STUDY PROTOCOL

The effects of a dietary regimen characterized by a high consumption of cruciferous vegetables on recurrence rate in patients with Intermediate, High and Very High risk Non-Muscle Invasive Bladder Cancer

P.I. Prof. Cosimo De Nunzio

INFORMED CONSENT FORM (version 2.0 of September 13, 2025)

Official Title of Study:

The Effects of a High-Cruciferous Vegetable Diet on Recurrence Rate in Patients with Intermediate-, High-, and Very High-Risk Non-Muscle-Invasive Bladder Cancer (CRUCIAL-R)

RECRUITMENT DETAILS

You are invited to participate in a nonprofit, prospective, interventional study to evaluate the effects of a high-cruciferous vegetable diet on recurrence rate in patients with intermediate-, high-, and very high-risk non-muscle-invasive bladder cancer.

New evidence highlights the importance of nutrition in modulating cancer-related pathways, suggesting that specific dietary patterns and food choices may influence cancer risk. A protective role has been suggested for high consumption of vegetables, unsaturated fatty oils (Mediterranean diet), fruits, and flavonoids, while a diet high in saturated fats and meat has been linked to an increased risk of bladder cancer.

In particular, the consumption of cruciferous vegetables, such as broccoli, cauliflower, Brussels sprouts, cabbage, kale, turnips, and others, has attracted attention for their potential protective effects due to their bioactive compounds: isothiocyanates (ITCs). Dietary ITCs are a group of phytochemicals with multifaceted anticancer mechanisms, derived primarily from cruciferous vegetables (CVs) as glucosinolates and converted into ITCs by the action of the enzyme myrosinase. Organic ITCs, particularly allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC), and sulforaphane (SF), are among the most widely studied cancer chemopreventive agents.

This study is a prospective, nonprofit, interventional study and does not impact the standard treatment regimen of uro-oncology patients undergoing transurethral resection of the bladder (TURB).

Before deciding whether or not to participate in this study, we ask you to carefully read the following information, which outlines the study objectives and the implications of your potential participation.

Pre-assignment Details

There are adverse effects associated with the consumption of cruciferous vegetables. Iodine absorption is affected, and therefore they should be consumed with caution in patients with hypothyroidism. The consumption of cruciferous vegetables may be dangerous for certain patient groups. Therefore, if you decide to participate in the study, you may be excluded based on the Exclusion Criteria in the protocol. Hypothyroidism and the use of anticoagulants contraindicate your participation in the aforementioned study.

ARM GROUP 1

Patient with a normal dietary regimen

ARM GROUP 2

Patient with a diet characterized by a high consumption of cruciferae vegetables

PERIOD

3 years from 18th October 2025 to 18th October 2028

NUMBER OF PARTICIPANTS

250 patients

Exclusion criteria:

o Low-risk NMIBC

o Muscle-invasive bladder cancer

o Concomitant upper urinary tract urothelial bladder cancer

o Radiation therapy to the prostate and bladder

o Urinary tract infection

o Chronic urinary retention or indwelling catheterization

o Other concomitant oncological conditions

o Active oncological treatments in the last 12 months

o Persistent disease (defined as residual disease detected within three months of transurethral resection of the bladder (TURB) or at the first follow-up cystoscopy

o Specific dietary restrictions such as vegetarianism, celiac disease, or lactose intolerance

o Chronic renal failure

o Chronic inflammatory diseases such as Crohn's disease or ulcerative colitis

o Personal history of gastric resection

o Liver disease such as cirrhosis or elevated ALT/AST levels three times above normal

o Pathologies thyroid (hypothyroidism, ...)

Clinical evaluation:

Patients with intermediate/high- and very high-risk NMIBC will be enrolled. Each patient will be assessed for:

• Age

• Gender

• Employment status

• Smoking

• Comorbidities

• Concomitant treatments and therapies

• Weight, Height, BMI

• Standard clinical laboratory tests

o Hemoglobin serum level

o Creatinine

o Urinalysis

• EORTC/EAU risk stratification

• Tumor characteristics

o Stage

o Grade

o Size

o Multiple/single

o Concomitant Carcinoma in Situ (CIS)

• Surgery

o TUR-B

o Re-TUR-B

o Induction ± maintenance with Bacillus Calmette-Guerin (BCG) or other immunotherapies

• Follow-up

o Cystoscopy and cytology every 3 months

o Recurrence rate

o Number of recurrences

o Number of tumor lesions

o Diameter of tumor lesions

o Location of lesions in the bladder

• **Questionnaires** and tools for assessing diet and QoL:

o Short 15-Item Food Frequency Questionnaire

o EORTC QLQ-C30 questionnaire

o Self-reported CV intake (CV-FFQ)

Intervention:

Patients will be assessed for study eligibility at the screening visit (Visit 1) three weeks after TURB. Only patients meeting the inclusion criteria will be included: patients diagnosed with Intermediate/High and Very High risk NMIBC, according to the EAU NMIBC prognostic factor risk groups.

Patients will receive a diet high in Cruciferae vegetables (≥1 cup (250g)/day).

STUDY'S PURPOSE: To evaluate the effect on recurrence rate of a diet characterized by a high consumption of cruciferae vegetables versus the absence of such intake in patients with intermediate, high, and very high-grade NMIBC.

Outcomes

PRIMARY : RECURRENCE FREE SURVIVAL (RFS)

1. Primary objective: To evaluate the effect on recurrence rate of a diet characterized by a high consumption of cruciferae vegetables versus the absence of such intake in patients with intermediate, high, and very high-grade NMIBC.

2. Secondary objectives:

- To evaluate the effect on time to recurrence of a diet characterized by a high consumption of cruciferae vegetables versus the absence of such intake in patients with intermediate, high, and very high-grade NMIBC.

- To evaluate the impact of high consumption of cruciferae vegetables on quality of life (QoL).

PROCEDURES

Study Description

This study is a non-profit, prospective, interventional study and does not affect the normal

therapeutic pathway of uro-oncology patients undergoing transurethral resection of the bladder.

Participation will be voluntary.

If you agree to participate in the study, your data will be entered into an anonymized database. This study will NOT alter your clinical outcome in any way.

You will be randomized to one of the two study groups. One of the two groups, in addition to regular quarterly checkups as per standard clinical practice, will be prescribed a diet high in cruciferous vegetables. Both groups will be asked to complete questionnaires to assess their quality of life.

Statistical Analysis Plan

The anonymized data will be entered into a Microsoft Excel database and subsequently analyzed using STATA 14.1 software (College Station, TX, USA). A preliminary analysis of the various variables will be performed to assess whether they are normally distributed.

The differences between the two groups of patients analyzed (with and without cruciferous vegetables) will be assessed using the Kruskall-Wallis test for continuous variables and the chi-square test for discrete variables. Furthermore, the analyzed variables will be evaluated using univariate binary logistic regression to determine whether they can be considered risk factors. Statistically significant variables in the univariate analysis will then be evaluated using multivariate binary logistic regression. The accuracy of the multivariate model will be assessed using ROC curves, C-Index, calibration curves, and clinical benefit (decision curve analysis). The model will then be transformed into a graphical model (nomogram) so that the analysis can be applied clinically. Type I error will be assessed as the probability of rejecting the null hypothesis when it is true, with a significance level (α) set at 0.05 (as in most social and/or health study protocols). Setting an alpha of 0.05 ensures that type II error (β) is minimized and therefore the power of the test is maximized ($1-\beta$).

A power analysis was conducted before data collection using SPSS to determine the appropriate sample size. A test was performed with a Type I error rate (α) of 0.05 and a statistical power of 80% to ensure adequate power to detect a significant effect. The expected effect is a 40% relative reduction (10% absolute reduction) in the recidivism rate in the study group, given a recidivism rate in the general population (according to the EAU risk stratification) of 25% at 1 year. To detect this difference, 250 patients are needed, using the adjusted chi-square method.

POTENTIAL BENEFITS

The use of cruciferous vegetables as a food with beneficial health effects stems from new evidence highlighting the importance of nutrition in modulating cancer-related pathways, suggesting that specific dietary patterns and food choices may influence cancer risk. A protective role has been suggested for high consumption of vegetables, unsaturated fats (Mediterranean diet), fruits, and flavonoids, while a diet high in saturated fats and meat has been linked to an increased risk of bladder cancer. In particular, the consumption of cruciferous vegetables, such as broccoli, cauliflower, Brussels sprouts, cabbage, kale, turnips, and others, has attracted attention for their potential protective effects due to their bioactive compounds: isothiocyanates (ITCs). Dietary ITCs are a group of phytochemicals with multifaceted anticancer mechanisms, derived primarily from cruciferous vegetables (CVs) as glucosinolates and converted into ITCs by the action of the enzyme myrosinase. Organic ITCs, particularly allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC), and sulforaphane (SF), are among the most widely studied cancer chemopreventive agents.

Withdrawal from the study

Even after consenting to participate, you may withdraw from the study at any time without providing any justification. In this case, no additional data will be added to the database and, in accordance with current legislation, you may request the withdrawal of the data entered to prevent further analysis.

Privacy and Use of Clinical Information

To conduct the study, it will be necessary to use some information from your medical record. Your consent will authorize us to use this information, in accordance with privacy protection law (EU Privacy Regulation 2016/679 (GDPR) and subsequent amendments, as per the Guidelines of the Italian Data Protection Authority for the Processing of Personal Data in Clinical Trials of Medicinal Products (Official Gazette 190 of 14/08/2008) and any other provisions/authorizations of the Italian Data Protection Authority).

Please note that participants have the right to obtain updated information on the recorded data and the right to request correction of errors.

The data will be stored in an anonymized database with an individual identification number. The paper components will be stored for at least 15 years in the Urology Clinical Audit Department located on the 5th floor of the Urology Department in the clinical studies cabinet equipped with a security key.

Research Results

The results obtained from this study will be published in a leading medical journal.

We wish to reiterate that this consent refers only to your participation in the study and does not

in any way replace the Informed Consent for anesthesia and the surgical procedure, for which you will receive adequate information and which you will be required to sign separately.

Any questions regarding the study can be addressed to Prof. Cosimo De Nunzio, P.I., of the Urology Unit of the Sant'Andrea Hospital in Rome (tel. 0633777716 – email cosimo.denunzio@uniroma1.it).

If you have any questions regarding your rights as a study participant, please contact the CET Lazio Area 1 Secretariat and its email address: comitatoetico.lazioarea1@policlinicoumberto1.it

Thank you for taking the time to read this information sheet.

Adverse Events

All the adverse events that will occur in the study will be recorded in the case report form (CRF).

An adverse event (AE) is defined as a harmful clinical event occurring in a patient or a human volunteer involved in a clinical experimentation who received a drug that does not necessarily have a relationship with the treatment given. It is considered AE any medical occurrence including undesirable signs or symptoms or abnormal lab finding.

A Serious Adverse Event (SAE) is an AE that, independently from the dosage of the drug used, had one of the following characteristics:

1) Results in death
2) It's life threatening (i.e. the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
3) It's cause of hospital admission or increase the time of hospitalization
4) It's cause of congenital abnormalities at birth
5) It's cause of disability or severe and long lasting inability

Treatment Emergent Adverse Events (TEAE) is defined as any event not present prior to the initiation of the treatments or any event already present that worsens in the either intensity or frequency following exposure to the treatments.

A SUSAR (Suspected Unexpected Serious Adverse Reaction) is the term used to refer to an adverse event that occurs in a clinical trial subject, which is assessed as being unexpected, serious and as having a reasonable possibility of a causal relationship with the study drug. It is defined as an untoward and unintended response to a study drug, at any dose, which is not listed in the product information, and meets one of the above mentioned serious characteristics.

Generally AEs are collected after signing the informed consent form and could be related or unrelated to the study drug.

For any AE/SAE recorded during the study a causal relationship with the drug used will be assessed as follows:

- Very likely: the AE/SAE is temporally associated to the drug administration, cannot be explained by other clinical conditions and/or concomitant treatments, disappears with drug withdrawal and recurs after drug reintroduction;
- Likely: the AE/SAE is temporally associated to the drug administration, cannot be explained by other clinical conditions and/or concomitant treatments, disappears with the drug withdrawal. There is no information on drug reintroduction;
- Possible: the AE/SAE is temporally associated to the drug administration, cannot be explained by other clinical conditions and/or concomitant treatments, disappears with drug. There is no information available on the drug withdrawal and reintroduction;
- Doubtful: the AE/SAE could be temporally associated to the drug administration, but can be explained by other clinical conditions and/or concomitant treatments;
- Not correlated: the AE/SAE is not temporally associated to the drug administration and/or can be explained by other clinical conditions and/or concomitant treatments.
- An AE/SAE with causal relationship very likely, likely possible with the drug is considered correlated with it and is defined Adverse Reaction (ADR).

WHO defines ADR as “a response to a drug which is noxious & unintended and which occurs at doses normally used for prophylaxis diagnosis or therapy of a disease or for modification of a physiological function”.

The difference between AE and ADR is that AE event does not imply causality but not for ADR, a causal rule is suspected.

An ADR unexpected is defined an ADR that for nature and severity is not listed in the product information (Reference Safety Information (RSI)/Investigator’s Brochure (IB)).

Patient information sheet

Study Title: The Effects of a Diet High in Cruciferous Vegetables on the Recurrence Rate in Patients with Intermediate, High, and Very High-Risk Non-Muscle-Invasive Bladder Cancer (CRUCIAL-R)

Principal Investigator: Prof. C. De Nunzio

UROLOGY C.U.; cosimo.denunzio@uniroma.it; Tel. 0633777716

I, the undersigned ..... born in .....

on ....., address .....

Telephone .....

Email.....

I DECLARE

✓ that I am a candidate for transurethral resection of the bladder

✓ that I voluntarily participate in the study in question, aimed at evaluating the effects of a diet characterized by a high consumption of cruciferous vegetables on the recurrence rate in patients with intermediate-, high-, and very high-risk non-muscle-invasive bladder cancer.

✓ that I have received comprehensive explanations from Dr. _____ regarding the request to participate in the research, particularly regarding the purposes and procedures;

✓ that I have had sufficient time to carefully read, understand, and if necessary have an explanation of the contents of the attached information sheet, which I have signed for acknowledgment and which confirms what has been explained to me verbally, in particular that the trial will be conducted in compliance with international ethical codes;

✓ that I have had the opportunity to ask questions and received satisfactory answers regarding the entire

trial, particularly regarding possible diagnostic and therapeutic alternatives and the

consequences of not performing the proposed procedure;

✓ that I have been informed of any reasonably foreseeable risks or discomfort;

✓ that I consent/decline to the physician in charge informing my family doctor;

✓ I consent to monitors, audits, and national and foreign regulatory authorities having direct

access to my clinical records for monitoring and verification purposes;

✓ I understand that participation is voluntary, with the assurance that refusal to

participate will not affect my receiving the most appropriate treatment;

✓ I may withdraw from the trial once it has begun at any time, without negative consequences

in receiving the most appropriate treatment and without the obligation on my part to justify my decision, unless the withdrawal is due to the onset of disorders or unwanted and/or unexpected effects, in which case I hereby undertake to promptly communicate their nature and extent to the investigating physician.

✓ My medical records will remain strictly confidential and the data will be used for the purposes indicated in the study (EU Privacy Regulation 2016/679 (GDPR));

✓ That I will be informed of any new data that may affect the risks or benefits, or of any changes to the protocol that may affect them;

✓ That I have the right to access the documentation concerning me and the assessment expressed

by the Ethics Committee, to which I may appeal if I deem it appropriate.

✓ That a copy of the informed consent and the documentation I have reviewed will remain in

my possession; that for any problems or further information, I may contact the Principal Investigator:

Prof. C. De Nunzio

Address: Sant'Andrea University Hospital of Rome

Via di Grottarossa 1035, 00185, Rome

Telephone number: 0633777716

Email: cosimo.denunzio@uniroma1.it

Privacy and use of clinical information

To conduct the study, it will be necessary to use some information from your medical records. Your consent will authorize us to use this information in accordance with the right to privacy protection, EU Privacy Regulation 2016/679 (GDPR) and subsequent amendments, as per the Guidelines of the Italian Data Protection Authority for the Processing of Personal Data in the Context of Clinical Trials

of Medicinal Products (Official Gazette 190 of 14/08/2008) and any other prescriptions/authorizations of the Italian Data Protection Authority itself.

I understand that for any problems or further information, I can contact the UROLOGY Investigator, Professor C. De Nunzio (063377716 – email cosimo.denunzio@uniroma1.it) and the Data Protection Officer of the Sant'Andrea Center: dpo@ospedalesantandrea.it (Attorney Isabella Lucati).

Therefore, I confirm that I have received comprehensive answers to all my questions and, having acknowledged the situation described,

I FREELY, SPONTANEOUSLY, AND IN FULL CONSCIENCE CONSENT TO THE TRIAL

PROPOSED TO ME.

Date .....

Patient's signature .....

Doctor's signature .....

Approval by our local Ethical Committee (CET Lazio Area 1) on October 16th 2025