MELFIB STUDY

Measuring effects of high-fiber dried chicory root on the gut microbiota of patients with an intermediate to high-risk cutaneous melanoma: an explorative study

RESEARCH PROTOCOL

Hoofdaanvrager: Prof. dr. G.A.P. Hospers
Universitair Medisch Centrum Groningen

February 2025

'*Measuring effects of high-fiber dried chicory root on the gut microbiota of patients with an intermediate to high-risk cutaneous melanoma: an explorative study.*'

Protocol ID	**19789**
Short title	Explorative study on addition of high-fiber dried chicory root in patients with an intermediate to high-risk cutaneous melanoma.
Version	4
Date	February 27, 2025
Principal investigators (in Dutch: hoofdonderzoekers/uitvoerders)	**Prof. Dr. G.A.P. Hospers** University of Groningen University Medical Center Groningen Department of Medical Oncology g.a.p.hospers@umcg.nl Phone: +31652724185
Investigators (in Dutch: uitvoerders)	**Emily Oosterhout, PhD candidate** University of Groningen University Medical Center Groningen Department of Medical Oncology e.oosterhout@umcg.nl Phone: +31652764949 **Prof. Dr. B.L. van Leeuwen** University of Groningen University Medical Center Groningen Department of Surgical Oncology b.l.van.leeuwen@umcg.nl Phone: +31631623706 **Prof. Dr. R.K. Weersma** University of Groningen University Medical Center Groningen Department of Gastroenterology and Hepatology r.k.weersma@umcg.nl Phone: +31655256256
Sponsor (in Dutch: verrichter/opdrachtgever)	**University Medical Center Groningen (UMCG)** Hanzeplein 1, 9713 GZ Groningen Phone: +31503616161
Subsidizing parties	**Seerave foundation** **WholeFiber Holding BV** (in kind)
Independent expert	**Dr. T.J.N. Hiltermann** University of Groningen University Medical Center Groningen Department of Pulmonology and Tuberculosis t.j.n.hiltermann@umcg.nl Phone: +31652724086

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Department: **Prof. Dr. J.A. Gietema** University of Groningen University Medical Center Groningen Department of Medical Oncology		
Principal Investigator: **Prof. Dr. G.A.P. Hospers** University of Groningen University Medical Center Groningen Department of Medical Oncology		

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	**Adverse Event**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
ELISA	**Enzyme-Linked Immuno Sorbent Assay**
FDR	**False Discovery Rate**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
METc	**Medical research ethics committee (MREc); in Dutch: medisch-ethische toetsingscommissie (METc)**
PBMC	**Peripheral Blood Mononuclear Cells**
PIF	**Patient Information Form**
(S)AE	**(Serious) Adverse Event**
SCFA	**Short Chain Fatty Acid**
SD	**Standard Deviation**
SEM	**Standard Error of the Mean**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Dietary fibers are digested in the gut by specific bacteria, which can produce short-chain fatty acids (SCFAs). These SCFAs together with dietary fiber on its own are hypothesized to have a beneficial effect on the immune cell composition, fecal calprotectin levels, stool pattern and general well-being. In intermediate to high-risk cutaneous melanoma (minimal stage II A) very often tumor infiltrating lymphocytes are found, which sometimes leads to spontaneous remission of these tumors. What is unclear, and in our view should be the first small step and the objective of this study, is to determine whether changes in the gut microbiome in this group of patients (with skin melanoma) is possible at all, if these changes in the gut microbiome lead to an increased production of SCFA and whether the changes in gut microbiome; immune cell composition and SCFA can indeed be measured.

Objective: Exploring the effect of supplementation with daily addition of prebiotic dried chicory root (WholeFiber™) for 6 weeks on fecal SCFA levels, gut microbial composition and immune cell composition in minimal stage II A melanoma patients following surgical treatment.

Study design: Supplementing with WholeFiber™ for 6 weeks two times a day (10 g per portion). During 3 visits questionnaires, measurements and (blood and fecal) samples will be taken.

Study population: The research population will be recruited at the University Medical Center Groningen (UMCG) and consists of 20 patients aged ≥18 years, who have undergone surgical treatment of a minimal stage II A melanoma, with no concurrent adjuvant treatment or autoimmune disease.

Intervention: Subjects will receive 2 sachets of 10 g WholeFiber™ per day for 6 weeks (equals 17 g fiber). In the first week of the intervention, the subjects will take 1 sachet of 10 g WholeFiber™ (equals 8 g fiber).

Main study parameters: The main study parameter is determining the effect of WholeFiber™ for 6 weeks on fecal SCFA levels in this patient group. Secondary (exploratory) parameters are gut microbial composition changes, immune cell composition, stool patterns, fecal calprotectin levels, and side effects after intervention.

Nature and extent of the burden and risks associated with participation, benefit, and group relatedness: WholeFiber™ is a dried vegetable (chicory root) that for 85% consists of prebiotic fibers, safe for consumption and is commercially available. Increasing dietary fiber intake is beneficial for overall health and may give some abdominal complaints at the start. Patients will visit the study site three times for blood tests and fecal samples will be collected at home. The collected fecal samples will be brought to the study visit by the participant to ensure the lowest impact for the subjects.

1. INTRODUCTION AND RATIONALE

1.1. Gut microbiome and dietary fiber

Chronic diseases such as diabetes, inflammatory bowel disease and cancer have dramatically increased over the past century, many of which are associated with changes in the gut microbiome[1]. This suggests that certain lifestyle changes may disrupt gut homeostasis and cause microbial dysbiosis; a condition typically characterized by the growth of pathogens at the expense of commensal bacteria when compared to a symbiotic microbiome[2]. One of the main factors affecting the composition of the gut microbiome is diet. Research has shown that the occurrence of beneficial microbes is often linked to food items rich in poly-unsaturated fatty acids, dietary fiber, and polyphenols[6]. These compounds are found in great amounts in the Mediterranean diet[3].
Despite these well described correlations, the associated dietary patterns are not yet tested in a clinical setting. Prospective clinical studies using a prescribed diet are hard to perform and to analyze[4]. Therefore, use of add-on dietary fiber could serve as an alternative approach to study these so-called beneficial dietary patterns.

1.2. Short-chain fatty acids, inulin and the immune system

Dietary fibers are digested in the intestine by specific microbes, which produce short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate as a byproduct. SCFAs have beneficial effects on gut health by acting as anti-inflammatory compounds and increasing gut-barrier function[5].
They also help modulating immune responses by interacting with various immune cells[6]. SCFAs such as acetate, propionate and butyrate have been identified to promote generation of colonic regulatory T cells[9]. Regulatory T cells produce several inhibitory cytokines including TGF-ß, IL-35 and IL-10 (*Figure 1*)[7,8]. SCFAs can also enter systemic circulation, thus affecting immune cells beyond the gut[9].
The dietary fiber inulin also plays an important role in regulating immune cell differentiation. The prebiotic fiber present in chicory root has been shown to increase the production of TNF-α and IFN-γ by CD4+ T cells (*Figure 1*)[10]. These immune cells are also known as T helper (T_H) cells, which are important in almost all adaptive immune responses. Inulin also directly affects CD8+ T cells and $\gamma\sigma$ T cells, which are innate immune cells who can directly kill infected, cancerous, or non-self-cells[11–13].

1.3. Gut microbiome and cancer

A recent review shows indications of gut OncoMicrobiome signatures, suggesting that patients with melanoma may have a different microbiome[14]. Additionally, there are ongoing studies on altering the microbiome in melanoma patients undergoing immunotherapy with the goal to make immunotherapy more effective. The methods that are studied now are fecal transplantation and nutritional modulations[15–17]. We know that the intake of dietary fiber can affect gut microbial composition[18]. Therefore, our proposal is to conduct this exploratory study using dietary fiber in patients with “early melanoma” to investigate the axis between fiber intake, gut microbiome and immune cell composition. In these patients, there are fewer confounders (no immunotherapy, colitis, steroids, etc.), which may contribute to better insight into the microbiome in these patients, the effect of increasing fiber on the gut microbiome and metabolites, and the immune cell composition. Moreover, we are gaining more information about the “healthy microbiome” through data from the Lifelines database in several ongoing studies, secondly these data could serve as a control. For example, we are investigating whether there is a relationship between gut microbiome and the development of melanoma (and other tumors) using the link between the Lifelines database and the Pathological-Anatomical National Automated Archive (PALGA) database.

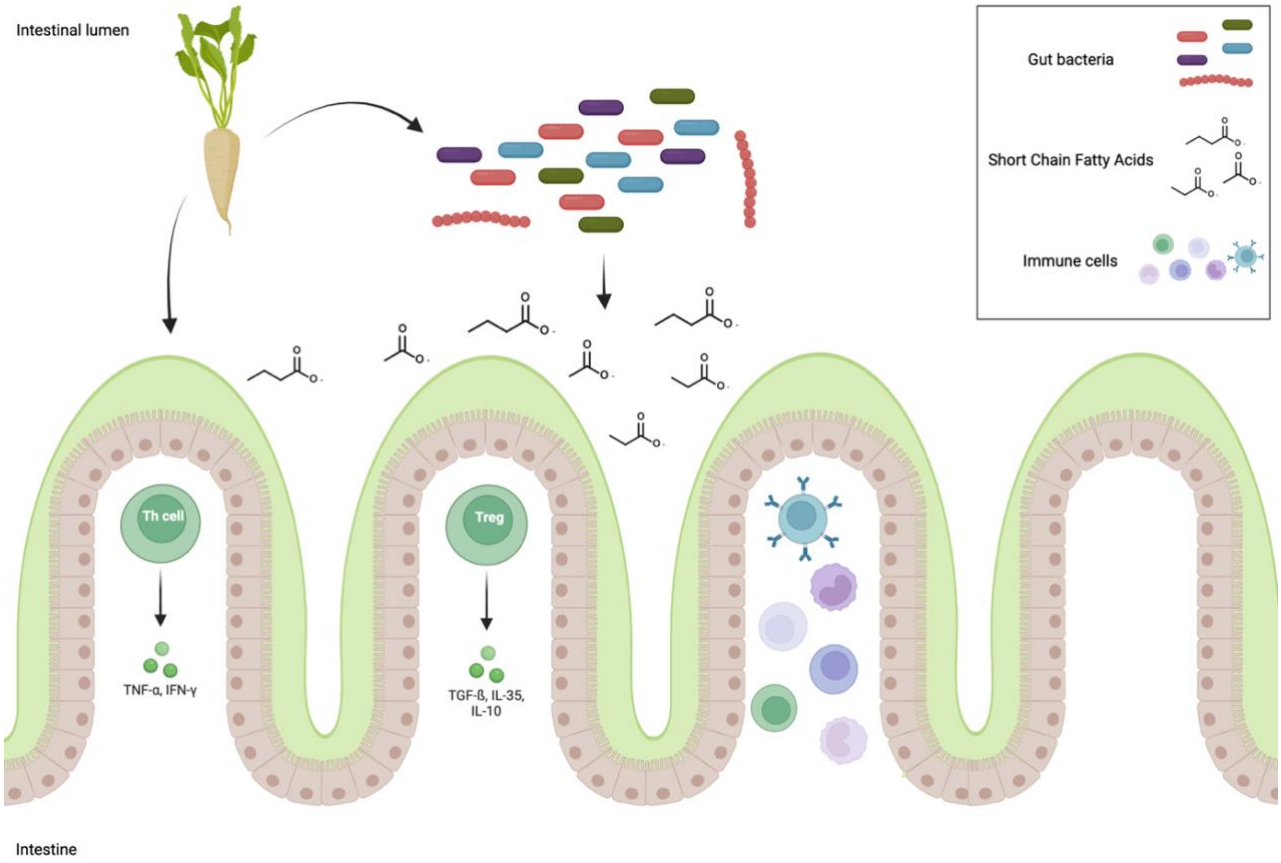

Figure 1: Proposed mechanism of action of WholeFiber™ on cellular level in the gut.

1.4. Target population: stage II melanoma

In intermediate to high-risk cutaneous melanoma (minimal stage II A) lymphocytes are often found, which may lead to spontaneous remission of these tumors[19]. In line with these findings, ICI treatment has been shown to be effective in this patient group as the 5–15-year survival rate of patients with this tumor type in stage IV strongly improved after ICI treatment[20]. This type of cancer treatment helps immune cells recognize tumor cells, enabling targeted elimination. Therefore, it can be beneficial to explore the effects of an inulin rich fiber in this population, as this has been shown to affect immune cell composition.

1.4.1 The Dutch population does not meet fiber intake recommendations

A recent study by Reynolds et al investigated the relationship between carbohydrate quality and global human health and found that implementation of recommendations to increase dietary fiber intake benefits human health[21]. The current guideline for fiber consumption in the Netherlands is a minimal intake of 14 grams of fiber per 1000 kcal consumed in adults aged 21-50, which translates to about >25 grams of fiber per day for women and >38 grams of fiber per day for men[22]. Interestingly, the general Dutch population does not meet these recommendations, as the mean fiber intake in the this population was measured at 20.3 g/day by the National Food Consumption Survey of 2019-2021[22].

Implementation of high fiber food sources in meals throughout the day would pose a solution for the stated ‘fiber intake gap’. However, it remains difficult to increase fiber intake, even with personalized advice, and easy-add-ons in the diet such as a supplement are suggested to have a better adherence[4]. Therefore, there is a need for an easy solution to increase fiber intake it in an effective way to meet the recommended dietary intake of fiber[21].

1.4.2 Product: dried chicory root

In this study, we propose to use WholeFiber™ as an easy add-on to the daily diet. WholeFiber™ is 100% dried root vegetable, chicory root, in which 85% of its dry weight consists of fiber. By using 20 g of the product daily the earlier mentioned 'fiber intake gap' can already be tackled. The fiber composition of chicory root contains multiple intrinsic prebiotic fibers, mainly inulin, and some pectin, hemi-cellulose, and cellulose. Furthermore, this dried vegetable is rich in polyphenols and potassium.

The dried vegetable WholeFiber™ has been used in a recent 5-week randomized controlled trial with Dutch adults at high risk of developing type 2 diabetes. In the study, 15-30 g/day of WholeFiber™ has been shown to significantly modulate the gut microbiota composition[18]. More specifically, they measured a great impact of WholeFiber™ on butyrate-producing bacteria such as *Bifidobacterium* and *Anaerostipes* (3-4-fold increase), as well as increased SCFA levels in feces (+26%)[18].

In a different trial where 15-30 g of WholeFiber™ was consumed daily for 12 weeks, an improvement of insulin sensitivity, plasma triglycerides, fat cells and liver fat (https://classic.clinicaltrials.gov/ct2/show/NCT04714944) in 40 overweight insulin resistant participants was measured. Furthermore, similar impact of WholeFiber™ on the gut microbiota, e.g. increase in *Bifidobacterium* and *Anaerostipes,* and SCFA levels was seen (data not published yet). Both studies show that WholeFiber™ modulates the gut microbial composition and promotes SCFA production, which can be explained due to the intrinsic nature of the fibers in WholeFiber™. These fibers are still in the original plant cell structure, as the chicory root is hardly processed[17]. Therefore, WholeFiber™ partly reaches the distal colon, where fibers have the largest impact on the gut microbiota, SCFA levels and immune cell composition[23].

We are planning to conduct this study in patients with a cutaneous melanoma (T3a, T3b, T4a, T4b, N0 or N+, M0, Stage IV with no evidence of M+ disease), that have undergone curative surgical treatment and will not undergo (neo)adjuvant treatment. The product that we are going to use in this trial, WholeFiber™, is hypothesized to influence the microbial composition in a way that specific microbes, which are reported to produce SCFA, are favored on short term[16,19]. The goal of the study is to test the effect of the inulin rich dietary fiber without any conditions that are hypothesized to influence its effects[24,25]. The proposed amount of 20 g has been used in previous studies using WholeFiber™, where no adverse effects were reported[26].

1.5 Future perspectives: moving towards advanced melanoma treated with immune checkpoint inhibitors

The discovery of the link between the gut microbiome, diet and response to immune checkpoint inhibitors (ICI) in stage IV melanoma patients have shown that the gut microbiome is not only a biomarker for treatment response but can also be a modifiable treatment target[27,28]. Interestingly, the immune system plays an important role in patients with melanoma tumors.

What is unclear, and in our view should be the first small step and the objective of this study, is to determine whether changes in the gut microbiome in this group of patients (with skin melanoma) is possible at all, if these changes in the gut microbiome lead to an increased production of SCFA and whether the changes in gut microbiome; immune cell composition and SCFA can indeed be measured.

2. OBJECTIVES

The primary objective of this study is to investigate the effect of dried chicory root (WholeFiber™) for 6 weeks on fecal SCFA levels in intermediate to high-risk melanoma patients after surgical treatment with no concurrent adjuvant treatment.

Secondary objectives

- Exploration of the gut microbial composition of surgically treated intermediate to high-risk cutaneous melanoma patients before intake and potential compositional changes after using WholeFiber™ for 6 weeks.
- Exploration of the effect of WholeFiber™ use for 6 weeks on immune cell composition, stool patterns, fecal calprotectin levels and side effects in intermediate to high-risk cutaneous melanoma patients after surgical treatment, with no concurrent adjuvant treatment.

3. STUDY DESIGN

This study will be an explorative study at the University Medical Center Groningen (UMCG). All measurements, in detail described in chapter '8.3 – Study procedures', will be performed in all patients. The effect of oral intake of dried chicory root (WholeFiber™) in intermediate to high-risk cutaneous melanoma after surgical treatment, with no concurrent adjuvant treatment, will be tested. Inclusion of patients in the study can take place within 16 weeks after surgery, the baseline visit (T0) can take place later than 16 weeks after surgery. This is in line with the exclusion criterium that patients can't use antibiotics in the 3 months prior to inclusion. WholeFiber™ will be used two times a day (10 g per portion) by the patients for 6 weeks. In the first week of the intervention WholeFiber™ will be used one time a day (10 g per portion) to let the study participants adjust to the product. The study consists of 4 visits, including the informed consent visit. The estimated visit time is 60 minutes, apart from the inclusion visit which will take approximately 30 minutes.

Three timepoints are included in this study (*Figure 2*):

- Baseline (T0), where patients will collect a fecal sample at home, a blood sample will be drawn, and patients will fill in questionnaires.
- Mid-Trial (T1), collection of a fecal sample at home, a blood sample will be drawn, and patients will fill in questionnaires.
- End of Trial (T2), collection of a fecal sample at home, a blood sample will be drawn, and patients will fill in questionnaires.

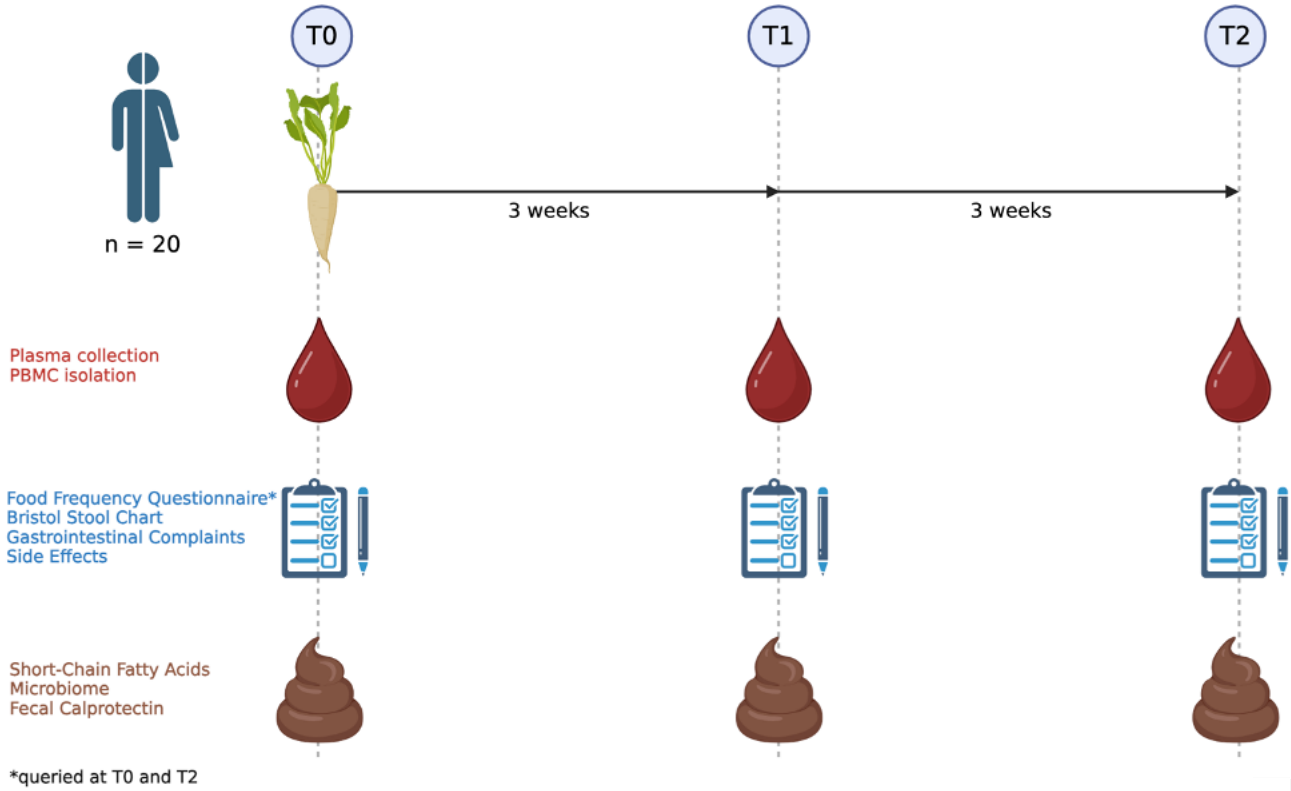

Figure 2: Trial design with 3 timepoints and specification about the measurements + questionnaires per time point.

4. STUDY POPULATION

4.1. Population (base)

The research population consists of intermediate to high-risk cutaneous melanoma patients who received surgical treatment at the University Medical Center Groningen (UMCG), that will not receive adjuvant treatment during the study period. Patients will be notified about the study at the UMCG by their treating physician and informed about the study by the coordinating investigator.

4.2. Inclusion criteria

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

- Age ≥18 years;
- The participant understands the study and can provide written informed consent;
- The participant received surgical treatment of an intermediate to high-risk cutaneous melanoma (T2a, T2b, T3a, T3b, T4a, T4b, N0 or N+, M0, Stage IV with no evidence of M+ disease);
- Being able to read and speak Dutch;
- Willing to come to the UMCG for practical reasons (visiting the study site);
- Willing to continue their regular lifestyle patterns during the study.

4.3. Exclusion criteria

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

- Receiving concurrent adjuvant treatment, adjuvant treatment after the study period is allowed.
- Having a medical history that may impact study outcomes, such as a diagnosis of diabetes mellitus type 2, heart disease, renal disease, autoimmune disease;
- Any clinically significant or unstable medical disorder involving the gut, including celiac disease, inflammatory bowel disease, short-bowel syndrome or acute/chronic pancreatitis;
- Having an ileostomy or colostomy, as this greatly impacts bowel function and gut microbial composition;
- Use of antibiotics in the 3 months prior participation in the study;
- Use of prednisolone or other immunosuppressive medication;
- Use of tube feeding or sib-feeding;
- Being pregnant or lactating;
- Participation in another interventional study at the same time;
- Unable or unwilling to comply to study rules.

4.4. Sample size calculation

A sample size calculation was not performed for this study since it is an explorative study. We do not aim to reach statistical significance, paired differences with a 95% confidence interval will suffice to reach the aim of the study. The main aim is to explore the effects of the intervention on our parameters and answer tolerability questions. Since this is an explorative study, inclusion of 20 participants should be sufficient[10].

5. TREATMENT OF SUBJECTS

We intent to use the food product WholeFiber™ (WholeFiber Holding BV, Espel, Netherlands) in the proposed trial. This product is commercially available on the Dutch market. The product consists of 100% chicory root and contains all the macro and micro-nutrients found in this root. WholeFiber™ is a vegetable product with high levels of prebiotic intrinsic dietary fibers (~85%) mainly consisting of inulin, and some pectin, hemi-cellulose and cellulose that is derived from the root vegetable, chicory roots. The dose will be 20 g/day corresponding to 17 g/day of dietary fiber, except for the first week where 10 g/day will be used. Besides dietary fibers, it contains low levels of protein (5%), fat (<1%), mono- and disaccharides (3%), organic acids (2%) and minerals (2%).

6. INVESTIGATIONAL PRODUCT

6.1. Name and description of the investigational product

WholeFiber™ is a vegetable product with high levels of prebiotic intrinsic dietary fibers (~85%) mainly consisting of inulin, and some pectin, hemi-cellulose and cellulose that is derived from chicory roots. For the production, the roots have been cut in small cubes and dried in specific conditions, resulting in granules with a size of ~4mm. Chicory roots are a major source of inulin and is therefore widely used for inulin extraction[23]. WholeFiber™ is produced in the Netherlands and commercially available. WholeFiber Holding BV is a certified food producer and is FSC22000 certified.

6.2. Summary of findings from non-clinical studies

Multiple mouse and rat studies have been performed, investigating the effect of dietary inulin on the ability to prevent colonization by pathogenic species[24,25]. Inulin-type fructans increase height of villi, depth of the crypts, number of goblet cells and thickness of the epithelial mucus layer in a study comparing germ-free rats and rats with a human fecal flora[26]. An important finding of this study was the observation that inulin-type fructans stimulated the number of bifidobacteria in the mucosa-associated flora, as assessed by molecular techniques based on fluorescent in situ hybridization with 16s rRNA-targeted probes. One *in vitro* fermentation study has been published using WholeFiber™ that found an increase in butyrate production, a SCFA that is produced by the gut microbiota and is associated with positive health effects[27].

6.3. Summary of findings from clinical studies

WholeFiber™ has been investigated in a five-week investigator-blinded randomized controlled trial including 55 subjects at risk for type 2 diabetes mellitus by Wageningen University[16]. Subjects first consumed 2 weeks of 15g/day of WholeFiber™ or 8 g of maltodextrin, followed by a three-week consumption of 30 g/day of WholeFiber™ or 16 g/day of maltodextrin. WholeFiber™ strongly modulated gut microbiota composition (7% variation, $p = .001$) and dramatically increased relative levels (3-4-fold) of *Anaerostipes* and *Bifidobacterium spp*., in a dose- dependent, reversible manner. Fecal SCFA levels such as butyrate also increased dramatically (+25.8%, $p = .023$). Furthermore, stool softness and frequency increases, and glycemic coefficient of variation decreased. In another trial by Maastricht University, 40 subjects at risk of type 2 diabetes consumed 15-30 g/day of WholeFiber™ for 12 weeks in a double-blind randomized controlled trial and was compared to maltodextrin. It was found that WholeFiber™ improved insulin sensitivity (measured by a 2-step hyperinsulinemic clamp), plasma triglycerides and liver fat content, which was not the case for placebo (in-house data, not published yet). Data-analysis of this trial is still ongoing as data collection was completed in March 2023 (https://classic.clinicaltrials.gov/ct2/show/NCT04714944). The WholeFiber™ intervention was well tolerated in both trials. Therefore, we propose that using 20 g of the product daily will be tolerated in our patient group.

6.4. Summary of known and potential risks and benefits

WholeFiber™ consists for 85% out of dietary fibers. Research participants who do not normally have a high-fiber diet may experience gastrointestinal symptoms (e.g. bloating, flatulence, diarrhea, constipation, or cramping) for a maximum of two weeks when they consume WholeFiber™. These discomforts are minor and should be limited to the first few days after starting to use the product. These discomforts should not last longer than a few hours per day. In the previous clinical trials, the participants did not report any major AEs in any of the study visits. Potential benefits are related to reported benefits of increasing fiber intake (improving stool pattern, improving glycemic control, and modulating the gut microbiota).

6.5. Description and justification of route of administration and dosage

The product is processed into food cubes and will be delivered in a commercially available package containing 350 g. The sachets containing a dose of 10 gram will be packaged by the research team member together with the participant during the first visit. This will be taken twice a day, adding up to a daily dose of 20 grams. In the first week participants will take a daily dose of 10 grams, to enable the participants to adjust to the product. Each sachet of WholeFiber™ consists of 10 grams WholeFiber™. In the study of Puhlmann et al (2022) and the Maastricht Study, subjects consumed 30 g/day of WholeFiber™, which was well tolerated. Furthermore, the mean fiber intake in the Dutch population was measured at 20.3 g/day in the national food consumption survey of 2019-2021[14], using 20 g of WholeFiber™ daily will add enough fiber to the participant's diet to meet the recommended daily fiber intake (35-40 g/day). Participants can choose how they consume WholeFiber™, this can be done as is or add/sprinkle it over their meal. Advice will be given how the product can be used for optimal compliance.

6.6. Dosages, dosage modifications and method of administration

Each sachet of WholeFiber™ consists of 10 grams of WholeFiber™. Subjects will consume 2 sachets per day for 5 weeks, resulting in 20 g/day of WholeFiber™. In the first week of the intervention period the participants consume 1 sachet per day, resulting in 10 g/day of WholeFiber™. The participant will be advised to add the product to various dishes according to one's taste. Subjects can use WholeFiber™ in the recipes if they wish, but it can also be consumed as is. Advice will be given on consumption of WholeFiber™.

6.7. Preparation and labelling of Investigational Medicinal Product

Preparation and the labeling of WholeFiber™ (350 g) will be conducted at WholeFiber Holding BV (Espel, the Netherlands), the same packaging is used as the commercially available product from WholeFiber Holding BV. Packaging of the individual sachets (supplemented by WholeFiber™) will be performed by the patient together with a member of the research team using a basetech scale (conrad article number: 1283451-83) to weigh out the portion size (10 g). Packaging of the products in sachets will be done according to the relevant GMP guidelines and takes place during the first visit. The sachets will be labelled with: Study name, content sachet (WholeFiber™), research institute, expiration date and storing conditions. Subjects will receive an additional document stating the use of WholeFiber™ and the ingredients of the sachets. The outer box will contain contact details of the investigators.

6.8. Drug accountability

Patients receive the product at the first visit. The sachets (supplemented by WholeFiber™) will be filled by the patient together with a member of the research team using a basetech scale (conrad article number: 1283451-83) to weigh out the portion size (10 g). At home, subjects are asked to store the sachets at room temperature for the duration of the intervention period. Each subject will receive 90 sachets (including 6 reserve sachets). At each visit, the patient is asked to bring the remaining sachets, and the researcher will count how many sachets are left. A calculation is made to estimate whether all sachets are used on each day.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1. Study parameters

8.1.1. Main study parameter

Effect of a high fiber prebiotic vegetable (WholeFiber™) for 6 weeks on fecal SCFA levels in intermediate to high-risk cutaneous melanoma patients, with no concurrent adjuvant treatment. HPLC ion chromatography system (Metrohm AG, Herisau, Switzerland) will be used to determine the levels of SCFA. The concentrations of SCFA; butyrate, propionate, and acetate will be measured with a conductivity detector. SCFA will be measured at baseline, halfway intervention and after 6 weeks.

8.1.2. Secondary study parameters

Discovering the gut microbial composition changes measured in intermediate to high-risk cutaneous melanoma patients with no concurrent adjuvant treatment, after using WholeFiber™ for 6 weeks. This analysis will be based on whole genome sequencing of fecal samples performed at the UMCG according to standardized protocol.

Exploring the effect of WholeFiber™ use for 6 weeks on immune functioning, stool patterns, fecal calprotectin levels and side effects in this patient group. Peripheral blood mononuclear cells (PBMCs) and bloodplasma will be isolated to determine immune cell composition before and after the intervention period. Fecal calprotectin will be determined using the respective ELISA kit. Calprotectin is released when neutrophils enter the gut wall and are activated during the inflammatory process[28]. Lastly, eating patterns, fiber intake before the interventional period, stool patterns and side effects will be identified using the Food Frequency Questionnaire[29], Bristol Stool Scale[30], Gastrointestinal Symptoms Rating Scale[31] and Health Related Quality of Life[32] questionnaires.

8.1.3. Other study parameters

Anthropometry measurements at the baseline visit such as height, weight, BMI, waist circumference. We will extract demographic and disease progression data from patients' medical records. If data is missing in the medical records, additional information will be acquired by interviewing the patient during the clinical visit(s).

8.2. Randomization, blinding and treatment allocation

Not applicable, as it is an explorative study with one arm, where we are also interested in the tolerability of the product by this patient group.

8.3. Study procedures

8.3.1. Screening

Surgically treated intermediate to high-risk cutaneous melanoma patients with no concurrent adjuvant treatment will be notified about this explorative study by their treating physician. When the patient is interested, the coordinating investigator can explain the study and provide the patient with the patient information form (PIF). The patient and the study coordinator can discuss about the next steps in study participation. Written informed consent for participation in the trial must be obtained before performing any study specific screening tests or evaluation. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before enrollment.

8.3.2. Assessment of medical history and demographics

Medical history includes clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and all medications (e.g. prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 3 months prior to the screening visit. Demographic data will include age, sex, BMI, and self-reported race/ethnicity.

8.3.3. Blood sample collection

Blood will be collected at the medical oncology department of the UMCG using a standardized protocol. The drawing of blood will be performed by trained employees and the time of sampling will be recorded on a blood drawing form. Blood will be drawn from the antecubital vein in the arm. During each visit, 10 ml of blood will be collected for PBMC isolation and plasma measurements. Specific collection tubes that are used are CPT tubes.

Blood will then be transferred to the laboratory site and PBMC isolation will take place immediately. The average amount of cells that can be isolated is 1.30*106 PBMCs/ml. PBMCs and plasma will then be stored at -80ºC, at the same laboratory site as the isolation, until analysis. PBMCs will be tranfered to liquid nitrogen storage 24 hours after isolation of the PBMCs.

8.3.4. Laboratory assessments

Samples of venous blood drawn at each visit will be analyzed at the study site's local laboratory. Local laboratory assessments will include PBMC isolation and storage of plasma. By using flow cytometric analysis the immune cell composition will be determined (Table 1).

Table 1: Flowcytometry panel PBMC analysis

Marker	Fluorochrome	Clone	Cell Type	Marker Type
CD24	BUV395	ML5	Monocytes	Surface
CD27	BUV661	M-T271	Memory T cells	Surface
CD45	BUV737	HI30	Leukocytes	Surface
CD38	BUV805	HB7	Activated T cells	Surface
CD3	APC-H7	SK7	T cells, γδ T cells	Surface
CD45RA	BV711	HI100	Naive T cells	Surface
CD4	BV785	OKT4	Helper T cells	Surface
FoxP3	AF488	259D	Regulatory T cells (Tregs)	Intracellular
CD8	PerCP/Cy5.5	SK1	Cytotoxic T cells	Surface
CD14	APC	HCD14	Monocytes	Surface
TNF	PE	MAb11	Inflammatory cytokine	Intracellular
IFNγ	PE/Cy7	4S.B3	Cytokine in T cells	Intracellular
CD25	PE	BC96	Regulatory T cells (Tregs)	Surface
TCR γδ	FITC	B1.1	γδ T cells	Surface
CD11c	BV605	3.9	Dendritic cells, monocytes	Surface
CD16	BV510	3G8	Non-classical monocytes	Surface

8.3.5. Fecal sampling

Three tubes of feces will be collected prior to the WholeFiber™ intervention, mid-trial, and at the end of the 6-week intervention period. After instructions, patients will collect their feces at home in a standardized manner. See section 15.2 for a detailed description of the stool sampling.

The stool samples must be stored in a (home) freezer within 10 minutes after collection. At the study visit, the stool samples will be brought by the participant in a cooler bag. These procedures are based on protocols used in Lifelines. After processing, the samples will be stored at -80ºC at the Microbiome Hub (UMCG).

8.3.6. Analyses of fecal material

Collected tubes with feces samples will be stored (at -80°C) until a sufficient batch size is present for further analyses (calprotectin, microbiome, SCFAs). Analyses of the microbiome will be conducted within the UMCG following standardized procedures. The composition and function of the gut microbiome will be analyzed prior to, mid-trial, and after the WholeFiber™ intervention using whole genome sequencing. Temporal changes in the gut microbiome following the WholeFiber™ intervention can be traced up to species and even strain level by using novel algorithms[33]. Analyses and interpretation of the microbiome will be in the context of patient characteristics (age, sex, BMI), and use of medication, including vitamins, probiotics, and homeopathic compounds.

HPLC ion chromatography system (Metrohm AG, Herisau, Switzerland) will be used for the short chain fatty acid analysis. The concentrations of SCFA (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, 2-methylbutyric acid, caproic acid) will be measured with a conductivity detector. The 6.1005.200 Metrosep Organic Acids column (Metrohm AG) with 5mm particle size and dimensions of 7.8 mm by 250 mm will be used for differentiating fatty acids. The mobile phase is 5mM HNO_3 and acetonitrile v/v ratio of 98:2, respectively, with a flow rate of 0.6 ml per minute.

Fecal calprotectin levels will be measured in the collected fecal samples (baseline, mid-trial, end of intervention) using Enzyme-Linked Immuno Sorbent Assay (ELISA).

8.3.7. Questionnaires

Subjects will complete the questionnaires prior to the study visit. After enrolment in the study, they will receive the questionnaires via post (physical or online), which need to be filled in prior to every study visit. This will take up to one hour. These questionnaires include:

- Food Frequency Questionnaire[29,34] (FFQ)
- Health Related Quality of Life[32] (HR-QoL)
- Bristol Stool Chart[30]
- Gastrointestinal Symptom Rating Scale[31] (GSRS)

8.3.8. Storage of samples

All patient related material (blood and feces) will be l be anonymized by using a unique patient identification code (see also section 12.1)

8.4. Withdrawal of individual subjects

Subjects can leave 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. If subjects leave the study, their results (blood samples, fecal samples, and questionnaires) will be used for the analyses unless the patient specifically states that we may not use the collected data and material when signing the informed consent.

8.5. Specific criteria for withdrawal

Reasons for withdrawal from the study may include but are not limited to the following:

- Subject withdrawal of consent at any time;
- Any medical condition that the investigator determines may jeopardize the subject's safety if he or she continues in the study;
- Investigator determines it is in the best interest of the subject;
- Subject non-compliance.

8.6. Replacement of individual subjects after withdrawal

If subjects withdraw before the intervention has started, they will be replaced.

8.7. Premature termination of the study

The study is a short intervention study and we do not expect a premature termination. Since WholeFiber™ is suitable for human consumption, safe, and commercially available, we do not expect any negative events. Consumption of the product may result in uncomfortable though harmless effects such as increased flatulence or bloating. Research has shown that these adverse events are highly variable among people.

9. SAFETY REPORTING

9.1. Temporary halt for reasons of subject safety

In accordance with section 10, subsection 4 of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize the health or safety of the subjects participating in the study. 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 primary investigator will take care of the fact that all subjects are kept informed about the decisions.

9.2. AEs, SAEs and SUSARs

9.2.1. Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator, will be recorded.

9.2.2. 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 hospitalization or prolongation of existing inpatients' hospitalization;
- 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.

The investigator 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.

9.3. 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.

10. STATISTICAL ANALYSIS

Statistical analyses will be performed using R. In general, continuous data will be presented as mean ± standard deviation (SD) or median and interquartile range when skewed, and categorical data as counts and percentages. To correctly model the paired pre-post study design, we will use a linear mixed-effects model (*lme4*-package, version 1.1-35.2, Bates et al., 2015). This is performed by adding a patient-specific intercept and slope. As this is an exploratory stud, mean differences with a 95% confidence interval will be considered as statistically significant. A false discovery rate (FDR) of *5%* will be used in regression analyses, regarding SCFA and bacterial taxa, to correct for multiple testing. We will adjust for differences in age, sex, BMI, and fiber intake before the start of the intervention by including these as independent variables in the linear mixed-effects models described below.

10.1. Primary study parameter(s)

This study population consists of one group, where the primary endpoint is defined as the effect of WholeFiber™ for 6 weeks on fecal SCFA levels in surgically treated intermediate to high-risk cutaneous melanoma patients with no concurrent adjuvant treatment. The study is considered to have a positive outcome when paired differences are measured in fecal SCFA levels, comparing pre- and post-intervention. This will be analyzed using a linear mixed-effects model, modeling the fecal SCFA levels post-intervention as the dependent variable and fecal SCFA levels pre-intervention together with patient-specific intercepts and slopes to take paired sampling into account. To further study the role of the gut microbiota, the relationship between post-intervention fecal SCFA levels and gut microbiota composition will be tested using a similar linear mixed-effects model. In this model, the post-intervention fecal SCFA levels will be modeled as the dependent variable and relative abundance of specific bacterial species together with patient-specific intercepts and slopes as independent variables.

10.2. Secondary study parameter(s)

To analyze changes in gut microbiota in surgically treated intermediate to high-risk cutaneous melanoma patients using WholeFiber™ for 6 weeks, we will use a similar linear mixed-effects model as described above. In this model, the relative abundance of bacterial species post-intervention will be used as the dependent variable and their relative abundance pre-intervention together with patient-specific intercepts and slopes as independent variables. Furthermore, the effect of WholeFiber™ for 6 weeks on the microbial diversity will be evaluated using both alpha and beta diversity metrics. Alpha diversity metrics quantify within sample diversity and can be compared across groups. Beta diversity compares between sample diversity and is often calculated by computing a dissimilarity index (e.g. Bray-Curtis).

10.3. Other study parameters

Other study parameters such as anthropometry data or data from medical records will not be statistically tested but is used to describe the study population.

11. ETHICAL CONSIDERATIONS

11.1. Regulation statement

The study will be conducted in accordance with this protocol as well as the principles of the Declaration of Helsinki (64th WMA general assembly; October 2013), the ICH-GCP guidelines and other applicable laws and regulations, and in accordance with the principles of the Medical Research Involving Human Subjects Act (WMO). The study will start after approval of the medical ethical committee (METc) is obtained. The investigators in this study declare to have no conflict of interest.

11.2. Recruitment and consent

All participants are recruited from the UMCG. Also, patients who participated in earlier research projects may be re-invited. Patients who seem to meet the inclusion criteria are invited by their treating physician to consider participation. The researcher decides if the patient can make a well-informed decision. The primary investigator provides information to the patient using the PIF and arranges a first inclusion visit with the patient (either on site or virtual). The patient is a given 2 weeks to consider participation. During this time, the patient is encouraged to council for advice using various sources. After one-week, additional questions can be answered and if the patient opts to participate the informed consent is signed. Documented informed consent must be obtained from all patients prior to inclusion in the study. Informed consent will be asked in writing, and in the following manner: the patient will sign and personal date the informed consent form first. When the investigator has signed and dated the consent form afterwards, the patient will receive a copy. Finally, the general physician of each patient will be informed about the enrolment of the patient to the study by a standard letter. The informed consent procedure takes place conform Good Clinical Practice (GCP). Documented informed consent must be obtained from all subjects prior to inclusion in the study. All subjects will be informed about their right to withdraw their agreement at any time without resulting in any claim of damage and about the possibility to ask for supplementary information from an independent physician.

11.3. Benefits and risks assessment

Participating in this study has a potential health benefit. It is known that an increased intake of fiber has a positive effect on the abundance of beneficial bacteria. In patients with melanoma, it is hypothesized that there's an imbalance in the bacterial composition in the gut, leading to a reduction in beneficial bacteria. If melanoma patients experience a rise in beneficial bacteria, making the gut microbial composition more like the microbiome of healthy people, it may result in a more favorable disease outcome (reduced reoccurrence of the removed cutaneous melanoma).

Participants could experience some bloating or flatulence, which are GI symptoms that are generally related to an increased fiber intake, when they start with WholeFiber™. These discomforts are limited to the first few days of use and should not last longer than a few hours per day. In the previous clinical trials, using 30 g of the product, the participants did not report any major AEs in any of the study visits. The collection of feces and blood samples entails no risks for the subjects.

11.4. Compensation for injury

The investigator has a liability insurance, which is in accordance with article 7, subsection 6 of the WMO. The sponsor(s) (also) has/have 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 23rd June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450,000 (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3,500,000 (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5,000,000 (i.e. five million Euro) for the total damage incurred by the organization 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.
Note: an exemption for the insurance obligation, due to the negligible risk of this study is requested.

11.5. Incentives

Travel costs will be compensated: the subjects will receive a compensation of 0.21 euro/km when travelling by car. In addition, participants will receive a ticket for free parking at the UMCG on study days. The total amount of money will be paid after completing the study, and after the last samples have been collected. The dietary supplement will be given to the participant free of charge. Participating in the study will not result in costs for the participant.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1. Handling and storage of data and documents

The handling of personal data complies with the Dutch 'GDPR (General Data Protection Regulation) and AVG (Algemene Verordening Gegevensbescherming). All related documents and records will be kept and archived for at least 15 years. The collection and processing of participants' personal information will be limited to what is necessary to ensure the study's scientific practicability, the evaluation of efficacy, adherence, side effects and the investigational product's safety. Information collected about participants during this clinical investigation will be treated confidentially. The investigator or her co- workers will collect data and transfer it without recording the patient's name or date of birth. Instead, data will be coded with a participant identification number. This identification number consists of the study name and the participant number (e.g. MELFIB-001, MELFIB-002, etc.).

The file with the key to the code will be managed by one person. The source documents will be kept in a locked file cabinet with limited access of the research personnel. In accordance with national laws and guidelines and the specifications of the ICH-GCP guidelines, the investigators are obligated to archive all documents pertaining to the study for the legally required period.

The acquired data and examination results will be entered into an electronic case record form (eCRF) that is accessible via the internet. Investigators will receive personal usernames and passwords for this purpose, and data will be encrypted for transfer. It will be agreed before the start of the study which documents serve as source documents for all data included in the eCRF.

12.2. Monitoring and quality assurance

Associated investigators will be carefully selected and comprehensively informed and trained regarding Good Clinical Practice (GCP), all study procedures and the required examinations and documentation.

The quality of data acquisition will be confirmed by regular monitoring visits as described in the Monitoring Plan. The monitor is independent from the study team and is not involved in the inclusion of participants and the design and implementation of the study. All monitoring activities will be in line with national laws and guidelines and the specifications of the ICH-GCP guidelines.

According to the NFU Richtlijn Kwaliteitsborging Mensgebonden Onderzoek (versie September 2023) this study can be identified as a low-risk study. Study monitors will visit the study site minimal once per year of which a minimal of two visits have to be on-site during the research period. Monitors will have access to all documents that are needed to perform their task according to the above-mentioned guidelines. Monitors will check whether requirements to conduct the study are met and study procedures are followed correctly, and will check the study site's documentation, the participants' source data, eCRF entries, and the correct maintenance of the Investigator Site File. Investigators will permit trial-related monitoring, audits, ERB reviews and regulatory inspections, providing direct access to source data and study documents.

12.3. 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 submitted for approval to the METc and to the competent authority. For non-substantial amendments, only a notification will be sent to the accredited METc, which will be recorded and filed by the investigator. Examples of non-substantial amendments are typing errors and administrative changes like changes in names, telephone numbers and other contact details of involved persons mentioned in the submitted study documentation.

12.4. Annual progress report

The 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.

12.5. 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 investigator 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.

12.6. Public disclosure and publication policy

The sponsor of this study is the UCMG, where the main goal of the study is to contribute to research and to publish the results. The results of the study will be disclosed unreservedly, both positive and negative trial results must be disclosed. In general, the results of this research will be submitted for publication in an international peer-reviewed journal adhering to applicable privacy laws and regulations. Publication strategy will be determined by the principal investigator.

13. STRUCTURED RISK ANALYSIS

13.1. Potential issues of concern

a. Level of knowledge about mechanism of action

The gut microbiome composition of patients with an immunogenic tumor as an intermediate to high-risk cutaneous melanoma (minimal stage II A) is currently unknown. However, a link between the gut microbiome, diet and response to immune checkpoint inhibitors in melanoma highlights the gut microbiome as a potential biomarker of response to treatment[1]. The gut microbiome can be modified through diet, and therefore is a potential therapeutic target.

A higher intake of dietary fiber is linked to positive changes in the gut microbiota and an increase in SCFA-production[35–39]. Dietary fibers can't be metabolized by human enzymes and therefore are fermented in the colon by the gut microbiota, which are able to produce SCFA. Daily intake of 15 g WholeFiber™ for 2 weeks has been shown to greatly modulate the gut microbiota and impact SCFA-production in adults with prediabetes[7]. In our study, we aim to explore the effects of a WholeFiber™ intervention in surgically treated intermediate to high-risk cutaneous melanoma patients, with no concurrent treatment, on fecal SCFA levels and gut microbial composition.

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

WholeFiber™ has been tested before in a clinical trial in Dutch adults with prediabetes with positive effects, and a daily dosage of 30 g was well tolerated[16]. Currently, WholeFiber™ is under investigation in healthy adults with constipation complaints (HappyFiber study, NL80274.091.22, currently recruiting).

c. Analysis of potential effect

We expect that the WholeFiber™ intervention will greatly modulate the gut microbiota and increase fecal SCFA levels. The gut microbial composition and SCFA production are hypothesized to play an important role as potential therapeutic target for patients with an immunogenic tumor classified as an intermediate to high-risk cutaneous melanoma (minimal stage II A). However, this is an explorative study. If indeed we see these effects, this needs to be further tested in a large randomized controlled trial.

d. Study population

For this study, we will recruit around 20 surgically treated intermediate to high-risk cutaneous melanoma patients, with no concurrent adjuvant treatment.

e. Interaction with other products

WholeFiber™ is a dried vegetable that is safe for consumption and commercially available. We do not expect an interaction with other products as this is a food product.

f. Predictability of effect

In the previous study of Puhlmann et al. (2022) an increase of +26% in total SCFA levels was measured within two weeks after 15 g/day consumption of WholeFiber™ in adults with prediabetes. Furthermore, a 3-4-fold increase in butyrate-producing bacteria *Anaerostipes* and *Bifidobacterium* was observed. A similar effect can be expected in this study population. No previous studies have been performed before on the effects of WholeFiber™ in a population with cutaneous melanoma patients, however this study aims to explore these effects.

g. Can effects be managed?

Not applicable, WholeFiber™ is a dried vegetable, which is commercially available and proven safe for consumption.

13.2. Synthesis

All subjects included in this study are surgically treated intermediate to high-risk cutaneous melanoma patients, with no concurrent adjuvant treatment and no autoimmune disease. No children will be included in this study. Pregnancy is one of the exclusion criteria in this study.

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15. APENDIX

15.1. Flow chart of study procedures

This table describes the detailed measurements included per visit. An overview of the sampling timeline is depicted in figure 2.

	Screening	Baseline	Mid-trial (3 week)	End-trial (6 week)
Signed Informed Consent Forms[1]		X		
Eligibility criteria	X			
Medical, surgical, and cancer histories, including demographic information[2]		X		
Clinical Evaluation/Anamnesis [3]		X	X	X
Concomitant medication[4]	X			
Weight and height[5]		X		
Blood sampling[6]		X	X	X
Feces sampling[7]		X	X	X
FFQ		X		X
HR-QoL		X	X	X
Bristol Stool Chart + GSRS		X	X	X

[1]Written informed consent can be obtained up to 30 days prior to study entry and is required for performing any study-specific tests or procedures. Results of standard-of-care tests or examinations performed prior to obtaining informed consent and within 28 days prior to study entry may be used for screening assessments rather than repeating such tests. The formal written consent for this trial of a subject must be obtained before initiation of any study-specific procedures.

[2]Cancer history includes date of diagnosis. Demographic information includes sex, age, and self-reported race/ethnicity, smoking history.

[3]CTCAE version 5.0

[4]Concomitant medications include any prescription medications or over-the-counter medications, and nutritional supplements. At screening, any medications the subject has used within the 3 months prior to the baseline visit should be documented.

[5]Weight and height will be assessed on the baseline visit.

[6]Blood sampling of 10 ml whole blood to collect PBMCs and plasma for further analysis.

[7]Feces sampling will be collected in a standardized manner as described in section 15.2 Feces sampling, participants will collect 3 frozen samples.

15.2. Fecal sampling

In deze instructie leest u welke materialen u van het onderzoeksteam ontvangt voor het verzamelen van ontlasting. In de beschrijving leest u stap voor stap hoe u de ontlasting thuis verzamelt. U wordt aangeraden de instructie eerst helemaal door te lezen voordat u daadwerkelijk de ontlasting gaat verzamelen.

Heeft u vragen over het verzamelen van ontlasting? Neem dan gerust contact met ons op.

U verzamelt de ontlasting thuis op één moment in de drie dagen voorafgaand aan de dag dat u een onderzoek afspraak heeft. Indien u bijvoorbeeld op woensdag een afspraak heeft; kunt u op zondag, maandag of dinsdag de ontlasting verzamelen. U vult alle buisjes in één keer.

Het opvangen en bewaren van de ontlasting verloopt in zes stappen.

- Opvangen van de ontlasting
- Verzamelen van ontlasting in de pipet
- Overbrengen van ontlasting in de pipet naar buisje
- Verzamelen van ontlasting met gebruik van een schepje
- Buisjes verzamelen in het zwarte zakje
- Bewaren van het zwarte zakje met de buisjes in de vriezer

In dit document worden de stappen nader toegelicht.

Materialen

Om de ontlasting te kunnen verzamelen ontvangt u de volgende materialen (controleer uw ontlasting verzamelpakket):

- 1x Grijs opvangbakje
- 1x Handschoenen
- 1x Ontlasting opvanger
- 1x Schaar
- 3x Buisjes
- 1 Buisje met schepje
- 3x Plastic pipet
- 1x Zwart zakje
- 1x Doorzichtig zakje
- 1x Koeltas
- 1x Ontlastingsverzamelformulier

Stap 1: Opvangen van de ontlasting

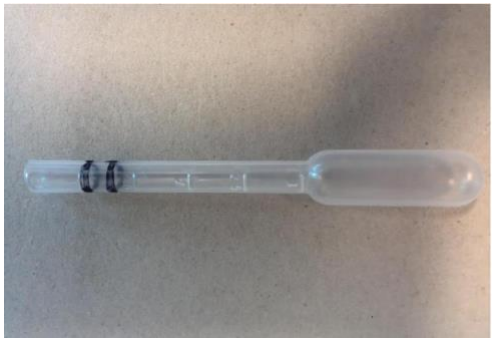
Om ontlasting op te vangen, gebruikt u de meegeleverde ontlasting opvanger.

- Vouw voorzichtig de ontlasting opvanger open in de richting van de pijltjes.
- Bevestig deze met de plakstroken op het achterste deel van de wc-bril, zonder dat de plakstrip met het water in contact komt.
- Vang de ontlasting op.

Stap 2: Verzamelen van ontlasting in de pipet

Na opvangen van de ontlasting, kunt u met de pipet de ontlasting verzamelen. Gebruik voor deze stap de handschoenen.

- Knijp het ballonnetje bovenaan de pipet voorzichtig in en houd het uiteinde van de pipet in de ontlasting.
- Druk de pipet tot ongeveer het eerste zwarte streepje in de ontlasting en laat het ballonnetje voorzichtig los. Door het loslaten van het ballonnetje kan de ontlasting een beetje omhoog gezogen worden, dat is niet erg.
- Veeg de buitenkant van de pipet af met wc-papier.



Stap 3: Overbrengen van ontlasting in de pipet naar buisje

Herhaal stap 2 en 3 zodat u in totaal 3 kleine buisjes met een pipet ontlasting heeft verzameld.

- Breng het uiteinde van de pipet over in het kleine buisje met de schroefdop. Probeer hierbij geen contact te maken met de schroefdraad.
- Knip het stukje pipet dat in het buisje zit af bij het tweede zwarte streepje.
- Het stukje pipet wat overblijft kunt u weggooien in de prullenbak.

Stap 4: Verzamelen ontlasting met gebruik van een schepje

- Draai de dop met het schepje van de buis af.
- Steek het schepje in de ontlasting.
- Schep de ontlasting op.
- Breng het schepje naar het buisje en vul het buisje maximaal tot de helft. Probeer hierbij geen contact te maken met het schroefdraad van het buisje.

Stap 5: Buisjes verzamelen in het zwarte zakje

- Doe de drie kleine buisjes en het buisje met schepje rechtop in het zwarte zakje.
- Vul het ontlastingsverzamelingsformulier in en steek deze ook in het zwarte zakje.
- Doe het zwarte zakje in het doorzichtige zakje, zorg ervoor dat uw gegevens zichtbaar blijven.

Stap 6: Bewaar het zwarte zakje met de buisjes in de vriezer

- Plaats de verzamelde materialen direct in de vriezer.
- Bewaar het zwarte zakje in de vriezer tot de onderzoek afspraak, u neemt het zwarte zakje in de koeltas mee naar de afspraak.

Het verzamelen van ontlasting is nu klaar en de ontlasting opvanger kan nu doorgespoeld worden. Wacht voor het doorspoelen een ogenblik, zodat het papier wat water opneemt en makkelijker door te spoelen is. Het grijze bakje kan in de afvalbak of container.