RESEARCH PROTOCOL

‘The chemopreventive effect of Lithium on adenoma development in patients with familial adenomatous polyposis (FAP); a pilot study’

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PROTOCOL TITLE 'The chemopreventive effect of Lithium on adenoma development in patients with familial adenomatous polyposis (FAP); a pilot study'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
AMC	Amsterdam UMC, location AMC
AR	Adverse Reaction
APC	Adenomatous Polypolis Coli
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CEMM	Centre for Experimental and Molecular Medicine
CRC	Colorectal Cancer
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
FAP	Familial adenomatous polyposis
FDA	The Food and Drug Administration
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
HRQoL	Health Related Quality of Life
IB	Investigator's Brochure
IC	Informed Consent
ISC	Intestinal Stem Cell
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
LGI	Lower Gastrointestinal
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
NSAID's	Non-Steroidal Anti-Inflammatory Drugs
(S)AE	(Serious) Adverse Event

SPC	Summary of Product Characteristics; in Dutch: samenvatting van de productkenmerken
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
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen
WT	Wilde Type

SUMMARY

Rationale: Familial adenomatous polyposis (FAP) syndrome is characterized by the development of numerous colorectal polyps. If left untreated, these patients have a chance of nearly 100% of developing colorectal cancer (CRC) at a young age. Therefore, guidelines recommend a prophylactic colectomy during early adulthood. Even after colectomy, most patients will develop adenomas in the retained rectum or ileoanal pouch requiring further endoscopic surveillance. In a recent study in mouse models, a chemopreventive effect of Lithium was observed on the spread of *Apc* mutated cells within the crypts of normal intestinal mucosa, suggesting polyp formation can be prevented. Lithium is used to treat patients with bipolar disorders but has never been investigated in patients with FAP aiming to reduce polyp burden. We hypothesize that Lithium could reduce the spread of *APC* mutated cells within the crypt of normal intestinal mucosa potentially reducing polyp burden in patients with FAP.

Objective: The aim of this study is to investigate the effect of low-dose Lithium on stem cell dynamics, the number and size of polyps and, to assess safety outcomes of this drug in FAP patients.

Study design: A prospective phase II, single arm pilot trial, with a duration of 18 months. The drug will be administered between month 6 and 12.

Study population: Twelve patients with FAP between the age of 18 and 35 not having undergone a colectomy (yet), having a genetically confirmed *APC* mutation and a family history with a classical FAP phenotype.

Intervention: All patients will be treated with Lithium with an oral dose of 300mg a day for six months, achieving a therapeutic serum level between 0.2-0.4 mmol/L.

Main study parameters/endpoints: The main outcome parameter is the effect of Lithium on the spread of *APC* mutant cells within intestinal crypts over time by using an *APC* specific marker *NOTUM* (a significance reduce of fixed crypts and reduction of fixed clone size of 50%).

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: A physical examination and an endoscopy with biopsies will be performed at baseline and every six months (four in total). Laboratory testing will be done at baseline and every two months during Lithium treatment. Patients will be interviewed by phone and Lithium side effect questionnaires will be obtained at baseline and during Lithium treatment. Lithium serum levels will be measured at day 12 and 22 after start of the study drug (at month 6). When the therapeutic range has been achieved, serum level testing will be done every month. Most relevant side-effects that could potential occur include polyuria, hyperparathyroidism and hypothyroidism. Most side effects are dose-dependent and will be

regularly monitored. Patients with FAP could potentially benefit from a chemopreventive therapy such as Lithium to postpone or even avoid invasive types of surgery.

1. INTRODUCTION AND RATIONALE

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder with a germline mutation in the tumor suppressor gene, Adenomatous Polyposis Coli (APC), with an estimated prevalence of 1 out of 5.000 – 7.000 births (1). Although FAP is a hereditary disease, a de novo mutation in the *APC* gene is responsible for approximately 25% of FAP cases (2). FAP is characterized by the early onset of hundreds to thousands of polyps in the colon and rectum. If left untreated, the risk of developing colorectal cancer (CRC) is nearly 100% (3). Since the development of adenomatous polyps to CRC takes fifteen to twenty years, most untreated patients with FAP will develop CRC between the age of 35 and 45 (1).To prevent CRC, guidelines suggest prophylactic surgery in these patients dependent on polyp burden, personal preference and family history (4). These invasive surgeries are mostly performed in the twenties and are known for the risk of postoperative comorbidities (5, 6). Even after surgery, new polyp formation still occurs in the remaining part of the intestine, resulting in further need of endoscopic surveillance and potential interventions (7, 8). Therefore, patients with FAP could benefit from therapy preventing polyp formation resulting in postponing or even avoiding prophylactic surgery. Up to date, several studies on (chemo)preventive therapy for FAP patients were conducted but due to lack of a significant effect and adverse events (AE's), no chemopreventive therapy for patients with FAP is routinely used in daily practice (9-11).

A recent study showed a promising chemopreventive effect of Lithiumchloride in *Apc*-mutant mouse models (12). *APC* is a critical component of the Wnt signaling pathway, an essential signaling route involved in the maintenance of intestinal stem cells (ISCs). Loss of *APC* function results in unrestrained activation of the Wnt signaling pathway which can lead to the development of premalignant adenomas (13). Given the time it takes to develop CRC and the short lifespan of most intestinal cell types, it is generally assumed that cancer is initiated in the long-lived ISC compartment (14). Previous work demonstrated that ISCs that have acquired an *Apc* mutation have a competitive benefit over wild type (WT) ISCs (15). In addition, recent work has revealed that *Apc* mutant ISCs act as supercompetitors, that actively disadvantage WT ISCs. This supercompetitive advantage is due to the expression of of Wnt antagonists (e.g. *Notum, Wif1*, *Dkk2*) by *Apc* mutants to which they themselves are insensitive, but result in active replacement of WT ISCs that are heavily dependent on Wnt signaling. When all WT ISCs are replaced, the mutant clone is fixed within the crypt and will initiate adenoma formation (12). Intervening before fixation of the mutant clones within a crypt can act as a therapeutic window. In a recent study, van Neerven et al. discovered a benefit of the use of Lithiumchloride in mouse models based on *Apc*-inactivation (12). This study could trace mutant ISCs by labeling these cells for Wnt antagonist *Notum*, and analyzing their spread through the crypts over time. Their competitive benefit was determined by quantifying the distribution of *Notum* clones in crypt bottoms over time. In addition, this study revealed that boosting the Wnt pathway in WT ISCs using lithiumchloride rendered them resistant to the Wnt antagonists secreted by *Apc*-mutant cells. As a result,

lithiumchloride administration diminished the competitive advantage of *Apc* mutants, thereby reducing crypt fixation and thus preventing adenoma formation (12).

Until now, no clinical data on Lithium use in patients with FAP is available. The aim of this study is to investigate the effect of low-dose Lithiumcarbonate on stem cell dynamics and number of polyps and to assess safety outcomes of this drug in FAP patients.

2. OBJECTIVES

Primary Objective:

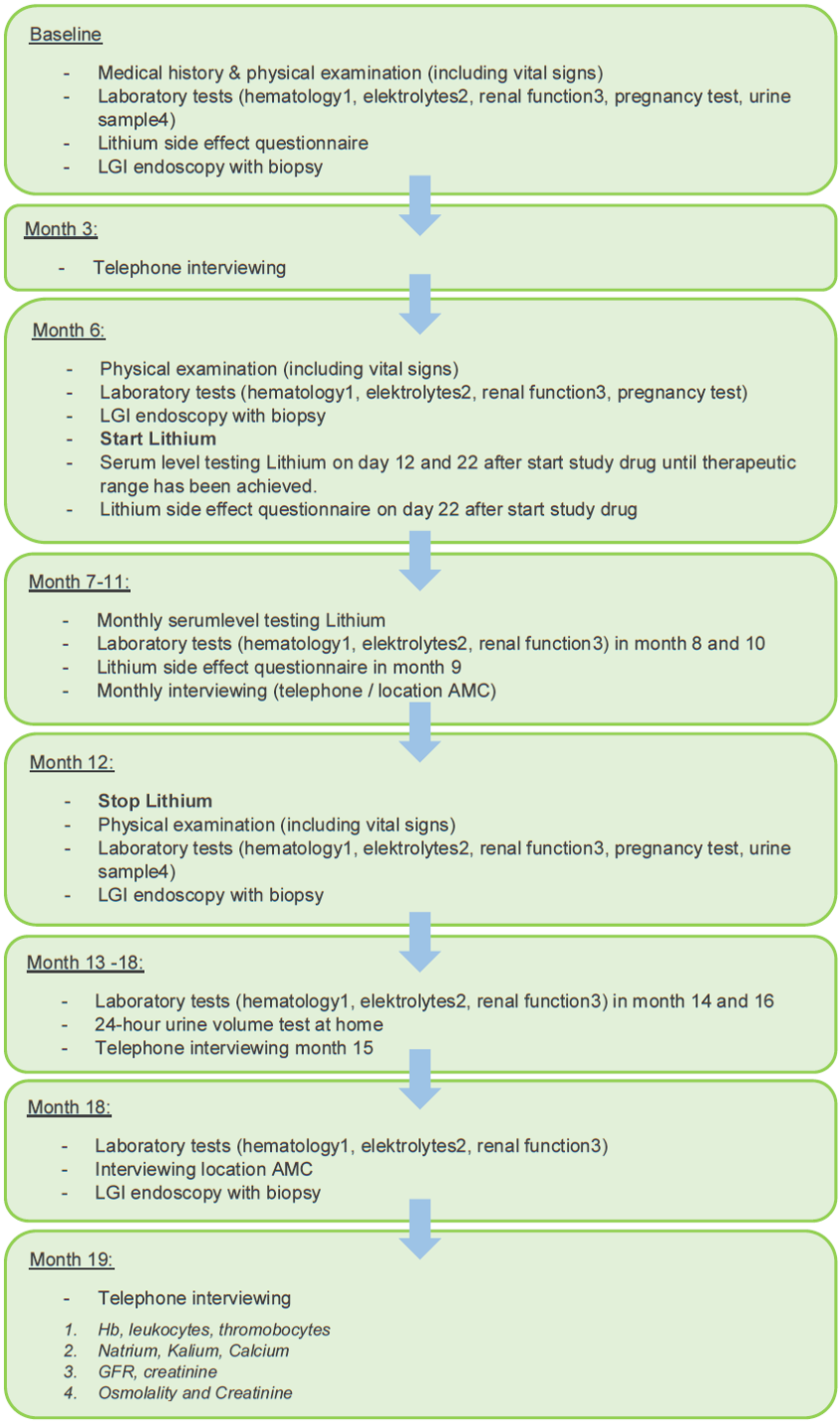
- To evaluate the effect of low-dose Lithiumcarbonate on the spread of *APC* mutant cells within intestinal crypts of FAP patients over time. This will be assessed by using an *APC* mutation specific marker (*NOTUM*) and is defined as a significant reduction of fixed crypts and reduction of fixed clone size by 50%.

Secondary Objective(s):

- To evaluate the effect on number of polyps and size, determined as sum of diameter of all polyps.
- To evaluate side effects of Lithiumcarbonate in FAP patients using Lithium side effects questionnaires;
- To assess the safety of low-dose Lithiumcarbonate use by evaluating adverse events, findings during physical examination and laboratory tests.

3. STUDY DESIGN

This study is a phase II, single arm pilot trial. Patients will be recruited at the Amsterdam UMC, location AMC (AMC). The duration of this study will be 18 months, and patients will undergo four colonoscopies in total (at t=0, t=6, t=12, t=18). During each colonoscopy biopsies from normal intestinal mucosa will be collected to determine stem cell dynamics. Safety outcomes will be monitored by regular physical examination, laboratory testing and telephone interviews. A safe therapeutic range will be maintained due to measuring serum levels of Lithiumcarbonate, and if necessary doses will be adjusted.



4. STUDY POPULATION

4.1 Population (base)

Patients (male and female) diagnosed with FAP, between the age of 18 and 35, not having undergone a colectomy (yet), are eligible for this study. We aim to include 12 patients for this pilot study, and patient recruitment will take place from the large cohort of FAP patients at the AMC. For this study no control group will be included, as the patients represent their own control in the non-treatment phase. To evaluate the effect of Lithiumcarbonate on the stem cell dynamics, regular biopsies of normal intestinal mucosa will be collected from each patient. International guidelines recommend (prophylactic) colectomy to be performed based on polyp burden, personal preference and family history, and this is most often performed when patients are in their twenties (4). Therefore, in the interest of studying the (chemo)preventive effect of Lithiumcarbonate in FAP patients, participants will be included up to an age of 35. In preparation of this study, a focus group meeting with 9 patients with FAP was organized to discuss the patients' perspective on this study. Patient representatives emphasize the major need of (preventive) therapy for FAP. We believe patients will be motivated to participate in a study aiming to delay polyp growth and consequently surgery (7, 8), and to be able to include twelve patients.

4.2 Inclusion criteria

Patients must meet all of the following criteria for inclusion in the study:

- Male or female between the age of 18 and 35 years;
- Confirmed *APC* germline mutation and one of the following:
    - Minimum of 100 colorectal adenomas
    - Minimum of 50 colorectal adenomas and a positive family history of a classical FAP phenotype (>100 colorectal adenomas);
- Intact colon;
- Participant is willing and able to give informed consent for participation;

4.3 Exclusion criteria

Patients that meets any of the following criteria will be excluded from participation in this study:

- Participation in any other clinical intervention study; observational trials accepted;
- Lithium use prior to participation of the study;
- Pregnancy, breast-feeding or no use of anticonception;
- No normal intestinal mucosa left for normal tissue biopsy;
- Indication for colectomy within 2 years;
- Known renal impairment, defined as GFR < 60 ml/min;
- Known severe cardiac disorder;
- Known severe brain injury;

- Hypothyroidism;
- Hyponatremia, defined as Na < 130mmol/L;
- Positive family history of Brugada syndrome
- Co-medication known for interacting with Lithium (please see Table 3).
- Regular NSAID use (defined as more than twice a week for 4 consecutive weeks) within 3 months prior to baseline;
- Use of immunosuppressive or anti-inflammatory drugs within 3 months prior to baseline;
- Use of any other FAP directed drug therapy within 3 months prior to baseline (use of any alternative supplements e.g. turmeric or fish-oil must be noted in questionnaire).

4.4 Sample size calculation

Since this study is not yet been performed in humans with FAP, this study will be a pilot study. We believe a number of 12 patients is sufficient for the explorative purpose of this study. By performing a paired analysis, the effect of variation between individuals will be reduced, and we expect sufficient power to draw significant results. To detect a reduction of 50% (power stat. 80%, p=0.05) in fitness of *APC*-mutant cells, we need to analyse sizes of *APC* mutant clones within 11 partially populated crypts. To ensure sufficient material we need 840 crypts per patient per time point. A typical biopsy yields 100-250 crypts, therefore we will perform 12 biopsies in total (2 biopsies per segment, 6 segments) per patient per time point. In that way, sufficient data will be obtained to confirm or refute the study hypothesis.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The investigational product of this study is Lithiumcarbonate. Lithiumcarbonate is a Gsk3β inhibitor, which stimulates intrinsic Wnt signalling. The therapeutic range of Lithium administered in patients suffering from bipolar disorder is 0.60 – 0.80 mmol/L (16). In the recent study of van Neerven et al., a chemopreventive effect of lithiumchloride on clonal fixation of *Apc*-mutant cells in mice was seen using a significantly lower serum level of 0.2 mmol/L (12). Toxic effects of lithiumcarbonate are mostly seen at serum levels 1.0mmol/L or higher (16-20). To limit adverse events and side-effects, we aim to use the lowest effective dose and for this reason we maintain a Lithiumcarbonate serum level of 0.20 – 0.40 mmol/L to analyse the (chemo)preventive effect of Lithiumcarbonate in patients with FAP. Serum levels of Lithiumcarbonate will be monitored at day 12 and day 22 after start of treatment (blood sample will be collected 12h after oral Lithiumcarbonate dose). When a therapeutic range is reached, serum levels will be monitored once a month. If necessary, adjustments will be made by the investigator based on serum levels, but also laboratory tests and side effects. As described in chapter 4, no control group will be included, thus no placebo or comparator will be analysed in this study, and participant serve as their own control in the non-treatment phase.

5.2 Use of co-intervention

Lithiumcarbonate passes the placenta and is associated with congenital malformations (21). Therefore, adequate anticonception must be used during participation of this study until 6 weeks after Lithiumcarbonate use. Lithiumcarbonate passes during lactation, therefore breastfeeding during participation of this study is also not allowed (22).
Known medications that interact with Lithiumcarbonate are ACE inhibitors, angiotensin II receptor antagonists, diuretics, and non-steroidal anti-inflammatory drugs (NSAIDs), as shown in table 3. These drugs should be avoided. During the trial, use of new medications should always be discussed with the study team and should not be started without their knowledge. If medication that interacts with Lithiumcarbonate is prescribed and urgently needed, serum levels of Lithiumcarbonate and laboratory tests will be regularly performed.
Patients will undergo colonoscopies at baseline, at 6 months, at 12 months and at 18 months follow-up. European guidelines suggest colonoscopy once a year or two yearly depending on polyp burden (4). Thus, colonoscopies and planned biopsies within this study should be considered as additional interventions.

Furthermore, it is known that some natural products, such as turmeric, caffeine and eicosapentaenoic acid can play a role in the Wnt pathway (23-25). For that reason, these factors will be included in the analysis.

5.3 Escape medication

Since NSAID's interacts with Lithiumcarbonate, the use of NSAID's is contra-indicated (see Table 3). In case of pain, administration of paracetamol is allowed.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product

The investigational drug is LithiumCarbonate. The Food and Drug Administration (FDA) approved Lithium in 1970. Since that moment in time it is used to treat patients suffering from mood disorders, such as bipolar disorders and unipolar depressive disorders. The exact mechanism of action in the treatment of bipolar diseases is still unknown, but it is suggested that Lithiumcarbonate enters competition with magnesium ions resulting in inhibition of several enzymes (26, 27). However, previous studies show that Lithiumcarbonate interacts with glycogen synthase kinase 3 (GSK-3β), which plays a role in the Wnt signalling pathway in stem cells (26-28). When GSK-3β is inhibited using Lithiumcarbonate, it will induce transcription due to β-catenin (12, 26). For further information see the SPC text of Lithiumcarbonate.

6.2 Summary of findings from non-clinical studies

This study is based on findings of polyp growth of studies in mouse models, and Lithiumcarbonate is not yet investigated for this effect in FAP patients. It is suggested that CRC develops when *APC* is inactivated by biallelic mutation, subsequently these *APC* mutant stem cells outcompete WT stem cells within the crypt and induce in that way polyp formation (15). The underlying mechanism remained unclear but van Neerven et al. recently described how these mutant stem cells outcompete WT stem cells. Following *APC* inactivation, the Wnt signaling pathway is activated. Subsequently, the mutant stem cells secrete Wnt antagonists (e.g. Notum, Wif1, Dkk2) (12). These Wnt antagonist will inhibit the Wnt signaling pathway in neighboring WT ISCs. This negative-feedback loop results in induction of differentiation, forcing WT ISCs out of the crypt.

Since the mutant stem cells are insensitive to this upregulation of Wnt inhibitors, they outcompete the WT stem cells by the ability of replacing them within the crypt. When all WT ISCs are replaced by *Apc* mutant stem cells, the mutant clone is permanently fixed within the crypt. When a clone is fixed, replacement by a healthy ISC is no longer possible, resulting in polyp formation (12). Therefore, chemoprevention strategies must be applied before fixation of a clone takes place. Recently, novel work has demonstrated a reduction in crypt fixation after administration of LithiumChloride. Lithiumcarbonate could diminish the competitive advantage of *Apc* mutant ISCs by downstream activation of Wnt signaling in WT ISCs by interacting with intracellular GSK-3β. The inhibition of GSK-3β by Lithiumcarbonate induces transcription through β-catenin which leads to downstream activation of Wnt signaling in the WT ISCs and by that desensitize WT ISCs to WNT antagonists (12). In this way WT ISCs are able to compete with mutant ISCs for a position within the crypt, leading to a reduction of fixed mutant crypts.

6.3 Summary of findings from clinical studies

Until now, no study on Lithiumcarbonate in FAP patients is conducted. However, as mentioned in 6.1, Lithiumcarbonate is used as treatment for mood disorders since 1970. Many studies have been conducted on side-effects, contra-indications and optimal therapeutic range (17-20). For further information please see the SPC text of Lithiumcarbonate and chapter 6.4-6.6. Although no data is available of Lithiumcarbonate use in FAP patients, several studies in patients using Lithiumcarbonate for bipolar disorders demonstrated a reduced cancer risk in patients with long-term use of Lithiumcarbonate (29, 30).

6.4 Summary of known and potential risks and benefits

Please see the SPC text of Lithiumcarbonate. Lithiumcarbonate is known for nephrotoxicity and several other side-effects. Most of these potential risks are dose-dependent and are mainly seen in patients with serum concentrations starting from 0.6-0.8mmol/L (18, 20). Table 1 shows the adverse events of Lithiumcarbonate in relation to serum levels at which they occur. The most common side-effects (occurring >10%) are weight gain, polyuria, polydipsia and tremor of the hands. Less often side-effects (occurring 1-10%) are nausea, vomiting and diarrhea, which occur at start of treatment. Later hypothyroidism and electrocardiographic changes may appear (17). Additionally, a decrease in renal function is associated with Lithiumcarbonate use, however chronic renal failure is correlated with long-term Lithiumcarbonate use and associated with high serum levels (>1.0mmol/L) (19, 20). Therefore Lithiumcarbonate is contra-indicated in patients suffering from severe renal impairment and also contra-indicated in known severe cardiac disorders (31). For this study, patients will only be included when having a normal renal function (GFR > 60 ml/min). As in this study only a low dose of Lithiumcarbonate (aiming a serum level between 0.2-0.4mmol/L) will be administered for a short period of time (6 months), potential risks and side-effects of Lithiumcarbonate will be minimized. During the trial, serum levels of Lithiumcarbonate and renal function will be measured, in case of eGFR < 60 ml/min Lithiumcarbonate will be stopped (31). However, patients of whom Lithiumcarbonate treatment has been discontinued, will not be excluded from the study, colonoscopies with biopsies will still be performed (see chapter 7.3).

During this trial, patients will undergo four colonoscopies with biopsies of normal mucosa. Colonoscopy is associated with a minimal risk of complications. Bleeding after biopsy has been reported to occur in up to 0.36%. Perforation has been reported in 0.03-0.11%,

depending on whether or not interventions were performed (32). Potential complications are cited in the written patient information. Furthermore, colonoscopies will be performed by experienced experts at our center.

In patients with FAP, at a certain moment in time a subtotal colectomy or a proctocolectomy with ileoanal pouch anastomosis or an end-ileostomy is performed due to high polyp burden and the risk of developing CRC (4). Surgery is invasive and comes with risks of complications and reduced functional outcome (5, 6). Nevertheless, polyp formation will continue in the remaining proximal and distal intestinal tract, and thus endoscopies are indicated for further surveillance (7, 8). The aim of this study is to evaluate the preventive effect of Lithiumcarbonate on polyp formation in patients with FAP. Based on the study in mice, we expect a significant reduction in polyp burden (12). Such a preventive therapy for polyp formation may postpone or prevent colectomy in patients with FAP.

Table 1. Side-effects of Lithiumcarbonate ***(SPC)***

System/Organ	Therapeutic range	Serum level 1.5-2mmol/L	Serum level > 2mmol/L	Independent of serum level or correlation unknown
Blood and lymphatic disorders	Mild agranulocytosis	Leukocytosis		
Endocrine disorders	Hypothyroidism with or without goiter*			Lowering Protein Binding Iodine, increased uptake of radioactive iodine, euthyroid goiter, hypothyroidism*, hyperthyroidism, hypercalcemia, hypermagnesemia, hyperparathyroidism diabetes insipidus
Feeding and metabolic disorders	Polydipsia	Thirst		
Psychological disorders			Confusion**, Apathy	Delirium

Nervous system disorders	Tremors of the hands, short-lived muscles shocks in arms or legs, often at night, difficulty concentrating and memory problems (especially particularly in elderly patients)	Tremors of the hands	Hyperreflexia, drowsiness, coma	changes in the EEG, vertigo, speech disorders, metallic taste, benign intracranial hypertension*, extrapyramidal disorders*, epileptiform insults*. encephalopathy, cerebellar syndrome, nystagmus, falls, peripheral neuropathy*. malignant neuroleptic syndrome** serotonin syndrome***
Ocular disorders				eye irritation (reversible in most cases), papilledema ****, exophthalmia *****
Cardiac disorders	Changes in ECG**			Changes in ECG, arrhythmia (in particular bradycardia), sinus node dysfunction, cardiomyopathy, atrioventricular block**
Gastrointestinal disorders	nausea, vomiting and diarrhea, usually at the start of treatment	Anorexia, xerostomia, nausea, vomiting, diarrhea		Abdominal pain, hypersalivation
Cutaneous and subcutaneous disorders	Increase of sebum production,			Leg ulcera, exacerbation of psoriasis, pruritus,

	alopecia, the occurrence or worsening of acne or psoriasis			myxedema is rarely seen, allergic rash Frequency not known: lichenoid drug eruption
Musculoskelatal and connective tissue disorders		Muscle weakness	Muscle fasciculation, fasciculations, hypertonia	Rhabdomyolysis
Renal and urinary system disorders	Polyuria, Long-term administration of lithium in high doses can have a detrimental effect on the kidneys and lead to formation of microcysts in the kidneys*.	Polyuria		Microcysts, oncocytoma and renal carcinoma of the Ductus Bellinitype (in long-term administration) (frequency not known)
Pregnancy, perinatal period and puerperium				Neonatal abstinence syndrome (frequency not known)
General disorders and disorders at administration site	Weight gain			Weight gain, peripheral edema, urticaria and angioedema attributed to one of the excipients.

** See SPC text of Lithium.*

*** When co-administering antipsychotics and lithium.*

**** Serotonin syndrome may be accelerated by concurrent use of serotonergic antidepressants.*

***** In some cases without increased intracranial pressure, leading to possible vision loss (generally reversible).*

****** Not always associated with disorders of the thyroid gland.*

6.5 Description and justification of route of administration and dosage

We aim to minimize side-effects by administering the lowest Lithiumcarbonate dose that we expect to be effective. As described in 5.1 and 6.4, serum levels between 0.2-0.4mmol/L will be targeted. Lithiumcarbonate will be administered through oral tablet once a day. Patients

will be treated with a starting dose of 200mg, after 5 days the dose will be increased to 300mg once a day. Serum levels will be measured at day 12 and day 22 after start of treatment. When a therapeutic range is reached, serum levels will be measured once a month. Blood samples for determining serum levels must be collected 12h after oral administration of Lithiumcarbonate. If the serum level is not within the therapeutic range, dose adjustments will be made. Serum levels will be monitored 12 days after dose adjustments, until the therapeutic range is reached or side-effects diminished.

6.6 Dosages, dosage modifications and method of administration

See chapter 6.5

6.7 Preparation and labelling of Investigational Medicinal Product

Lithiumcarbonate will be labelled by the AMC Hospital Pharmacy according to GMP guidelines (GMP-annex 13). The funding of the drugs will be provided by the study sponsor. Since Lithiumcarbonate is a registered drug, the label text originated from the producer will be used. An additional label related to the study will be added with the following information: 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

Storage and disposition of Lithiumcarbonate will be done by the AMC Hospital Pharmacy according to patient name and patient number. Medication will be prescribed per 60 tablets. The first dispense will take place directly after the second colonoscopy in the AMC (t=6m). The tablets will be distributed at the AMC hospital Pharmacy every two months at time of consultation in the AMC by the study doctor, since it is not possible for a participant to collect a study drug at a local pharmacy. 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

- Clone sizes will be quantified as proportions of the crypt circumference positive for *NOTUM* (in parts of eight, 1:8 to 8:8). When a whole crypt is positive for *NOTUM* (8:8), this crypt is fixed (crypt fixation) (12).

7.1.2 Secondary study parameters/endpoints

- Difference in number and size of polyps between baseline and end of study
- Patient reported side effects of Lithiumcarbonate using a Lithium side effect questionnaire (see appendix 2, chapter 14.2)
- Safety outcomes by analysing reported adverse events, physical examination and laboratory findings.

7.1.3 Other study parameters

Other assessments in questionnaire (please see chapter 14.3, appendix 3: patient diary):

- Number of cups of coffee per day (decaffeinated coffee not included)
- Turmeric use (gram per day)
- Number of fish oil capsules per day (amount of eicosapentaenoic acid in terms of mg)
- History of smoking, if yes number of pack years

7.2 Randomisation, blinding and treatment allocation

In this study, no randomisation will take place, every participant is given the same treatment. All crypt analysis data and adenoma counts will be scored blindly.

7.3 Study procedures

Baseline and follow-up measurements and monitoring

- Medical history at baseline.
- Physical examination (including vital signs) at baseline and every six months (at time of endoscopy).
- Clinical tests including pregnancy test at baseline, month 6 and month 12 (at time of endoscopy).
- Laboratory testing at baseline and every two months: haemoglobin, thrombocytes, leukocytes, GFR, creatinine, urea, sodium, potassium, calcium and TSH (if deviating T4).
- Testing spot urine sample at baseline and month 12: urine osmolality and creatinine.

- Testing serum levels of Lithiumcarbonate at day 12 and 22 after start of treatment (blood sample will be collected 12h after oral administration of Lithiumcarbonate). When a therapeutic range is reached, serum levels will be monitored once a month in certified laboratory.
- Check-ups through telephone interviews to evaluate adverse events and side effects using a questionnaire at baseline and during treatment with Lithiumcarbonate (see appendix 3, chapter 14.3). During Lithiumcarbonate treatment telephone interviewing will be done monthly to evaluate Lithiumcarbonate dose through serum level testing.
- Colonoscopy with biopsies at baseline and every six months (a total of four endoscopic procedures) performed at the AMC by experienced experts.
- 24-hour urine volume test in month 13 (test can be done at home).

Assessment of size of fixed clones

For each patient crypts will be retrieved form normal tissue biopsies through colonoscopy (t=0, t=6, t=12, t=18 months). *APC* mutant stem cell dynamics will be determined in these biopsies by tracing the spread of the *APC* mutant cells using specific expression of *NOTUM* by RNAscope (in situ hybridization). *NOTUM*-positive crypts will be quantified by determining the proportion of the crypt circumference positive for *NOTUM* (in parts of eight, 1:8 to 8:8). This will result in an average clone size distribution for each patient per time point, as well as the proportion of fixed crypts (8:8). By analyzing the differences in clone size distribution before, during and after Lithiumcarbonate treatment we aim to observe a relative reduction in average clone size of 50% during the lithiumcarbonate treatment, as well as a reduction in crypt fixation of 50%.

Assessment of degree of polyposis

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

<u>Baseline and follow-up colonoscopies</u>

A full colonoscopy will be performed at baseline, and at months 6, 12 and 18 to assess polyp burden and to take biopsies. During colonoscopy, Boston Bowel Preparation Scale (BBPS) should be at least 6 and cecal intubation achieved. Video recording starts at withdrawal of the endoscope form the cecum, which is performed fluently in a spiral fashion aiming to visualize the entire colonic mucosa. The biopsy forceps will be used to assess the size of polyps if estimated ≥ 3mm. Polyp burden will be assessed, based on number of polyps and size (please see table 2), for the 6 different segments: cecum, ascending colon, transverse colon, descending colon, sigmoid and rectum (including retroflexion). When moving from one segment to the next segment, the endoscopist will be asked to stop the video and start a new video for the next segment. In that way, there will be a separate video for each segment to

make sure the two separate reviewers (explained later) will assess the exact same segment. For each colonoscopy, the table below will be used to calculate the sum of diameter of polyps to determine the total colorectal polyp burden. It is important that the endoscopist does not go back and forth with the endoscope during video recording and only uses white light endoscopy. During video recording, no polypectomies are performed.
After video recording, NBI is allowed and polypectomies are performed at the discretion of the endoscopist. Polyps ≥5mm should be excised at all colonoscopies. Also, two random biopsies of normal mucosa will be taken in each segment, resulting in a total of 12 biopsies of normal intestinal mucosa during each endoscopy. In addition, 3 random polyps retrieved from a random segment will be excised for determination of histology.

After month 18, in total 4 clustered colonoscopy video recordings of each participant are available (4x6 of each segment per participant). The 4 videos of the participant will be reviewed by two separate independent expert endoscopists in a random order and they will assess the polyp burden using the table below. For each segment there wil be a separate video, as explained above. The assessment of the first and second reviewer will be compared to identify possible disagreements. If there is a disagreement in polyp burden assessment of more than 30%, a third independent reviewer will be engaged, after which the average of these three reviewers will be used for further analysis.

Table 2: Total polyp burden, based on size and number of polyps.

Segment	Number of polyps
Cecum	☐ 1-2mm ☐ 3-5mm ☐ 6-10mm ☐ >10mm
Ascending colon	☐ 1-2mm ☐ 3-5mm ☐ 6-10mm ☐ >10mm
Transverse colon	☐ 1-2mm ☐ 3-5mm ☐ 6-10mm ☐ >10mm
Descending colon	☐ 1-2mm ☐ 3-5mm ☐ 6-10mm ☐ >10mm
Sigmoid	☐ 1-2mm ☐ 3-5mm ☐ 6-10mm ☐ >10mm
Rectum	☐ 1-2mm ☐ 3-5mm ☐ 6-10mm ☐ >10mm
Total polyp burden	☐ **1-2mm** ☐ **3-5mm** ☐ **6-10mm** ☐ **>10mm**

7.4 Withdrawal of individual subjects

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

7.4.1 Specific criteria for withdrawal

If any exclusion criteria will be met during the study, a participant will be withdrawn from treatment.

7.5 Replacement of individual subjects after withdrawal

Subjects who withdraw from the trial after start of Lithiumcarbonate will not be replaced.

7.6 Follow-up of subjects withdrawn from treatment

In every participant withdrawing from the study for any reason, further surveillance will be scheduled according to standard care for FAP patients.

However, when patients withdraw from the study after start of Lithiumcarbonate treatment, the time of withdrawn will be handled as timepoint t=12m (please see the flowchart). Subsequently, the patient will follow the consecutive procedures as described in this protocol.

When withdrawn is conducted due to side-effects of the use of Lithiumcarbonate, follow-up will be performed weekly through telephonic interviewing until symptoms disappear.

7.7 Premature termination of the study

Since Lithiumcarbonate is a long-used medicine and has been well investigated, adverse events leading to premature termination of the study is not to be expected. No criteria for early termination have been established.

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/her 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:

A. the event must be serious (see chapter 9.2.2);
B. 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;
C. 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

The safety committee will consist of all involved investigators in this study [redacted]. Safety monitoring will be conducted through telephonic interviewing, physical examination and lab and serum level testing. Serious and non-serious adverse events of any kind will be collected by the PhD

candidate and reported to the safety committee immediately. Classifying as serious or non-serious adverse event, deciding on the toxicity grade, the relatedness to Lithiumcarbonate will be done by the safety committee. As well as subsequent measures to be taken. Since the effect of Lithiumcarbonate has not been studied in FAP patients, a safety committee is needed.

9. STATISTICAL ANALYSIS

To describe the study population, baseline characteristics and changes in outcome parameters during follow-up, descriptive statistics will be used in this study. SPSS for Windows software (Chicago, IL, USA) version 26.0 will be used for these analyses.
If other statistical analysis will be used, this will be mentioned in the study paper.

9.1 Primary study parameter(s)

Primary analysis for the primary outcome parameter will be done by analyzing the data using two-sided student's t-test. will be performed. The limit for the statistical significance will be established at $p < 0.05$ with a confidence interval of 95%.

9.2 Secondary study parameter(s)

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

9.3 Other study parameters

Not applicable.

9.4 Interim analysis

Not applicable.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This 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, the study will not start before formal approval has been granted. Informed consent form by all patients is required. 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

Patients will be recruited from the AMC. Patients will receive information about the study written on paper and/or mail and it will be explained in person by the study doctor (a PhD candidate) or a colleague doctor. The patients will be informed about the study by telephone or at the outpatient clinic. After explanation, informed consent can be given at the outpatient clinic by the participant. No time limit will be set on considering participation. Withdrawal of informed consent can be done by the participant at any time and for any reason. Please see the patient information letter and informed consent (files are attached).

10.3 Objection by minors or incapacitated subjects

Minors and/or incapacitated adults will not be approached for participation of this study.

10.4 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). 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.

10.5 Incentives.

Included participants will receive no reimbursement for entering this study. Travel costs will be compensated.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Adenomatous tissue collected endoscopically, as part of routine care, will be stored according to regulations of the department of pathology in the AMC. Normal intestinal mucosa collected endoscopically, as part of study material to assess *NOTUM* distribution size e.g., will be analysed in the Center for Experimental and Molecular Medicine (CEMM). Source documents and CRFs will be stored by the project leader for 15 years after closure of the trial. Collected samples will be stored for 5 years. In case of informed consent is reached for participation in a biobank, samples will be stored for 15 years. 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 Algemene Verordening Gegevensbescherming, AVG).

11.2 Monitoring and Quality Assurance

For this study, monitoring was requested from Clinical Monitoring Center (CMC). After approval by the METC, a monitoring intake visit will be scheduled to set up a monitoring plan.

11.3 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.

11.4 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.

11.5 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.

When this drug seems to be effective, there is a strong wish from patients to continue taking Lithiumcarbonate. For those participants it will be possible to continue Lithiumcarbonate off-label after the study has ended. Non-registered drugs can be prescribed by the clinician through a 'named patient program' (in Dutch: op artsenverklaring). Subsequently each request have to be approved by the Inspection of Health care and Youth (in Dutch: Inspectie Gezondheidszorg en Jeugd, IGJ) for each individual. Whether this off-label drug will be reimbursed depends on each health insurance company.

11.6 Public disclosure and publication policy

This study will be executed by the following team: [redacted]

[redacted]

There will be no conflicts of interest regarding to publication of expected results from this study. Scientific results will be published in scientific journals and will be presented on congresses (national and international). In case of co-operation between departments, the first and subsequent authors will be determined according to the contribution to the (part of the) project of the involved departments. Published data will be impossible to relate back to the participant.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Lithiumcarbonate is been used to treat patients suffering from bipolar disorders. It is suggested that Lithium enters competition with magnesium ions which leads to inhibition of several enzymes (26, 27). Furthermore, Lithiumcarbonate interacts with glycogen synthase kinase 3β (GSK-3β), which plays a role in the Wnt signalling pathway in ISCs (26, 27). When GSK-3β is inhibited, for example when Wnt ligands binds to its receptor or through Lithiumcarbonate administration, it will activate transcription of Wnt target genes through nuclear re-localisation of β-catenin (12, 26). In a recent study, lithiumchloride administration resulted in downstream activation of Wnt signalling in WT ISCs, thereby desensitizing the WT ISCs to Wnt antagonists secreted by *Apc* mutants (12). As a result, lithiumchloride prevents the fixation of Apc mutant cells in the crypt thereby reducing polyp formation in *apc*-mutant mice (12). This suggest that intervening before clones are fixed within the crypt, polyp burden can be reduced and time to progression to dysplasia can be prolonged.

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

Since 1970 Lithium is approved by the FDA and used to treat patients suffering from bipolar disorders. Details about the exposure of human beings with Lithiumcarbonate are clearly described in the SPC text. As previous mentioned, Lithiumcarbonate has never been investigated in patients with FAP. However, in the study of van Neerven et al. an effect of Lithiumchloride was seen on stemmness and clonogenicity in organoids deriving from human colon that were incubated with FAP conditioned medium (12).

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

Up to now, Lithiumcarbonate has never been investigated in patients with FAP. As previous described, the primary outcome and effect on number of polyps has been studied in mouse models.

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

Please see the SPC text and chapter 12.1a for explanations of working mechanisms. For known side-effects, please see Table 1. Drugs that could interact with Lithiumcarbonate are described in Table 3 (please see the appendix).

e. Analysis of potential effect

Side-effects can be found in Table 1. As mentioned in chapter 6.4 the occurrence of side-effects is dose-dependent (18-20). Most side-effects are mainly seen in serum concentrations starting from 0.6-0.8 mmol/L (18, 20) . Toxicity effects of Lithiumcarbonate

(such as nephrotoxicity) is associated with serum concentrations starting from 1.0 mmol/L (19, 20). In the study on mice, a significant effect of Lithiumchloride on *NOTUM* clone size distributions within the crypt was seen using a serum level of 0.2 mmol/L (12). We believe using a serumlevel between 0.2-0.4 mmol/L in the participants of this study, side-effects and toxicity will be minimalized and still a promising effect can be expected.

f. Pharmacokinetic considerations

Please see the SPC text for pharmacokinetics of Lithiumcarbonate.

g. Study population

Research subjects will contain patients diagnosed with FAP, between the age of 18 and 35 years. The colon of these patients must be intact, patients who underwent a colectomy of any kind will be excluded. No exclusion will be made based on sex or race. Female participants with a pregnancy wish are not allowed to participate in this study. During participation participants must use adequate contraception.
Due to the high risk (nearly 100%) of developing CRC, patients with FAP are likely to undergo colectomy in the twenties to prevent the developing of a tumour (3, 4). These types of surgery are known for its high risk of comorbidities (5, 6). Even after surgery adenoma formation is seen in these patients, and by that CRC can still develop (7, 8). These patients would benefit from a medical treatment preventing polyp formation, and in that way postpone or even prevent invasive types of surgery. Until now, no such chemopreventive therapy is available.

h. Interaction with other products

Please see table 3 for drugs that interact with Lithiumcarbonate. The use of any of these products is not allowed when enrolled in this study, as mentioned in the exclusion criteria (see chapter 4.3). If any of these drugs needs to be started during the study, it must be in consideration with the investigator at any time. Dependent on the expected interaction and indication of the supplementary drug, additional serum level testing will be conducted or the patient will be withdrawn from the study.

i. Predictability of effect

The primary study outcome will be measured by analyzing *NOTUM*^{pos}/*APC* clone size distributions within the crypt, defined before the start of six months treatment with Lithiumcarbonate and after treatment. We believe by using such a quantifiable outcome measurement, results will provide relevant translational data. The combination of the secondary study outcome parameters, obtained through endoscopic measurements and laboratory findings, with the primary study outcome parameter will provide both clinical and translational information. In case this study shows an effect on *NOTUM* expansion and safety outcomes have been achieved, a larger-scale clinical trial of Lithiumcarbonate in FAP patients can be conducted whereas Lithiumcarbonate can be administered for a longer period of time.

j. Can effects be managed?

A hospital pharmacist, a nephrologist and a psychiatrist all with an expertise in Lithiumcarbonate use will be actively involved in this study. Through telephonic interviewing, physical examination and laboratory testing side effects or adverse events can be detected early. If needed, dosage of Lithiumcarbonate will be adjusted. Participants will be instructed to contact the investigator when side-effects or adverse events occur. In case of an emergency, the patient can contact the gastroenterologist that is on call. Contact details will be given.

k. Covid-19 analysis

Concerning the pandemic due to the covid-19 virus, risks of any further developments should be taken into account. In case of increasing covid-19 cases or new restrictions, site visits will be minimized and study medication can be sent to the patients home. Also the time frame for planning study procedures (e.g. colonoscopy) can be extended since it will be more difficult to plan the procedures within a window of 7 days. We are aware that due to the pandemic and possible restrictions, the trial can take longer.

12.2 Synthesis

Lithiumcarbonate is known for its narrow therapeutic dose range and toxicity, as mentioned in chapter 6. However, these SE and toxicity is associated with long-term use and high serum-levels of Lithiumcarbonate (18-20). In this study a serum level between 0.2-0.4 mmol/L will be used to treat the participants with a regularly monitoring of serum levels. Further safety monitoring will be achieved through telephonic interviewing, physical examination and laboratory testing. By doing so, SE and toxicity can be minimalized or caught at an early stage. Patients will be excluded based on renal function, cardiac disorders, laboratory abnormalities and the use of co-medication that can interact with Lithiumcarbonate (for further exclusion criteria, see chapter 4.3).

Colonoscopy is associated with minimal risk of complications. Bleeding after biopsy has been reported to occur in up to 0.36%. Perforation has been reported in 0.03-0.11%, depending on whether or not a biopsy was performed (32). Potential complications are cited in the written patient information. Furthermore, colonoscopies will be performed by highly experienced experts.

Included participants in this study are likely to undergo prophylactic surgery within a short time, these types of surgery are associated with a high risk of comorbidities (5, 6). If a chemopreventive effect is seen in this study, patients with FAP would benefit from therapy with Lithiumcarbonate where these types of invasive surgery could be postponed or even avoided.

We believe, by implementing the risk management as described above, treatment with Lithiumcarbonate in FAP patients is justifiable. Results of this study can be used to set up

a larger-scale clinical trial with a longer follow-up period to determine the effect of Lithiumcarbonate in FAP patients.

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14. APPENDIX

14.1 Appendix 1: Drugs that interact with Lithium

Table 3. Drugs that interact with Lithium.

Interactions that may increase lithium concentrations	
Angiotensin-converting enzyme (ACE) inhibitors	
Angiotensin-II receptor antagonists	
Antibiotics: metronidazole, tetracyclines, cotrimoxazol, trimethoprim	
Diuretics: thiazides, spironolactone, furosemide	
Non-steroidal anti-inflammatory drugs (NSAIDs)	
Selective serotonin re-uptake inhibitors (SSRI's)	
Other: topiramate, medicines that affect electrolyte balance (e.g. corticosteroids)	
Interactions that may decrease Lithium concentrations	
Carbonic anhydrase inhibitors	
Sodium bicarbonate and sodium chloride containing products	
Xanthines: theophylline, caffeine	
Other: urea	
Interactions that can cause toxicity with normal Lithium concentrations	
Antipsychotics: haloperidol, olanzapine, clozapine	
Calcium channel blockers (verapamil, diltiazem)	
Tricyclic antidepressants	
Others: Carbamazepine, phenytoin, methyldopa, clonazepam	
Interactions that can lead to QT Prolongation (with high concentrations of Lithium)	
NYHA-klasse Ia- en III-anti-aritmica	
Others: arsenic trioxide, chloroquine, chlorpromazine, claritromycine, domperidone, droperidol, erytromycin, haloperidol, methadone, pentamidine, pimozide, sotalol	
Other interactions	
Neuromuscular blocking agents	Lithium may prolong the effects of these agents.
Triptans, Selective serotonin re-uptake inhibitors (SSRI's)	Simultaneously use may accelerate serotonin syndrome

14.2 Appendix 2: Side effects questionnaire

Bijwerkingen van Lithium

Deze lijst is alleen voor lithiumgebruikers.

Geef aan in welke mate u last heeft van de genoemde bijwerkingen, het gaat uitsluitend om uw eigen beleving.

	nee	licht, nauwelijks hinder	matig met enige hinder	sterk met veel hinder
Hoofdpijn	0	1	2	3
Duizeligheid	0	1	2	3
Vermoeidheid	0	1	2	3
Wazig/dubbel zien	0	1	2	3
Concentratiestoornissen	0	1	2	3
Geheugenstoornissen	0	1	2	3
Misselijkheid	0	1	2	3
Droge mond	0	1	2	3
Dorstgevoel	0	1	2	3
Toegenomen urineren	0	1	2	3
Verminderde eetlust	0	1	2	3
Toegenomen eetlust	0	1	2	3
Diarree	0	1	2	3
Obstipatie	0	1	2	3
Sexuele functiestoornissen	0	1	2	3
Transpireren	0	1	2	3
Tremor van handen	0	1	2	3
Spierzwakte	0	1	2	3
Coördinatiestoornissen	0	1	2	3

14.3 Appendix 3: Patient diary

Datum: …../…../…..	**Periode**: Visite maand … - Visite maand …
Subjectnummer:……..	

Patiënten dagboek

Beste deelnemer,

Sommige zaken (zoals voedingsmiddelen of roken) kunnen mogelijk invloed hebben op de ontwikkeling van poliepen. Om hiermee rekening te kunnen houden tijdens dit onderzoek is het belangrijk een goed beeld te krijgen van uw wekelijkse inname/gebruik van deze producten.

Noteer per week de hoeveelheid die u in die week ingenomen heeft per onderdeel.
Of u wel of niet rookt hoeft u maar eenmalig in te vullen. Dit dagboek wordt iedere 6 maanden verzameld.

Roken

Vraag	Antwoord (kruis het juiste antwoord aan of vul het getal in)	
1. Rookt u? (indien ja ga verder naar vraag 2)	☐ Ja	☐ Nee
2. Hoeveel jaren rookt u?	_____ Jaren	
3. Hoeveel sigaretten rookt u per dag?	_____ Sigaretten	
4. Rookt u….?	☐ Sigaretten	☐ Shag

Datum: …../…../….. **Periode**: Visite maand … - Visite maand …

Subjectnummer:……..

Voedingsmiddelen

Week	**Voedingsmiddel**	**Hoeveelheid (per week)**
Week 1	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 2	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 3	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 4	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
	Kurkuma (geelwortel)	_____ Theelepels

Datum: …../…../….. **Periode**: Visite maand … - Visite maand …

Subjectnummer:……..

Week 5	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 6	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 7	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 8	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 9	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels

Datum: …../…../….. **Periode**: Visite maand … - Visite maand …

Subjectnummer:……..

Week 10	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 11	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels
Week 12	Koffie (décafe niet meegerekend)	_____ Kopjes
	Visolie (omega3)*	_____ Capsules/tabletten
	Kurkuma (geelwortel)	_____ Theelepels

* Indien u visoliesupplementen gebruikt, gelieve een foto van het doosje/potje te maken zodat u deze kunt tonen naan de onderzoeker.