biopsies that are taken and the tattoos are study related.

Assessment of degree of polyposis

For the primary outcome measure, the size of polyps will be measured as follows:

1. Size of polyps:

The polyp-size is estimated by the endoscopist using the open biopsy forceps (the most reliable method currently available). Two independent reviewers will also assess polyp size of marked polyps at the LGI endoscopy by viewing matching still images per patient on dual monitors. They are blinded for timing of endoscopy (baseline or follow-up). The mean size per polyp is calculated from the assessments of the three reviewers. If the assessment of the reviewers differs by more than 3mm from the assessment of the endoscopist, consensus is needed.

For the secondary outcome measures, the degree of polyposis will be measured as follows:

2. Number of polyps:

During endoscopy, the number of polyps is estimated by the endoscopist. At a later phase, two independent reviewers will also assess the number of polyps seen (categorized per 10 polyps) by viewing both videos (before and after treatment) per patient, blinded for the order. The mean number of polyps is calculated as a mean of all 3 assessments. If the assessment between reviewers differs by more than 10 polyps from the assessment of the endoscopist, consensus is needed.

3. Global polyp burden:

During endoscopy, the global polyp burden is estimated by the endoscopist. At a later phase, two independent reviewers will also globally assess polyp burden by viewing both videos per patient, blinded for order. The second video in the pair could take the value of −2 (much better), −1 (better), 0 (same), 1 (worse) or 2 (much worse) relative to the first video. Mean scores are calculated for each subject and averaged for the three reviewers. If the assessment of the reviewers differs by more than 1 point from the assessment of the endoscopist, consensus is needed.

7.3 Withdrawal of individual subjects

If they wish to do so, subjects can leave the study at any time for any reason without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

7.3.1 Specific criteria for withdrawal

Participants will be withdrawn from treatment under the following circumstances:
-If any of the exclusion criteria apply during the study

7.4 Replacement of individual subjects after withdrawal

If participants are withdrawn from the study due to any reason they will not be replaced.

7.5 Follow-up of subjects withdrawn from treatment

All participants that have been using sirolimus for at least 1 month and who are withdrawn for any reason, will undergo another sigmoidoscopy within 6 weeks after withdrawal. If patients withdraw due to side-effects of sirolimus, these patients will be contacted by telephone weekly until symptoms have disappeared.

7.6 Premature termination of the study

There are no criteria for early termination, as sirolimus has been investigated previously and therefore unknown adverse events (resulting in premature termination of the study) are not to be expected.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Subjects will be instructed to contact the investigator to report any symptom and the investigator will question each subject regarding symptoms at the time of each contact moment (at least monthly). All adverse events, including duration and severity, will be captured in the case report forms.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

8.5 Safety Committee

All investigators involved in this study (prof. dr. E. Dekker, Prof. dr. G.R. van den Brink, Dr. F. Bemelman and a PhD candidate) are the safety committee. As previously described, safety of this treatment will be monitored by performing regular lab and trough level testing, physical examinations and monthly telephone contacts. All serious and non-serious adverse events that are collected by the PhD candidate will immediately be reported to the safety committee. The

safety committee will decide whether it is a serious or non-serious adverse event, the toxicity grade of this event, the relatedness to the study drug and subsequent measures to be taken. This safety committee is needed as sirolimus has not been investigated previously for the indication as being used in this study.

9. STATISTICAL ANALYSIS

Descriptive statistics will be used to describe the study population, characteristics and changes in outcome measures during follow-up. SPSS for Windows software (Chicago, IL, USA) version 21.0. will be used for these analysis.

9.1 Primary study parameter(s)

Descriptive statistics will be used to describe the changes in outcome measures during follow-up.

9.2 Secondary study parameter(s)

Descriptive statistics will be used to describe the changes in outcome measures during follow-up.

9.3 Interim analysis (if applicable)

Not applicable.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (18th WMA General Assembly, Helsinki, Finland, June 1964), which was amended by the 64th WMA General Assembly, October 2013, Fortaleza, Brazil) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The protocol of this study will be submitted to the Medical Ethical Committee of the Academic Medical Center and will not start before formal approval has been granted. All patients are required to sign an informed consent form. The study will also be conducted in accordance with ICH Good Clinical Practice (GCP) and all applicable subject privacy requirements.

10.2 Recruitment and consent

Participants will be given oral and written explanation about the study, before they give written informed consent. Participants will be given as much time as possible to make a decision. Subjects are allowed to withdraw informed consent at any time for any reason if they wish to do so without providing arguments. The results will be published in a peer-reviewed scientific journal. Subjects will be approached for participation by the investigator or colleagues. This invitation could take place at the outpatient clinic or during telephonic contact. Participants will not be compensated for their travel costs.

10.3 Compensation for injury

The sponsor/investigator has a liability insurance, which is in accordance with article 7 of the WMO. The sponsor also has an insurance, which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study:

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.4 Incentives (if applicable)

Participants will not receive any reimbursements.

ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.5 Handling and storage of data and documents

Endoscopically collected material of adenomatous tissue, as part of routine care, will be stored according to regulations of the department of pathology in the AMC. Slides of this adenomatous tissue, used for the assessment of immunohistochemistry and cell proliferation rates, and endoscopically collected material of healthy intestinal mucosa, will be analysed and stored at the Tytgat institute. Source documents and CRFs will be stored by the project leader for 15 years after closure of the trial. Data of the subjects will be coded in order of participation. The code and the data are stored in different locations. The code can only be seen by the investigators. Qualified authorities can get insight in the code and data, but only when accompanied by the investigators. Informed consent forms are kept in separate files, to ensure the data security. The handling of personal data will comply with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, Wbp).

10.6 Monitoring and Quality Assurance

Not applicable.

10.7 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

10.8 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

10.9 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

10.10 Public disclosure and publication policy

This study will be executed by the following team: prof. dr. E. Dekker (gastroenterologist), prof. dr. G.R. van den Brink (gastroenterologist), dr. F. Bemelman (nephrologist), dr. L. Koens (pathologist) , a PhD candidate and a research nurse.

11. STRUCTURED RISK ANALYSIS

11.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Recently it was shown that the mTOR inhibitor sirolimus can cause tumour cells to undergo growth arrest and differentiation in APC-deficient mice (14). Signalling through the mTOR pathway is necessary for epithelial cell proliferation and tumour growth (15, 16). It was shown that mTOR complex 1 (mTORC1) activity is required for the proliferation of APC-deficient enterocytes. This complex mediates inhibition of eEF2 kinase, which is needed for the proliferation of APC-deficient cells. Sirolimus targets eEF2 resulting in growth arrest and differentiation of tumour cells. The effect of sirolimus on colonic adenoma regression was confirmed in other mice studies, with one of them showing beneficial effects in the small bowel (17-22). Sirolimus seems to inhibit polyp formation and could even lead to regression of polyps (21, 23). Moreover, it seems to increase survival and time to progression to dysplasia (20, 21).

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

As previous described, sirolimus is a registered drug for patients who have undergone a renal transplantation. Details about the exposure of human beings with sirolimus are clearly described in the SPC text. Sirolimus has never been investigated in patients with FAP. However, since February 2014 an American center has been recruiting 15 participants for a pilot study aiming to investigate if mTor pathway activation decreases in patients with surgically-resectable desmoid tumors that are removed following pre-operative treatment with sirolimus. That study is being conducted in children and young adults (up to 29 years), which includes patients with FAP. Results are expected in 2016 (25).

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

As far as we know, sirolimus has never been investigated in ex-vivo human cell material of FAP patients. However, as written in chapter 12.1a the primary outcome and some of the secondary outcomes were found effective in mouse models.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

See the SPC text and chapter 12.1a for explanations of working mechanisms. Known side-effects can be found in chapter 6.2 and all other drugs that could interact with sirolimus can be found in Table 1.

e. Analysis of potential effect

See chapter 6.2 for side-effects of sirolimus. The incidence of side-effects increases at a rising trough level of sirolimus. The dosing regimen as nationally recommended for patients with a renal transplantation reaches blood level concentrations sufficient to achieve a clinical result. As our proposed dosing regimen is similar to that but on the lower side, we believe effect on the intestinal mucosa can be expected with a limited risk of side-effects. No data from human studies is available on the required level of sirolimus in full blood to achieve intestinal effects. See chapter 6.4 for further risks and benefits.

f. Pharmacokinetic considerations

See the SPC text for pharmacokinetics of sirolimus.

g. Study population

The study population consists of patients with FAP, which is a potentially life threatening disease. The patients that will be included are likely to undergo a subtotal colectomy with ileoanal pouch anastomosis or an end ileostomy on a short term due to the severe degree of polyposis. These invasive types of surgery are accompanied with a significant risk of comorbidities (29). Even after surgery, patients will develop polyps in the remaining intestinal tissue and therefore surveillance sigmoidoscopies are needed to prevent the development of cancer (5, 30-32). There is currently no alternative treatment available for these patients. Therefore, a medical treatment to postpone or even prevent surgery could be beneficial. If a polyp reducing effect is shown in the intestinal tissue, this therapy could possibly also be beneficial in postponing a preventive colectomy in patients with FAP who have their colon in situ and in postponing a duodenectomy in patients with severe duodenal polyposis.

h. Interaction with other products

See the SPC text for interactions of sirolimus with other products. To avoid these interactions, the use of any of these products is not allowed when enrolling in this study (see exclusion criteria). If any of these products are started during the study, additional trough level testing will be done or patients will be withdrawn from this study (depending on the expected interaction).

i. Predictability of effect

The combination of endoscopic measurements and laboratory measurements will provide relevant data, both clinical and translational, that will help answering the research question. If no effects on polyp size are seen, laboratory measurements will provide us with useful translational information. If, for example, an effect is seen in cell proliferation or mTOR targets and the chosen dosing regimen is safe, a future study could evaluate the effect of a higher dose for a longer period of time.

j. Can effects be managed?

A renal transplantation physician, working at the AMC, is actively involved in this study. She is experienced in treating patients with sirolimus and therefore she will assist us if needed. Patients will be given contact details of the investigating team and in case of emergencies when the investigating team cannot be contacted, the patient can contact the gastroenterologist that is on call.

11.2 Synthesis

To reduce the risk of side-effects related to sirolimus, a relatively low trough level will be managed. Furthermore, regular telephonic contact, trough level testing, laboratory testing and physical examination will lead to an early detection of any side-effects. We believe this could help patients, who would otherwise need surgery on a short term. As of today no strict contraindications exist for the use sirolimus. However, we exclude patients using medications that could interact with sirolimus. Furthermore, patients with specific laboratory abnormalities as well as patients with specific conditions (such as a deep venous thrombosis or sucrose insufficiency) that could deteriorate due to the use of sirolimus or that could influence the effect of sirolimus are excluded.

We will use tattoos with SPOT™ for marking the polyp sites, which has been used for many years in colonoscopy practice (26). No side-effects of SPOT have been described in the literature. Furthermore, colonic biopsies are accompanied by a minimal risk of perforation, bleeding and infection. These complications have been described in some case reports after hot biopsy but mostly in patients with pre-existent ulcerative colitis or infectious colitis. Bleeding after hot biopsy has been reported to occur in up to 0.26% and perforation in up to 0.01% after hot biopsy. During our study only cold biopsies will be obtained. Only case reports have reported on complications after cold biopsy (27, 28). These complications are cited in the written patient information.

In our opinion, treatment with sirolimus could help patients, who would otherwise need surgery on a short term base associated with a significant risk of morbidity and mortality. By applying the above risk management, we believe this treatment is justified. Results of this pilot study could help in creating a future trial assessing the effect of sirolimus in patients with FAP.

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