Immunotherapy in patients with early dMMR rectal cancer

A Danish DCCG phase II trial

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Participating centres

Sponsor

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List of Abbreviations

AE	Adverse event
ALAT	Alanine aminotransferase/alanine transaminase
ANC	Absolute neutrophil count
ASAT	Aspartate transaminase
cCR	Clinical complete response
CEA	Carcinoembryonic antigen
cfDNA	Circulating free DNA
CRC	Colorectal cancer
CRP	C-reactive protein
CRT	Chemoradiotherapy
ctDNA	Circulating tumor DNA
CTIS	Clinical Trial Information System
CTLA	Cytotoxic T-lymphocyte-associated antigen
dMMR	deficient mismatch repair
EGA	European Genome-Phenome Archive
GDPR	General Data Protection Regulation
Hb	Haemoglobin
ICI	Immune checkpoint inhibitors
IV	Intravenous
LDH	Lactate dehydrogenase
LRR	Locoregional recurrence
MSI	Microsatellite instability
PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligand 1
Pl	Platelets
RBGB	Regionernes Bio- og Genom Bank
RT	Radiotherapy
SAE	Serious adverse event
SAR	Serious adverse reaction
SNV	Single-nucleotide variant
SOP	Standard operating procedure
SPC	Summary of product characteristics
SUSAR	Suspected unexpected adverse reaction
TCR-Seq	T-cell Receptor sequencing
TME	Total mesorectal excision
W & W	Watch and wait

Title

Immunotherapy in patients with early dMMR rectal cancer.

Introduction

Standard therapy in patients with resectable rectal cancer

Colorectal cancer (CRC) is the third most common cancer (1.8 million cases) and the third most common cause of cancer-related death (0.8 million deaths) worldwide in 2018 (1,2), and rectal cancer accounts for roughly one-third of CRC.
The main curative treatment modality for patients with rectal cancer is surgery, often combined with chemotherapy and/or radiotherapy (RT). The global recognition of total mesorectal excision (TME), that decreased locoregional recurrence (LRR) by itself, questioned the need for radiotherapy (RT) before or after surgery. Several randomized trials have demonstrated the importance of preoperative RT (short course RT or long course chemo-radiotherapy (CRT)) in reducing LRR, in patients with high-risk rectal cancer (3). However, RT or CRT does not improve overall survival, and in addition neoadjuvant RT/CRT followed by TME is associated with perioperative morbidity and the risk is increasing with age (4). Therefore, ongoing trials are testing other strategies, such as the omission of (C)RT or even avoidance of surgery (5,6).

Watch & wait strategy

Organ preservation has become a growing demand from many patient groups in recent year.
''Watch and wait'' (W&W) is a non-standard, non-operative approach for patients with locally advanced rectal cancer who have achieved a clinical complete response (cCR) after CRT or RT. In these patients, surgery is reserved only for patients with development of a local regrowth. The W&W approach was introduced into clinical practice by Habr-Gama in the early 1990s (5). In patients with low risk rectal cancer, induction therapy followed by local excision has become popular and is investigated in clinical trials. In stage 2 and 3 rectal cancer, an increasing number of reports have shown the possibility of achieving pathological complete response and this strategy is becoming increasingly popular (7–9).
In patients with proficient MMR, a cCR after pre-operative therapy is a surrogate for pathological complete response because studies have shown that a non-operative option in patients achieving cCR after pre-operative chemo-radiation results in an outcome that is similar to that in patients undergoing surgical resection (10).

Patients with MSI/dMMR

In many countries - including Denmark - screening for deficient DNA mismatch repair (dMMR) is recommended for all individuals with CRC. Around 15% of patients with resectable CRC harbor deficient MMR (dMMR); in colon cancers dMMR occurs in 15-20% of tumors, but prevalence of dMMR in rectal cancer is less frequent around 5-10%. The percentage of patients with dMMR decreases with stage, around 20% are dMMR in stage 2 but only around 5% in metastatic CRC (probably because fewer patients with dMMR experience recurrence).
In the last decade the optimal therapy in these patients with resectable tumours has been discussed. Patients with dMMR have a better prognosis and they do not benefit from adjuvant monotherapy with 5-FU (11,12).
In patients with chemo-refractory dMMR mCRC, the immune checkpoint inhibitors pembrolizumab, nivolumab, and the combination of nivolumab/ipilimumab have become the standard of care (13–15).
Results from the first line KEYNOTE-177 study showed that pembrolizumab significantly prolonged progression-free survival with fewer adverse events compared to combination chemotherapy (16). and recently EMA approved pembrolizumab in the first-line treatment of metastatic dMMR CRC. Response rate was 44% (11% complete response) in patients receiving pembrolizumab and 33% (4% complete response) in patients receiving chemotherapy. However, in patients receiving pembrolizumab 29% (compared to 12%) had progressive disease (PD) as best response.

Chalabi et al tested the benefit of a single dose of ipilimumab and nivolumab (day 1) and nivolumab (day 15) before surgery in 20 patients with stage 1-3 resectable dMMR colon cancer (17). A pathological complete response was achieved in 12 patients (60%) and a major pathological response in 19 (95%) patients. No patients had PD.
Only few cases evaluating immunotherapy in early dMMR rectal cancer have been reported (18,19). However, we have reported a case of clinical, pathological and serological complete response in a patient with T3dN1 dMMR rectal cancer after one cycle of therapy with nivolumab and ipilimumab – same dose and schedule as planned in present phase 2 trial (20). Two recent presentations (21,22) have shown that immunotherapy in patients with dMMR tumors may completely eradicate the primary cancer and regional lymph nodes leading to a possibility for organ-sparing medical treatments.
Based on the NICHE trial (17) and supported by two recent presentations (21,22), we are confident that a "watch and wait strategy" (no resection and no stoma) is safe in patients achieving complete clinical regression. In addition, we recommend further treatment as described below and re-evaluation in patients having definite but less than complete regression at four weeks.

In May 2022, a presentation with simultaneous NEJM publication (10,23) showed that 14/14 patients with dMMR rectal cancer obtained complete response after six months (9 cycles every 3 weeks) of immunotherapy (dostarlimab). Thus, we have now become confident that immunotherapy without surgery will be the "new standard", and we will recommend a W&W strategy in patients with rectal cancer obtaining major tumor shrinkage and these patients will be followed carefully with clinical and molecular evaluation (which was not part of the NEJM paper). No patient in the NEJM paper had progressive disease and therefore we recommend a second cycle of immunotherapy (instead of resection in unclear cases) and re-evaluation. We are confident that 1 or 2 cycles of immunotherapy will result in complete radiological, pathological, and molecular response in a substantial number of patients and this short duration of therapy will reduce toxicity and especially drug costs.

In conclusion, immunotherapy in patients with dMMR CRC tumors may completely eradicate the primary cancer and regional lymph nodes leading to a possibility for organ-sparing medical treatments, and we are confident that this new strategy of 1 or 2 cycles of immunotherapy will be the future standard of care, and in Denmark we have the chance to monitor these patients closely with clinical and high-level molecular follow-up.

In one of the most recent trial (22), the authors stated that dMMR characterizes 5-10% of all rectal cancers, and they included 11 patients at a single center. Therefore, we are confident that completion of the present trial (with a total of 39 Danish patients) is possible, because the trial is supported by DCCG and oncological and surgical departments from all five Danish regions will participate.

All patients must be evaluated and discussed at the local MDT after the evaluation (day 43 +/- 7 days) and eventually after re-evaluation.

Immune checkpoint inhibitors (ICI)

Human cancers are characterized by genetic mutations and harbor multiple genetic and epigenetic alterations that are potentially recognizable to the immune system. Despite this, the overwhelming state of affairs is one of immune tolerance. This immune tolerance develops due to multiple resistance mechanisms acquired by tumors, including local immune suppression, induction of tolerance and systemic dysfunction of cytotoxic immune cells signaling.

Ipilimumab is a fully humanized monoclonal anti-CTLA-4 antibody that acts as an antineoplastic ICI by selectively binding to cytotoxic T-lymphocyte-associated antigen 4, a molecule located on the surface of

cytotoxic T-cells, suppressing the immune response (17). Ipilimumab blocks CTLA-4, leading to a continuously active immune response in malignant cells.

Nivolumab is a highly selective fully humanized, IgG4 monoclonal antibody inhibitor of programmed death-1 (PD-1) (17). PD-1 is an inhibitory receptor expressed on the surface of T-cells, B cells, macrophages, and NK cells. Endogenous binding of PD-1 with one of its two ligands PD-L1 and PD-L2 results in production of an inhibitory signal which results in reduction of T-cell proliferation, cytokine production, and cytotoxic activity. This results in significant dampening of the immune response. Nivolumab acts to selectively block the receptor activation of PD-L1 and PD-L2, resulting in a release of PD-1 mediated inhibition of the immune response.

Based on these facts, we will conduct a clinical phase II trial in patients with stage 1-3 dMMR rectal cancer.

Study Rational

The purpose of this study is to evaluate the efficacy and tolerability of immunotherapy with nivolumab and ipilimumab in patients with stage 1-3 MSI/dMMR rectal cancer (Figure 1).

Primary objective:

Number of patients with clinical complete response evaluated day 93 (+/- 7 days) after one or two cycles of immunotherapy.

Complete clinical response will be defined as no visible or palpable tumor examined by rectal exploration (if low tumors), endoscopy and MR-scan. Patients with definite but less than complete regression BUT with a representative biopsy without viable tumor cells will also be classified as cCR in this trial.

Secondary objectives:

- Number of patients with complete biological response after 1 or 2 cycles of immunotherapy.
- Number of patients without any sign of recurrence after 12 months.
- Response rate according to mrTRG (24).
- Adverse events
- Correlation between bio-markers (ctDNA and CEA) and outcome.
- Quality of life (EORCT QLQ-C30 and EORCT QLQ-CR29)

Study Design

This is an investigator-initiated, multicenter phase II trial to investigate the efficacy of immunotherapy with nivolumab and ipilimumab instead of surgery in patients with stage 1-3 MSI/dMMR rectal cancer.

Patients will be treated with 1 or 2 cycles of combination immunotherapy:

Cycle 1: Nivolumab 3 mg/kg days 1 and 15 & ipilimumab 1 mg/kg day 1
Cycle 2: Nivolumab 3 mg/kg days 50 and 65 & ipilimumab 1 mg/kg day 50

4 weeks after completion of immunotherapy (day 43 +/- 7 days) all patients will be evaluated by

- Clinical examination including rectal exploration and flexible endoscopy with photos and biopsies
- Radiological examination with CT-scan of the thorax and abdomen and MR-scan of the pelvic
- Biological assessment with ctDNA and CEA measurements

The treatment strategy for all patients will be discussed at the local MDT immediately after the evaluation.

- In case of complete clinical-, radiological- and biological response a W&W strategy is recommended.
- In case of definite but less than complete regression, it is recommend to continued immunotherapy (day 50 and 65) and re-evaluation (day 93 +/- 7 days)

- In case of no response, resection is recommended. Any additional therapy will be discussed at the local MDT.

All patients, independent of whether the patients receive one or two cycles of immunotherapy, the patient must be evaluated by a second tumor re-staging procedure (day 93 +/- 7 days):

- Clinical examination including rectal exploration and flexible endoscopy with photos and biopsies
- Radiological examination with CT-scan of the thorax and abdomen and MR-scan of the pelvic
- Biological assessment with ctDNA and CEA measurements

The treatment strategy for patients will be discussed at the local MDT immediately after the re-evaluation

- In case of complete clinical-, radiological- and biological response a W&W strategy is recommend.
- In case of definite but less than complete regression, a recommendation (resection or closely monitoring) must be discussed at MDT and with the patient.
- In case of no response, resection is recommended, possibly preceded by pre-operative radio- or radiochemotherapy.

In all cases, we will follow the patients 24 months after inclusion in the study (Figure 1).

Based on the NICHE trial (17) and the study by Cercek et al (10) we are confident that a "watch and wait strategy" (no resection and no stoma) is safe in patients achieving complete clinical regression. In addition, we recommend further treatment as described above and re-evaluation in patients having definite but less than complete regression at four weeks.

Feasibility

The identification of dMMR in colon- and rectal cancer is a routine procedure in Denmark. Patients with stage I-III dMMR rectal cancer are a small subgroup of all patients with rectal cancer accounting for 3-5% of patients. Thus, it is expected that 30-50 patients per year nationally will be eligible for inclusion with an inclusion rate of 50% based on patient consent and eligibility criteria. To ensure the inclusion of the necessary number of patients within the timeframe, we have currently made agreements with eight Danish hospital centers regarding participation in the study: Aalborg University Hospital, Århus University Hospital, Odense University Hospital, Herlev University Hospital, Bispebjerg Hospital, Rigshospitalet, Zealand University Hospital and, Vejle Hospital, all covering colorectal and/or oncological services, however all institutions treating patients with rectal cancer will be invited to participate. The participating departments routinely treat multiple patients with rectal cancer and are familiar with conducting clinical studies.

Number of patients: 39 patients with non-metastatic rectal cancer and dMMR included in the study.

Recruitment start: November 2022

Recruitment completion: November 2024

Period of recruitment: 24 months

Study Population

Inclusion and exclusion criteria:

Inclusion criteria

- Age ≥ 18 years.
- Histologically verified non-metastatic rectal cancer stage 1-3.
- No indication for local therapy like TEM.
- Histologically verified dMMR or MSI.
- Performance status (WHO) of 0-1.
- No previous chemotherapy, radiotherapy or immunotherapy for colorectal cancer
- Adequate haematological function defined as neutrophils ≥ 1.5 x 10^9/l and platelets ≥ 100 x 10^9/l.
- Adequate organ function (bilirubin ≤ 1.5 x UNL (upper normal limit), GFR (may be calculated) > 30 ml/min.
- Women of childbearing potential must have been tested negative in a serum pregnancy test within five days prior to registration. Fertile patients must agree to use a highly effective method of birth control. (i.e., pregnancy rate of less than 1 % per year) (Appendix 1) during the study and for six months after the discontinuation of study medication.
- Has provided written informed consent prior to performance of any study procedure.
- Written informed consent must be obtained according to the local Ethics Committee requirements.

Exclusion criteria

- Any other condition or therapy, which in the investigator's opinion may pose a risk to the patient or interfere with the study objectives.
- Concomitant use of systemic glucocorticoids more than the equivalent dose to tablet prednisolone 10 mg/day. Treatment with systemic glucocorticoids must end no later than two weeks before inclusion.
- Subjects with active, known, or suspected autoimmune disease. Subjects with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enrol.
- Known allergy or intolerance to any of the drugs used (nivolumab and ipilimumab).

Study Procedures

Definition of populations to be analyzed

Intention-to-treat-population: Consist of all eligible patients who initiated immunotherapy.

Evaluation

The following is required prior to registration

- Informed consent.
- Confirmation of in- and exclusion criteria.

Within two weeks before inclusion

Medical history (with registration of symptoms) and physical examination.

Performance status, height, and weight.

Blood counts: Haemoglobin (Hb), leukocytes, absolute neutrophil count (ANC), platelet count (Pl).

Liver chemistry: bilirubin, ALAT or ASAT, LDH, alkaline phosphatase.
Renal chemistry: creatinine, GFR (measured or calculated), albumin.
Blood test for administration of immunotherapy – according to local guidelines (eg. cortisol, corticotropin and TSH + FSH and LH for females and testosterone for males).
Other blood tests: CRP, CEA.
Quality of life questionnaire.
If the subject's characteristics comply with all the clinical and laboratory criteria necessary for registration, the subject will be registered in a REDCap database.

Time line and evaluation:

Prior to day 1 cycle 1 (within three days)

- Clinical evaluation
- Blood samples for administration of immunotherapy
- Blood samples for biomarkers (ctDNA and CEA)
- CT (thorax and abdomen) scan within four weeks
- MR (pelvis) scan within four weeks

Prior to day 15 cycle 1 (within three days)

- Clinical evaluation
- Blood samples for administration of immunotherapy
- Evaluation of adverse events

Tumor re-staging - day 43 (28 days +/- 7 days after therapy day 15)

- CT (thorax and abdomen) scan to exclude extra-pelvic disease.
- MR (pelvis) scan to evaluate the response
- Physical examination including flexible endoscopi with photos and biopsies
- Liver and renal chemistry and other blood test
- Blood samples for biomarkers (ctDNA and CEA)
- Evaluation of adverse events

The treatment strategy for all patients will be discussed at the local MDT immediately after the evaluation (day 43-50).

Prior to cycle 2, day 1 of immunotherapy (day 50 total - within three days)

- Clinical evaluation
- Blood samples for administration of immunotherapy
- Blood samples for biomarkers (ctDNA and CEA)

Prior to cycle 2, day 15 cycle 2 (day 65 total - within three days)

- Clinical evaluation
- Blood samples for administration of immunotherapy

Re-evaluation:
Second tumor re-staging - day 93 (28 days +/- 7 days after therapy day 15 in cycle 2)

- MR (pelvis) scan to evaluate the response
- Physical examination including flexible endoscopi with photos and biopsies
- Liver and renal chemistry and other blood test
- Blood samples for biomarkers (ctDNA and CEA)
- Evaluation of adverse events

Post treatment evaluation/follow-up:

- Recording of toxicity until resolved to grade 1 or less.
- Clinical, biological and radiological tumour evaluation at months 4, 10, 16, and 24.
- Further follow-up according to institution guidelines

Registration

Patients will be registered before first cycle of therapy. Immunotherapy must be planned within 8 days after registration.

Data and registration are handled by Odense Patient data Explorative Network (https://open.rsyd.dk/) using REDCap (www. http://project-redcap.org/). The data management system ensures compliance with current legislation and regulations on data handling and data safety.

Collection, handling, and biobanking of biological samples

Collection and handling of blood samples

Collection of blood samples is performed in collaboration with Regionernes Bio- og Genom Bank (RBGB). At each blood draw, a total of 80 mL blood is collected. Plasma, serum, and buffy coat (nucleated blood cells) are extracted from the blood. Drawing, handling and storage of the blood is done in accordance to a standard operating procedure (SOP). Blood samples from each site will continually be sent to RBGB. From the extracted plasma, circulating free DNA (cfDNA) will be extracted and analyzed for presence of tumor-associated somatic mutations. The serum will be used for measurement of carcinoembryonic antigen (CEA), which is the only approved blood-based biomarker for monitoring tumor burden. The CEA marker will be used as a reference for the measurement of ctDNA as a marker of tumor burden. From the nucleated blood cells, constitutional DNA will be extracted. This DNA will be used as a reference when identifying the tumor-associated somatic mutations in the cfDNA and in the tumor DNA.
The tumor and normal mucosa tissue biopsies will be collected as part of the trial.

Research Biobank

All blood samples will be stored in a research biobank associated with the present study. Patients will only be included if they consent in writing to collection and biobanking of their blood, and clinical data.

Processing of personal data in the study
The General Data Protection Regulation, the Danish Data Protection act, the Health Act, and the Helsinki II declaration will be complied with unconditionally. The project will be registered on the Central Denmark Region internal inventory for research projects. Throughout the study, all clinical data and samples will be labeled with non-personal identifiers. The molecular (including sequence data) and clinical data produced in the study will be stored on keyword-protected and log-file operated servers operated by Aarhus University and Aarhus University Hospital.

Biobank for future research
At the end of the study, left-over biological materials, clinical data, molecular and sequencing data will be transferred to the "Colorectal Cancer biobank MOMA, AUH" with the aim to facilitate future research projects. The storage of tissue is done in accordance with the General Data Protection Regulation and the Danish Data Protection act. The "Colorectal Cancer biobank MOMA, AUH" has been approved by the Danish Data Protection Agency case no. 1-16-02-27-10. The biological materials, clinical data, molecular and sequencing data will remain in the biobank, until the approval expires (currently March 1st 2030) or until the

patients request their tissue and/or data re-moved. The patient may at any time request to have his or her samples removed from the "Colorectal Cancer biobank MOMA, AUH" and destroyed. New projects based on the biobank material will only be initiated after the pertinent permissions have been obtained from the relevant authorities and will be conducted in compliance with The General Data Protection Regulation and the Danish Data Protection Act.

In accordance with good academic practice and the requirements of the bodies funding the study and the scientific journals publishing the study results, the study data (health data and genomic data), in pseudo-anonymized format, will be transferred to, and stored in the secure database "European Genome-Phenome Archive, (EGA)" (https://ega-archive.org) and registered in REDCap Database. This will happen after the study has been completed. The purposes are to enable sharing of the data with other research groups, inside and outside Denmark, confirmation of the study findings as well as future research. The EGA provides the necessary security required to control access and maintain patient confidentiality, while providing access to those researchers and clinicians authorized to view the data. Data sharing will be conducted in accordance with the European data protection regulations, including The Danish Data Protection Act and the General Data Protection Regulation (GDPR).

Molecular analyses

T-cell receptor analyses

T-cell Receptor sequencing (TCR-Seq) will be performed to identify the role of the adaptive immune system in mediating the effect of the immunotherapy exposure explored in the trial. We will do TCR-Seq on DNA and RNA extracted from tumor tissue and from serially collected blood samples to 1) determine the TCR profiles in the tissues, 2) establish the TCR profiles of the blood, 3) monitor changes in the TCR repertoire over time and in response to treatment, and 4) to compare the profiles of blood and tissue.
For TCR-seq, we will use the AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel, which is a highly multiplexed targeted resequencing panel to measure T-cell diversity and clonal expansion by sequencing T-cell receptor (TCR) beta chain rearrangements. Data will be generated on the Illumina NovaSeq platform. Target coverage is >1,000x.

DNA sequencing

We will apply genomic sequencing (either exome- or whole genome sequencing) to tumor- and germline DNA to identify tumor-specific genomic changes and neoantigens. Furthermore, the genomic sequencing data will be used to establish tumor fraction, ploidy and clonality of the analyzed biological samples. Illumina TruSeq DNA Kit and NimbleGen SeqCap EZ v3.0 (or similar) will be used for generation of sequencing libraries and capture. Data will be generated on the Illumina NovaSeq platform. Target coverage is <100x.

RNA sequencing

Total RNA-seq will be used for identification of composition and gene expression of tumor-infiltrating immune cells associated with treatment and tumor evolution. Further, RNA-seq will be used to investigate T-cell receptor clonality. The ScriptSeq protocol (or similar) will be used for generation of libraries. Data will be generated on the Illumina NovaSeq platform. As coverage varies with expression level, there is no target coverage. However, we aim to generate 100 mio sequencing reads per sample.

Quantification of carcinoembryonic antigen (CEA)

Despite being an unspecific and insensitive marker of CRC, the blood-based protein biomarker CEA has been approved by both European and US regulatory authorities for surveillance after treatment for CRC. In this study CEA will be profiled in parallel to ctDNA and the performance of the two markers will be compared.

Bioinformatics and Data analyses

Multi-dimensional omics data from DNA- and RNA analyses on tumor and blood will be analyzed and integrated in collective, predictive models for treatment response and survival. Specifically, we will process the DNA and RNA sequencing data through well-established analytic pipelines at the Department of Molecular Medicine, AUH. For DNA, data will be aligned and calibrated by picard and GATK suites. SNPs will be identified by GATK HaplotypeCaller, and somatic mutations (SNVs) and indels will be identified with Mutect2 and Strelka2 (or similar software). RNA-Seq data will be mapped and quantified using Tuxedo Suite (eller similiar software). TCR-seq data will be processed through the tool VisTCR, which enables TCR sequence assembly, assignment to genomic V, D and J genes, extraction of CDR3 regions, establishment of TCR clonotypes and clonotype diversity measurements.

Storage of sequencing data

All sequencing data are processed and stored at GenomeDK. GenomeDK is a national high-performance computing facility for bioinformatics and life sciences managed by Center for Genome Analysis and Personalized Medicine, located at Aarhus University, Denmark. GenomeDK is used by AUH and the Central Denmark Region for processing and storing sequencing data produced in the relation to the treatment of patients. The present study uses the same security infrastructure and procedures as the Central Denmark Region.

Study Medication

Commercial formulations of nivolumab and ipilimumab will be used and administered. Administration of nivolumab and ipilimumab is standard at all the participating departments. There are no concomitant prophylactic medications.

Known toxicities/side-effects are listed in Summary of Product Characteristics (SPC) for the drugs used in the study.

Concomitant use of systemic glucocorticoids more than the equivalent dose to tablet prednisolone 10 mg/day is not permitted due to drug interactions.

Day 1 nivolumab is to be administered first. Patients will receive nivolumab at a dose of 3 mg/kg as a 30-minute IV infusion. The nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the next infusion.

The second infusion is ipilimumab and will start at least 30 minutes after completion of the nivolumab infusion. Patients will receive ipilimumab at a dose of 1 mg/kg as a 30-minute IV infusion.

On day 15, patients will receive nivolumab at a dose of 3 mg/kg as a 30-minute IV infusion.

Dose modification is not permitted. The nivulomab infusion at day 15 can be postponed up to 14 days if the investigator finds it indicated.

The medicine is handled and delivered at a local hospital pharmacy. Likewise, is the traceability is registered by the local hospital pharmacy.

Safety

Both nivolumab and ipilimumab have been administered safely at doses ranging up to 10 mg/kg. Overall, infusion reactions including high-grade hypersensitivity reactions have been uncommon across multiple clinical studies, and all have been managed by following the safety algorithms. Infusion duration of 30 minutes is not expected to present additional safety concerns. Both agents given as single agent are

uncommonly associated with infusion reactions, incidence less than 1% for ipilimumab and for nivolumab 3% and Grade 3 or Grade 4 hypersensitivity/infusion reactions are very rare. Subjects should be carefully monitored for infusion reactions during nivolumab/ipilimumab administration.

In the NICHE trial, the combination was well tolerated, and all patients underwent radical resections within the predefined six weeks after study inclusion, with primary anastomoses in all patients. The median duration from the first dose of nivolumab to surgery was 32 days. Five patients (13%) experienced grade 3–4 treatment-related toxicity. Two patients experienced a grade 3 rash (resolved on steroid treatment (one oral, one topical); 1 patient experienced a grade 3 colitis 2 months after surgery (a single dose of infliximab was given with resolution of symptoms within 3 days). Three grade 3–4 adverse events were asymptomatic increases in laboratory tests, which resolved spontaneously.

Reporting Safety Information

Safety

The safety evaluations will focus on the AEs and laboratory assessments. All patients included will be evaluated in the safety analysis. All AEs will be summarized and in addition, separate summaries Grade 3-4 AEs will be presented. All AEs will be registered in the RedCap Database

Monitoring Procedures

Investigators responsibilities

The investigator agrees to conduct the study in accordance with the Clinical Trial Protocol, ICH guidelines E6 – GCP and the applicable regulatory requirements. The investigator is required to ensure compliance with the protocol and other procedures provided by the Sponsor.

The sponsor or an authorized representative will evaluate and approve all investigators, who in turn will select their staff. The investigator should ensure that all persons assisting with the study are adequately informed about the protocol, amendments, and study treatments, as well as study-related duties and functions. The investigator should maintain a list of sub-investigators and other appropriately qualified persons to whom he or she has delegated significant study-related duties.

The investigator or a designated member of the investigator's staff must be available during monitoring visits to review data, resolve queries, and allow direct access to subject records (e.g., medical records, office charts, hospital charts, and study-related charts) for source data verification. The investigator must ensure timely and accurate completion of CRFs and queries.

For the purpose of ensuring compliance with the protocol, GCP, and applicable regulatory requirements, the investigator will permit auditing by the sponsor or its representative and inspections by regulatory authorities.

The investigator shall take appropriate measures required by the sponsor to take corrective actions for all problems found during audits and or inspections.

Monitor

The main duty of the monitor is to help the Investigator and the sponsor to maintain a high level of quality in all aspects of the trial. At regular intervals during the trial, each site will be contacted, through monitoring visits, letters, or telephone calls to review study progress, compliance, and follow up.

The study will be monitored by regular site visits. During site visits, the study monitor should review source documents (i.e. hospital charts and laboratory records etc.). Additionally, the monitor should observe study procedures and will discuss any problems with the investigator. Adequate time for these visits should be allocated by the Investigator. The investigator should also ensure that the monitor is given access to source documents of the subject which support data entered in the case report forms.

The investigator should also provide direct access to source data / documents for possible trial-related audits, EC review and regulatory inspections by national or foreign health authorities.

A manual describing the monitoring procedures will be worked out.

The following data will be monitored: Informed consent; in- and exclusion criteria; SAEs, and date of CR.

Protocol Amendments

Any amendment to this protocol must be approved by the Protocol Committee. Amendments will be submitted to the IRB/EC for written approval. Written approval must be obtained before implementation of the amended version occurs. The written signed approval from the IRB/EC should specifically reference the sponsor/investigator name, protocol number, study title, and amendment number(s) that is applicable. Amendments that are administrative in nature do not require IRB/IEC approval but will be submitted to the IRB/IEC for information purposes.

Case Report Form

An electronic CRF will be provided by the datacenter. It is the responsibility of the investigators to fully complete CRFs. The data collected can be found in the schedule of events (Table 1) The handling of data by the sponsor may generate additional requests to which the investigator is obliged to respond by confirming or modifying the data questioned.

Definitions

Adverse events (or adverse experience) (AE)

An AE is any untoward occurrence in a subject receiving a pharmaceutical product (and) which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Due to regulatory requirements, events occurring during pre- and post-treatment periods should also be designated as AEs. Therefore events occurring in the period between the signed informed consent and beginning of the study drug administration are to be designated as AEs.

Serious adverse event or reaction/experience (SAE)

All serious adverse events (SAE), i.e. adverse events that are life threatening, result in death, hospitalization or prolonged hospitalization, persistent or significant disability, results in congenital anomaly or birth defect, are to be registered in the eCRF and followed until the problem has been solved/stabilized or it has been established that participation in the study was not the cause.

Investigators must immediately and within 24 hours report (using REDCap) a new SAE to sponsor.

At the end of the trial, all recorded SAE´s will be reported to regulatory authorities through CTIS. Sponsor will report suspected unexpected serious adverse reactions (SUSARs) to EudraVigilance and EC within 7 days, if lethal or life threatening and within 15 days, if not life threatening. All serious adverse reactions (SARs) will be reported by sponsor in the annual safety report to the DHMA and EC once a year via CTIS.

The applicable SPCs for nivolumab and ipilimumab are used as reference documents in the assessment of adverse events.

Exempted from reportable SAEs

Planned hospitalizations and hospitalizations for reasons unrelated to the cancer or its treatment are also exempted from reporting. Generally, known toxicity to immunotherapy, complications of cancer, progressive disease, and events caused by progressive disease are exempted from reporting to regulatory authorities. In cases of doubt whether an event requires reporting, this will be left to the clinical judgement of the investigator or his/her delegates.
Any patient's death must be recorded in the eCRF.

Recording of Adverse Events

All AEs must be documented in the appropriate section of the electronic CRF (RedCap).

The following aspects must be recorded for each event in the CRF:

- A description of the AE in medical terms, not as reported by the subject
- The most severe degree

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination.

All AEs occurring during the study period and within 30 days must be recorded. The clinical course of each event should be followed until resolution, stabilization or until it has been determined that study treatment or participation is not the cause. SAEs which are still on-going at the end of the study period must be followed up to determine the final outcome.

Any SAE, which occurs after the study period and is considered to be possibly related to study treatment or study participation, should be recorded and reported immediately.

The grade as assessed by the investigator according to the definitions in NCI-CTC, version 5.0: Grade 1 - 5:

- Grade 1 = mild
- Grade 2 = moderate
- Grade 3 = severe
- Grade 4 = life-threatening or disabling
- Grade 5 = death related to AE

The causal relationship to therapy as assessed by the investigator; the decisive factor in the documentation is the temporal relation between the AE and therapy. The following judgments of the causality to therapy or study procedures are to be used:

Not Related: There is not a temporal relationship to study drug administration (too early, too late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

Not Likely: There is a temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the AE.

Possible: There is a reasonable causal relationship between the study drug and the AE. Dechallenge information (information referring to withdrawal of drug) is lacking or unclear.

Probable: There is a reasonable causal relationship between the study drug and the AE. The event responds to dechallenge (withdrawal of study drug). Rechallenge is not required.

Certain/Definite: There is a reasonable causal relationship between the study drug and the AE. The event responds to dechallenge and recurs with rechallenge, when clinically feasible.

Death on Study

Any death occurring between the study inclusion and 30 days following the last dose will be reported to the Regulatory Authorities and ethics committees according to local rules. Death occurring during the study follow-up period (i.e. later than 30 days after the last dose) need only to be reported as serious adverse event if it is thought that there was a possible relation to study drug(s) (possible, probable). All deaths should be reported on the death report form section in the CRF regardless of cause.

Follow-up

Patients withdrawn from the study treatment due to any AE will be followed at least until the outcome is determined even if it implies that the follow-up continues after the patient has left the trial, and where appropriate until the end of the planned period of follow-up.

Safety instructions specific to the trial

Adverse events will be recorded for all patients (including those withdrawing from the study treatment because of toxicity) for 24 months following the last dose of study drug. Adverse events related to study drug(s) that are observed, either during study treatment, or prior to the twenty-eighth day following the last dose of study drug(s), will be followed until resolution or stabilization.

Discontinuations

Following events are considered sufficient reasons for discontinuing a subject from study:

- Withdrawal of consent
- Death
- Lost to follow up
- Protocol violation
- Other (to be specified in the CRF)

The reason for study discontinuation should be recorded in the CRF and in the source documents.

At the time of withdrawal of consent, it should be determined whether the subject is withdrawing from treatment alone, or from treatment and collection of further data (eg. survival). Replacement of subjects is not planned, unless a subject wishes to withdraw entirely from evaluation of efficacy and AE. In this case the subject will be replaced.

Statistics

The most important end-point in the treatment of patients with resectable rectal cancer is OS but complete response rate and organ preservation (preservation of rectum without stoma, either with local excision or watch and wait strategy after neoadjuvant treatment) is also very important, and we will use cCR as the primary end-point.

Statistical analysis

Patients will be analyzed according to intention-to-treat. The number of patients (the sample size) is based on Simon's two stages Mini-max design (25). This design ensures early study termination if there is insufficient effect. Patients will be evaluated for the first time 4-6 weeks after completion of one or two cycles of immunotherapy. In studies testing a W&W strategy after CRT or RT, the cCR may be 30% (TREC trial).

A cCR less the 30% at the time of the first evaluation is not clinically relevant. Assuming a significance level at 0.05 ($\alpha = 0.05$) and a power at 80% ($\beta = 0.20$) it can be calculated that 19 patients should be included in the first part of the study. The enrolment will continue until 19 patients have been evaluated at day 93 (+/- 7 days). If five or less patients out of the first 19 consecutive patients achieves cCR at the second evaluation, we will reject our hypotheses and close the study after the first stage of accrual. If at least six or more patients achieve a cCR, a cCR rate of least 30% cannot be excluded and the study will continue until a total of 39 patients have been included. If 16 out of 39 patients achieve cCR, a cCR rate of 50% cannot be excluded, and it will be concluded that the treatment is effective enough to continue with future studies.

Ethics

The study will be conducted in compliance with the protocol and in accordance with the ethical principles put forward in the second Declaration of Helsinki and in accordance with GCP rules (ICH-GCP - online at http://www.ema.europa.eu/pdfs/human/ich/013595en.pdf). The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society. Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective tasks.

In this study we will include patients with dMMR rectal cancer. The benefit of pre-operative radiotherapy and chemotherapy is not well-known, and there is a risk that patients do not get any benefit but only toxicity and delay of surgery. In patients with stage 1-3 dMMR colon cancer, a single cycle of combination immunotherapy has produced complete elimination of tumor tissue in more than 50%. Therefore, we will offer a similar therapy in patients with rectal cancer. The patients with a complete clinical-, radiological- and biological response after immunotherapy can discuss a W&W strategy with the physician to avoid having a permanent colostomy. A comparable W&W strategy after pre-operative radiotherapy is becoming more and more popular in many countries including Denmark, but in patients with proficient (normal) MMR rectal cancer. The patients in this protocol seek alternative treatment possibilities (avoiding colostomy), and this study will provide a new treatment for these patients. In case of definite but less than complete regression after immunotherapy, the strategy can be surgical resection of the tumor - preceded by standard pre-

operative treatment (radio- or radiochemotherapy) or continued immunotherapy and re-evaluation at the discretion of the attending physician. In case of no response, we recommend resection, possibly preceded by pre-operative radio- or radiochemotherapy. Thus, the patients who do not respond adequately to the immunotherapy treatment will be offered alternative treatments.

Finally, a recently published systematic review have not found a significant association between the length of the treatment interval (time from diagnosis of cancer to surgery or neoadjuvant radiotherapy) and overall survival or cancer specific survival (26). Therefore, oncological treatment can be safely undertaken in this period further improve the long-term oncological outcomes and for some patients lead to a W&W strategy with sparring of the organ.

Genomic sequence analysis
In this study, we will perform extensive mapping of the human genome with the aim to: 1) determine the TCR repertoire, 2) establish the somatic tumor mutation profile and the neoantigens formed by the mutations, and 4) establish gene expression profiles. We will not search the data for germline genetic variation (e.g. SNPs) s. Consequently, the risk of incidental finding a potential clinically relevant, genomic variant related to inherited diseases is extremely low, and practically hypothetical. Nevertheless, in the unlikely event that we do identify a genetic variant with potential clinical relevance, then we will have its importance evaluated by an expert committee, appointed by Department of Molecular Medicine, Aarhus University Hospital.

The committee is appointed when needed and the members will be chosen according to the potential disease. The committee will include a molecular biologist, specialized in genetic sequencing, a medical doctor specialized in personalized medicine, a clinical geneticist specialized in inherited diseases, and a medical doctor specialized in the disease in question. If deemed relevant other specialists may be included.

This committee will assess if

1. The technological quality of the analysis is sufficient for a reliable result.
2. There is sufficient evidence in the literature for a clinical relevance (e.g. expected penetrance).
3. The sum of information justifies a relevant risk for a genetic disposition.
4. The disease, according to current standards, can be treated or prevented.

Based on the assessments, the committee decides whether the patient should be informed (by written letter) that the research has accidentally resulted in a finding with potential influence on his or her health. The patient will also be informed that further information and advice on the matter will be offered to him/her and/or potentially affected family members.

If the patient in the consent form opted out of receiving important health-related findings or has died, the committee will assess whether to contact relatives, with purpose of saving lives and preventing disability

Informed Consent

All patients will receive written and verbal information regarding the study at a prior interview, held in an uninterrupted location. This information will emphasize that participation in the study is voluntary and that the patient may withdraw from the study at any time and for any reason. All patients will be given the opportunity to ask questions about the study and will be given sufficient time to decide whether to participate in the study. Information will further be supplied from the Ethical Committee: ”Your rights as a study person in a biomedical investigation.”

Potential patients will be screened based on information from patient records including age, MMR-status, radiological information, indication for surgery, blood tests, and performance status. Screening of medical records will be done in accordance with “Sundhedsloven §46

Before performing any study-related procedures described in the schedule of study events (Table 1), the informed consent form will be signed and personally dated by the patient (or their legally acceptable representative and/or witness, as applicable) and by the person who conducted the informed consent discussion.

The patient information leaflet will include that the time of death will be provided to the Sponsor also after end of participation in the Main Study.

A copy of the patient information leaflet including the signed consent form will be provided to the patient.

Information of the Patient

All patients will be informed about:

- The aims of the study
- The possible adverse events
- The procedures and possible hazards to which the patient will be exposed
- Strict confidentiality of any patient data
- Medical records possibly being reviewed for trial purposes by authorized individuals other than their treating physician

Standard therapy in patients with resectable rectal cancer is resection. However, in patients achieving complete response to therapy (radiotherapy, chemotherapy, immunotherapy) a W&W strategy without immediate surgery is becoming popular. It is expected that the possible side effects will clearly be outweighed by the potential benefit of therapy used in this protocol.

Rights and responsibilities

At any time the patient has the right to withdraw from the investigation without this will have any influence on further treatment.

The patient must be aware that personal information will be examined closely under audit of relevant authorized personal, but that this personal information will be handled under strict confidentialiy. No personal information will be published. In this case the patient is guaranteed to remain anonymous.

Data that may identify the patient will be found in the hospital records. Material from the patient will only include what is mentioned in the section on biomarkers. Information may contain name, address, telephone number, medical history (e.g. historical/current disease details, blood sample results, hospitalizations) and will be handled according to legal obligations.

Time schedule

The trial may start when the protocol is approved by the Danish authorities. Target recruitment period is estimated to be 24 months and follow-up period/end date is estimated to be 24 months after inclusion of last patient. Last patient last visit (LPLV) is defined by the last access of medical records, which is estimated to be June 2026.

Patient insurance

Patients participating in the study are covered by national regulation.

Publications

A study report will be prepared after the end of the study. The Vancouver declaration should be followed in all publications based on this study, and we plan that the study will be published in international peer-reviewed journals. The study will be published once it is completed, and the final analysis has been performed. Any publication based on the data from this study proceeds from the investigator group, with specification of the participating clinics and responsible contacts. The names on the author list will be given according to the active participation in the design of the protocol, in the recruitment of eligible and evaluable patients, in the compilation of results and in the production of the article.

The protocol committee writes the first draft. The manuscript will be completed and submitted by the sponsor-investigator, who will also decide who will be the first author. Per Pfeiffer who wrote the first draft will be senior (last) author. Co-authors are oncologist/surgeons from those centres that have included at least 10% of the patients. In publication of tumor.biological sub-studies the researchers are the main authors and co-authors are the protocol committee and representatives from each centre participating in the tumour biological collection including at least 10% of the patients.

Irrespective of the outcome of this clinical trial, the sponsor will submit a summary of the results of the clinical trial to the EU database within one year from the end of the clinical trial in all Member States concerned. This summary will be accompanied by a summary written in a manner that is understandable to laypersons.

Economy

This is an investigator-initiated trial initiated nationally at all Danish oncology centers involved in the treatment of patients with rectal cancer. The trial is endorsed by the Danish Multidisciplinary Group for colorectal cancer (DCCG).

We will apply for funding to support clinical part and cost for conducting ctDNA analyses. All grants are transferred to and administered at separate hospital research accounts subject to public audit. There is no financial gain for the departments involved. None of the involved staff has any economic involvement in this study. Patients will not receive any compensation for participation in the study.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. november 2018;68(6):394–424.

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, m.fl. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. maj 2021;71(3):209–49.

3. Roeder F, Meldolesi E, Gerum S, Valentini V, Rödel C. Recent advances in (chemo-)radiation therapy for rectal cancer: a comprehensive review. Radiat Oncol. 10. november 2020;15(1):262.

4. Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A, m.fl. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 1. juli 2017;28(suppl_4):iv22–40.

5. Kennedy ED, Pooni A. Selection of Patients for Organ Preservation After Chemoradiotherapy: It Is Too Early to Exclude Any Method for Assessment of Response After CRT. Ann Surg Oncol. august 2020;27(8):2580–2.

6. Rega D, Granata V, Romano C, D'Angelo V, Pace U, Fusco R, m.fl. Watch and Wait Approach for Rectal Cancer Following Neoadjuvant Treatment: The Experience of a High Volume Cancer Center. Diagnostics (Basel). 21. august 2021;11(8):1507.

7. Fernandez LM, São Julião GP, Figueiredo NL, Beets GL, van der Valk MJM, Bahadoer RR, m.fl. Conditional recurrence-free survival of clinical complete responders managed by watch and wait after neoadjuvant chemoradiotherapy for rectal cancer in the International Watch & Wait Database: a retrospective, international, multicentre registry study. Lancet Oncol. januar 2021;22(1):43–50.

8. Dossa F, Chesney TR, Acuna SA, Baxter NN. A watch-and-wait approach for locally advanced rectal cancer after a clinical complete response following neoadjuvant chemoradiation: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. juli 2017;2(7):501–13.

9. van der Valk MJM, Hilling DE, Bastiaannet E, Meershoek-Klein Kranenbarg E, Beets GL, Figueiredo NL, m.fl. Long-term outcomes of clinical complete responders after neoadjuvant treatment for rectal cancer in the International Watch & Wait Database (IWWD): an international multicentre registry study. Lancet. 23. juni 2018;391(10139):2537–45.

10. Cercek A, Lumish M, Sinopoli J, Weiss J, Shia J, Lamendola-Essel M, m.fl. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. N Engl J Med. 5. juni 2022; 386: 2363-76.

11. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, m.fl. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 10. juli 2010;28(20):3219–26.

12. Sinicrope FA, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, m.fl. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. J Natl Cancer Inst. 8. juni 2011;103(11):863–75.

13. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, m.fl. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 28. juli 2017;357(6349):409–13.

14. Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, m.fl. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: KEYNOTE-164. J Clin Oncol. 1. januar 2020;38(1):11–9.

15. Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M, m.fl. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol. 10. marts 2018;36(8):773–9.

16. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, m.fl. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. N Engl J Med. 3. december 2020;383(23):2207–18.

17. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, m.fl. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat Med. april 2020;26(4):566–76.

18. Demisse R, Damle N, Kim E, Gong J, Fakih M, Eng C, m.fl. Neoadjuvant Immunotherapy-Based Systemic Treatment in MMR-Deficient or MSI-High Rectal Cancer: Case Series. J Natl Compr Canc Netw. juli 2020;18(7):798–804.

19. O'Connell E, Reynolds IS, McNamara DA, Prehn JHM, Burke JP. Microsatellite instability and response to neoadjuvant chemoradiotherapy in rectal cancer: A systematic review and meta-analysis. Surg Oncol. september 2020;34:57–62.

20. Tarpgaard LS, Andersen PV, Øgaard N, Demuth C, Andersen CL, Pfeiffer P. Complete pathological and serological response to immunotherapy in a patient with MMR-deficient early rectal cancer. Ann Oncol. juni 2021;32(6):805–6.

21. Ludford K, Cohen R, Svrcek M, Foo WC, Colle R, Parc Y, m.fl. Pathological Tumor Response Following Immune Checkpoint Blockade for Deficient Mismatch Repair Advanced Colorectal Cancer. J Natl Cancer Inst. 1. februar 2021;113(2):208–11.

22. Lumish MA, Cohen JL, Stadler ZK, Weiss JA, Lamendola-Essel MF, Yaeger R, m.fl. PD-1 blockade alone for mismatch repair deficient (dMMR) locally advanced rectal cancer. JCO [Internet]. februar 2022 [henvist 31. marts 2022];40(4_suppl):16–16. Tilgængelig hos: http://ascopubs.org/doi/abs/10.1200/JCO.2022.40.4_suppl.016

23. Cercek A, Lumish MA, Sinopoli JC, Weiss JA, Shia J, Stadler ZK, m.fl. Single agent PD-1 blockade as curative-intent treatment in mismatch repair deficient locally advanced rectal cancer. JCO [Internet]. 10. juni 2022 [henvist 28. august 2022];40(17_suppl):LBA5–LBA5. Tilgængelig hos: https://ascopubs.org/doi/10.1200/JCO.2022.40.17_suppl.LBA5

24. Siddiqui MRS, Gormly KL, Bhoday J, Balyansikova S, Battersby NJ, Chand M, m.fl. Interobserver agreement of radiologists assessing the response of rectal cancers to preoperative chemoradiation using the MRI tumour regression grading (mrTRG). Clin Radiol. september 2016;71(9):854–62.

25. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. marts 1989;10(1):1–10.

26. Franssen RFW, Strous MTA, Bongers BC, Vogelaar FJ, Janssen-Heijnen MLG. The Association Between Treatment Interval and Survival in Patients With Colon or Rectal Cancer: A Systematic Review. World J Surg. september 2021;45(9):2924–37.

Figure 1

dMMR early rectal cancer

Cycle 1 of **ipilimumab** + **nivolumab** (day 1) and **nivolumab** (day 15)

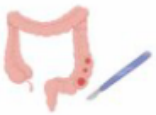
Tumor re-staging with clinical examination, CT- & MR scan, endoscopy and ctDNA (day 43 +/- 7 days)

Complete clinical-, radiological- and biological response a **W&W strategy** will be discussed with the patient.

Definite but less than complete regression, the strategy can be

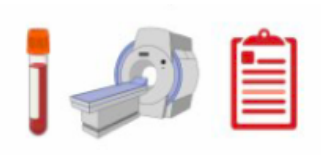
- Surgical **resection** of the tumor preceded by standard pre-operative treatment (radio- or radio-chemotherapy.
- Continued **immune-therapy, cycle 2** (day 50 and 65) and **re-evaluation** (dav 93).

No response, we recommend surgical **resection**, possibly preceded by pre-operative radio- or radiochemotherapy.



Re-evaluation (day 93) and MDT discussion

Follow-up at 4, 8, 12~~10~~, 16 and, 24 months



- Physical examination
- CT- & MR scan
- Blood test for immunotherapy
- QoL questionaries & toxicity assessment
- ctDNA assessment

Appendix 1

Highly Effective Methods of Contraception (< 1% failure rate)

Barrier/intrauterine methods	Hormonal methods (female partners [of childbearing potential] of male participants ONLY)
Total sexual abstinence (evaluate in relation to the duration of the clinical study and the preferred and usual lifestyle choice of the participant) Vasectomized sexual partner (with participant assurance that partner received post-vasectomy confirmation of azoospermia) Tubal occlusion Intrauterine device (provided coils are copper-banded) • Copper T intrauterine device • Progesterone T intrauterine device • Levonorgestrel-releasing intrauterine system [a]	Injection: Medroxyprogesterone injection[b] Levonorgestrel-releasing intrauterine system[b] Implants: Etonogestrel-releasing implants Intravaginal Devices: Ethinylestradiol/etonogestrel-releasing intravaginal devices Combined pill: Normal and low dose combined oral contraceptive pill Minipill: Progesterone-based oral contraceptive pill using desogestrel is currently the only highly effective progesterone-based pill Patch: Norelgestromin/ethinylestradiol-releasing transdermal system

[a] Also considered a hormonal method

[b] Hormonal contraception associated with inhibition of ovulation.

Male patients with a female partner of childbearing potential

- Non-sterilized male patients who are not abstinent and intend to be sexually active with a female partner of childbearing potential must use a male condom plus spermicide from the time of screening throughout the total duration of the study treatment and 6 months after last dose of study treatment. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Male patients should refrain from fathering a child or donating sperm during the study treatment and 6 months after the last dose of study treatment.

- Female partners (of childbearing potential) of male patients must also use a highly effective method of contraception throughout this period (see table above).

Table 1 Schedule of events

	Baseline	Cycle 1 Day 1	Cycle 1 Day 15	Evaluation & MDT (day 43 +/- 7 days)	Cycle 2 Day 1	Cycle 2 Day 15	Evaluation & MDT (day 93 +/- 7 days)	Follow-up
Medical history	X	X	X		X	X		X
Physical examination	X	X	X		X	X	X	X
PS and weight								
Endoscopy with photos and biopsies	X			X			X	X
CT scan	X			X				
MR scan	X			X			X	X
Blood tests for immunotherapy	X		X		X	X		X
Registration of toxicity	X		X		X	X		X
Blood samples for biomarkers	X			X			X	X
Quality of life questionnaire	X			X			X	
Nivulomanb		X	X		X	X		
Ipilimumab		X			X			

All patients

Some patients