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Neoadjuvant nivolumab and relatlimab in locally advanced MMR-deficient colon cancer: a phase 2 trial

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Mismatch repair deficiency (dMMR) is found in approximately 15% of non-metastatic colon cancers (CCs) and is characterized by a defective DNA mismatch repair system, resulting in hypermutated and highly immunogenic tumors. Although patients with dMMR CC have limited benefit from chemotherapy, these tumors have been shown to respond exceptionally well to neoadjuvant anti-PD-1 plus anti-CTLA-4, with high rates of pathologic responses. Here, based on data from melanoma studies, we postulated a high efficacy and favorable toxicity profile of anti-PD-1 plus anti-LAG-3. In the NICHE-3 study, a total of 59 patients with locally advanced dMMR CC were treated with two 4-weekly cycles of nivolumab (480 mg) plus relatlimab (480 mg) before surgery. Pathologic response was observed in 57 of 59 (97%; 95% confidence interval (CI): 88–100%) patients, meeting the primary endpoint. Responses included 54 (92%; 95% CI: 81–97%) major pathologic responses (≤10% residual viable tumor) and 40 (68%; 95% CI: 54–79%) pathologic complete responses. With a median follow-up of 8 months (range, 2–19), one patient had recurrence of disease. The treatment displayed an acceptable safety profile, with all-grade and grade 3–4 immune-related adverse events (irAEs) occurring in 80% and 10% of patients, respectively. The most common irAEs were infusion-related reactions (29%), thyroid dysfunction (22%) and fatigue (20%). In conclusion, our results show that neoadjuvant nivolumab/relatlimab induces high rates of pathologic responses and that further investigation of this treatment in larger studies is warranted. These data add to the body of evidence in support of neoadjuvant immunotherapy regimens in dMMR CC. ClinicalTrials.gov identifier: NCT03026140.

Immune checkpoint blockade (ICB) has become a mainstay of treatment for several malignancies in the metastatic setting, including mismatch repair-deficient (dMMR) colorectal cancer (CRC)[1,2]. Moreover, immunotherapy has been rapidly introduced into earlier stages of disease and is currently part of the standard of care for various tumor types[3–6]. To date, the highest response rates to neoadjuvant ICB have been observed in patients with non-metastatic dMMR CRC[7,8]. MMR deficiency is found in approximately 15% of non-metastatic CRC[9–11] and is characterized defective DNA repair machinery, giving rise to a hypermutated genome with frequent single-nucleotide and frameshift mutations, resulting in an abundance of neoantigens[12,13].

Recent data from the NICHE-2 study, in which patients with locally advanced dMMR colon cancer (CC) were treated with neoadjuvant nivolumab/ipilimumab, demonstrated an exceptional pathologic response rate of 98% in 111 patients, including a pathologic complete response (pCR) rate of 68% and no recurrences at

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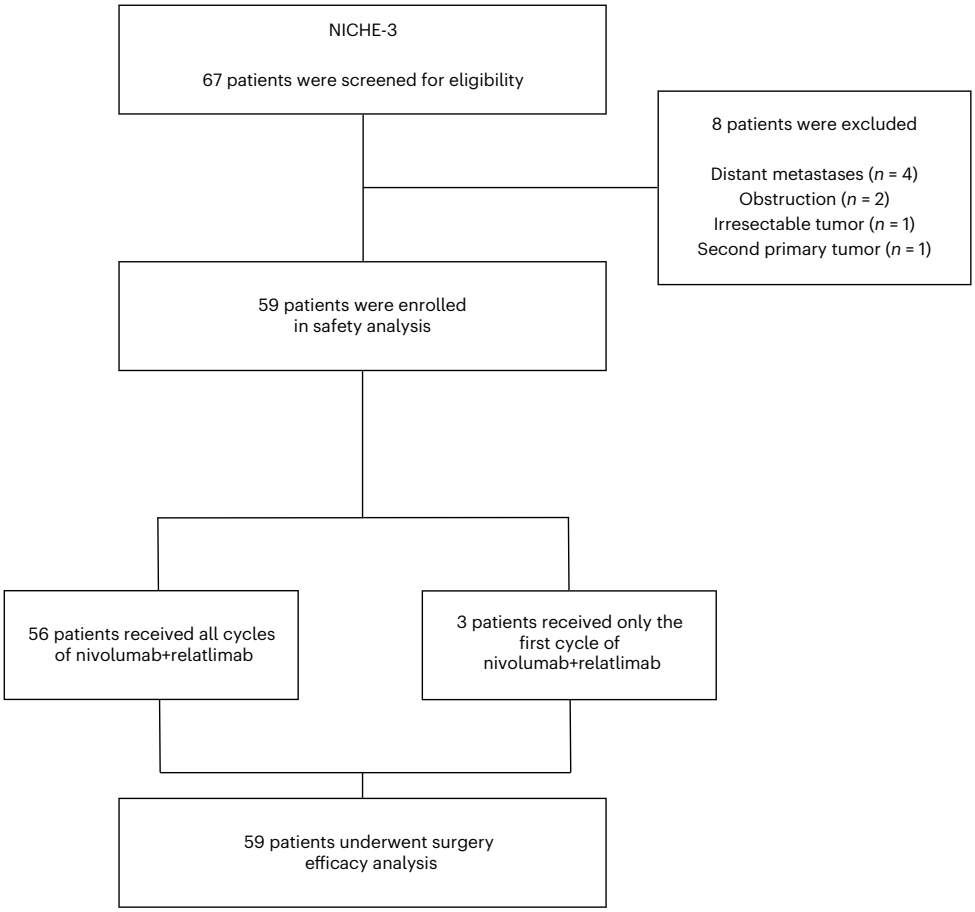

Fig. 1 | CONSORT diagram. CONSORT diagram with an overview of screening and enrollment of patients.

median follow-up of 26 months[8]. Several smaller studies provided further evidence in support of neoadjuvant ICB for patients with dMMR CRC, as demonstrated by high pathologic and clinical complete response rates[7,14,15]. Conversely, the FOxTROT study showed a pathologic response rate of only 7% in dMMR tumors after neoadjuvant chemotherapy[16].

The lymphocyte activation gene 3 (LAG-3) is an immune checkpoint often expressed by T cells after prolonged antigen stimulation and is indicative of T cell exhaustion. Binding of this inhibitory receptor to major histocompatibility complex II molecules reduces proliferation and activation of T cells while also promoting suppressive properties of regulatory T cells, thereby making LAG-3 an attractive therapeutic target[17]. In melanoma, efficacy of nivolumab/relatlimab has been shown in both advanced and early-stage disease, in addition to a favorable toxicity profile compared to studies with other combination regimens[18–20]. In dMMR CC, previous studies showed a high expression of LAG-3 in tumor-infiltrating lymphocytes and at the invasive front[21]. Based on these data, we hypothesized a high efficacy profile and potentially improved safety profile of neoadjuvant nivolumab/relatlimab in patients with dMMR locally advanced resectable CC. In the NICHE-3 study (NCT03026140), patients were treated with a short regimen of two cycles of nivolumab (480 mg) plus relatlimab (480 mg) on day 1 and day 29, followed by surgery within 8 weeks of enrollment (Extended Data Fig. 1). The primary endpoint was pathologic response. Pathologic response was defined as ≤50% residual viable tumor (RVT). The study would be considered successful if a pathologic response was observed in at least 47 out of 59 patients. Secondary endpoints included safety, major pathologic response (MPR; ≤10% RVT) and pCR (0% RVT) as well as disease-free and overall survival. Additional planned secondary endpoints not reported in this paper are circulating tumor DNA, transcriptomics and genomics analyses as well as radiographic and metabolic response assessment.

Results

Patient and tumor characteristics

Between 15 December 2022 and 4 April 2024, 67 patients were screened, and 59 were enrolled and started treatment. Eight patients who did not meet inclusion criteria failed screening due to metastatic disease ($n = 4$), clinical signs of obstruction ($n = 2$), second primary malignancy ($n = 1$) and irresectable tumor ($n = 1$). All 59 enrolled patients were included in the efficacy and safety analyses (Fig. 1). Baseline patient and tumor characteristics are reported in Table 1. The median age was 65 years (range, 21–85), and 54% of patients were female. Eleven patients (19%) had confirmed Lynch syndrome. No notable differences were observed between patients with Lynch-associated tumors and sporadic dMMR tumors, except for a lower median age in patients with Lynch syndrome (Extended Data Table 1). Most patients (37/59, 63%) had clinical stage III disease, and 68% of patients had cT4 tumors as assessed on computed tomography (CT) scans. Two patients had a synchronous colon tumor; in one patient, both tumors were dMMR; and one patient had an MMR-proficient (pMMR) tumor as a second primary tumor (Extended Data Table 2).

Safety

All 59 patients in the study underwent surgery, and tumor-free surgical margins (R0) were achieved in 100% of patients. The median lymph node yield was 33 (range, 9–104). Surgery was performed without treatment-related delays, according to protocol definitions, in 56 (95%) patients. Three patients had surgical delays ranging from 4 weeks to 26 weeks due to immune-related adverse events (irAEs).

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Table 1 | Baseline patient characteristics

Characteristics	Patients (*n*=59)
Age at enrollment (years)	
Median (range)	65 (21–85)
Sex (%)	
Female	32 (54%)
Male	27 (46%)
Race or ethnicity[a] (%)	
White	51 (86%)
Black	3 (5%)
Asian	1 (2%)
Other	4 (7%)
WHO performance status (%)	
0	42 (71%)
1	17 (29%)
Tumor stage[b] (%)	
cT2	1 (2%)
cT3 or cT3–4a	18 (31%)
cT4a	26 (44%)
cT4b	14 (24%)
Lymph node stage[c] (%)	
cN0	22 (37%)
cN+	37 (63%)
Primary tumor location (%)	
Right	48 (81%)
Transverse	6 (10%)
Left	5 (8%)
Lynch status (%)	
Lynch syndrome	11 (19%)
Sporadic MMR deficiency[d]	48 (81%)

[a]Other includes patients of Middle Eastern or North African descent and Hispanic or Mixed heritage. [b]As assessed by CT scan, staged according to the American Joint Committee on Cancer Staging Manual, 8th edition[38]. [c]Numbers may not add up to 100% due to rounding. [d]Sporadic MMR deficiency was defined as dMMR due to *MLH1*-promoter hypermethylation or due to somatic MMR mutations.

Table 2 | Immune-related adverse events

Adverse event	Any grade	Grade 3–4
	Number of patients (%)	
Any adverse event	47 (80%)	6 (10%)
General disorders		
Infusion-related reaction	17 (29%)	–
Fatigue	12 (20%)	–
Dry mouth	6 (10%)	–
Flu-like symptoms	2 (3%)	–
Malaise	2 (3%)	–
Endocrine disorders		
Thyroid dysfunction	13 (22%)	1 (2%)
Adrenal insufficiency	5 (8%)	–
Gastrointestinal disorders		
Colitis	3 (5%)	2 (3%)
Hepatitis	3 (5%)	3 (5%)
Abdominal pain	1 (2%)	–
Musculoskeletal disorders		
Arthralgia	1 (2%)	–
Myalgia	1 (2%)	–
Renal and urinary disorders		
Nephritis	2 (3%)	–
Skin and subcutaneous disorders		
Rash and dermatitis[a]	10 (17%)	–
Pruritus	5 (8%)	–
Alopecia	1 (2%)	–
Hyperhidrosis	1 (2%)	–
Sicca syndrome	1 (2%)	–

AEs considered at least possibly related to either nivolumab and/or relatlimab were classified as irAEs. irAEs were graded according to the CTCAE, version 4.0 (ref. 39). Percentages of all-grade events may total more than 100% due to more than one AE per person. [a]Includes patients with maculopapular rash, acneiform rash, eczema and dermatitis.

irAEs of any grade were observed in 47 of 59 (80%; 95% confidence interval (CI): 67–89%) patients (Table 2). The most frequently observed irAEs were infusion-related reactions (29%), thyroid dysfunction (22%), fatigue (20%) and rash/dermatitis (17%). Grade 3–4 irAEs occurred in six (10%; 95% CI: 4–21%) patients. Grade 3 events consisted of hepatitis (3%), colitis (3%) and hyperthyroidism (2%), and one patient had grade 4 hepatitis. There were no cases of myocarditis in our study.

In both patients with grade 3 hepatitis, this was observed after the first cycle of treatment, leading to omission of the second cycle and surgical delay in one patient. Liver enzymes normalized after treatment with steroids in both patients. The patient with grade 4 hepatitis was diagnosed after the second cycle of treatment, after presentation with a fever and markedly elevated liver function tests. Despite an initial improvement on steroid treatment, the patient developed grade 2 immune-related nephritis with subsequent elevation of liver enzymes, for which mycophenolate mofetil was initiated. Surgery was eventually performed after a delay of 26 weeks.

All three cases of colitis were confirmed through endoscopy and biopsies. In one patient, this resulted in omission of the second treatment cycle and a delay of surgery of 12 weeks. The other two patients developed symptoms after surgery. Both cases of grade 3 colitis were refractory to both steroids and infliximab, leading to initiation of tacrolimus with resolution of symptoms in one patient. The other patient had persistent diarrhea and endoscopic confirmation of persisting grade 3 colitis, upon which fecal transplantation was performed with quick and complete resolution of symptoms thereafter.

Endocrinopathies were observed in 17 (29%) patients, most of which were grade 1–2 events (16/17). These consisted of hypothyroidism (19%), adrenal insufficiency (8%) and hyperthyroidism (3%). One patient with grade 3 hyperthyroidism presented with nausea and tachycardia, resulting in hospital admission. Chronic hormone replacement was required in 15 (25%) patients and included 10 (17%) patients with thyroid hormone replacement therapy, four (7%) patients with adrenal hormone replacement therapy and one (2%) patient requiring both.

Grade 3–4 postoperative adverse events (AEs) were observed in four (7%; 95% CI: 2–16%) patients (Extended Data Table 3) and included ileus (3%), surgical site infection (2%), intra-abdominal abscess (2%), anastomotic leak (2%) and postoperative hemorrhage (2%).

Efficacy

With a median time from first cycle of immunotherapy to surgery of 7.6 weeks (range, 6.4–34.3), a pathologic response was observed in 57 of 59 (97%; 95% CI: 88–100%) patients, meeting the primary endpoint.

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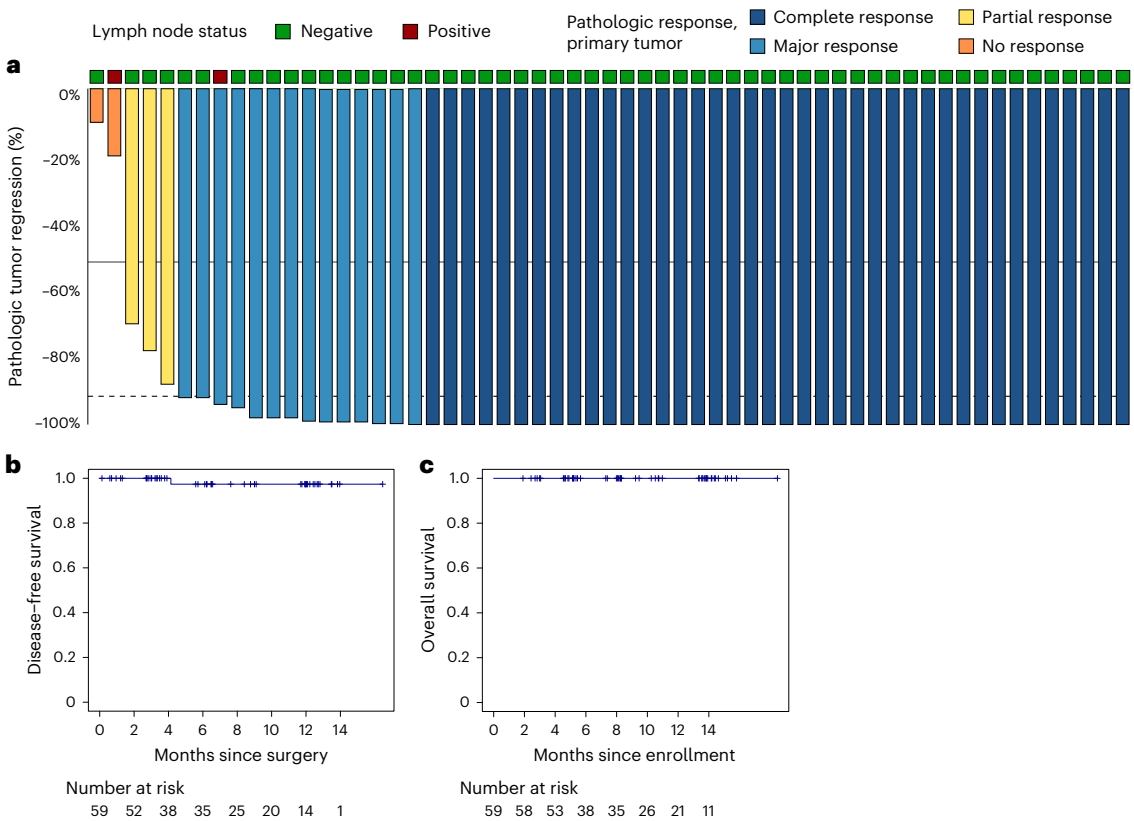

Fig. 2 | Pathologic response and outcome after neoadjuvant nivolumab plus relatlimab. a, Percentage of pathologic regression in the primary tumor bed shown per tumor. The horizontal black line depicts the threshold of 50% regression for a pathologic response, and the horizontal dashed line depicts the threshold of 90% regression for an MPR. Boxes above each bar indicate the corresponding pathologic lymph node status. **b**, Kaplan–Meier plot for DFS. **c**, Kaplan–Meier plot for overall survival.

Table 3 | Pathologic response in all treated patients (*n*=59)

Pathologic response (RVT)	Full cohort *n*=59	cT2–3 *n*=19	cT4a *n*=26	cT4b *n*=14	cN0 *n*=22	cN+ *n*=37
Yes (≤50%)	57 (97%)	19 (100%)	25 (96%)	13 (93%)	22 (100%)	35 (95%)
Major (≤10%)	54 (92%)	17 (89%)	25 (96%)	12 (86%)	20 (91%)	34 (92%)[a]
Complete (0%)	40 (68%)	14 (74%)	18 (69%)	8 (57%)	16 (73%)	24 (65%)
Partial (11–50%)	3 (5%)	2 (11%)	0	1 (7%)	2 (9%)	1 (3%)
No (>50%)	2 (3%)	0	1 (4%)	1 (7%)	0	2 (5%)[a]

[a]One patient had lymph node metastases in the resection specimen.

Of patients with a pathologic response, 54 of 59 (92%; 95% CI: 81–97%) had an MPR, defined as ≤10% RVT. This included 40 of 59 (68%; 95% CI: 54–79%) patients with a pCR (Fig. 2 and Table 3). Notably, all three patients who received only one cycle of nivolumab/relatlimab had an MPR, including one pCR. A partial pathologic response, defined as 11–50% RVT, was observed in three patients, with RVT ranging from 12% to 30%. Two patients with RVT of 80% and 90% were classified as non-responders. In one of these patients, surgery had been delayed for 6 months due to immune-related hepatitis and nephritis for which long-term immunosuppression had been administered. At 4 months after surgery, the patient had extensive, permanently irresectable liver metastases, confirmed to be MMR deficient and clonally related to the primary colon tumor, and was treated with chemotherapy.

Notably, pathologic response was observed regardless of clinical staging, with similar pCR rates in cT4a/b (65%) and cT2/3 (74%) tumors as well as in cN+ (65%) and cN0 (73%) disease (Fig. 3). The pCR rate was numerically lower in patients with an increased carcinoembryonic antigen (CEA) level (≥5 μg L^{-1}) at baseline (50% versus 74%).

Tumor-positive lymph nodes were histopathologically detected in two patients, including one non-responder and one responder with 6% RVT. In both patients, additional lymph nodes with complete tumor regression were found. Both patients refused adjuvant chemotherapy after counseling.

In the patient with two synchronous dMMR tumors, the largest tumor (cT4bN+) displayed a pCR, and the second tumor (cT3N0) displayed an MPR. The patient with a synchronous pMMR tumor had an MPR of the dMMR tumor (cT4bN+), whereas no regression was observed in the pMMR tumor (Extended Data Table 2). Of note, one patient with a pCR (ypT0N0) was diagnosed with a malignant peritoneal mesothelioma peroperatively, for which cytoreductive surgery with concurrent hyperthermic intraperitoneal chemotherapy was performed. The mesothelioma lesions showed histopathologic signs of response with the presence of tumor-infiltrating lymphocytes and tertiary lymphoid structures.

After a median follow-up of 8 months (range, 2–19), all patients were alive, and 98% (58/59) of patients were disease free (Fig. 2).

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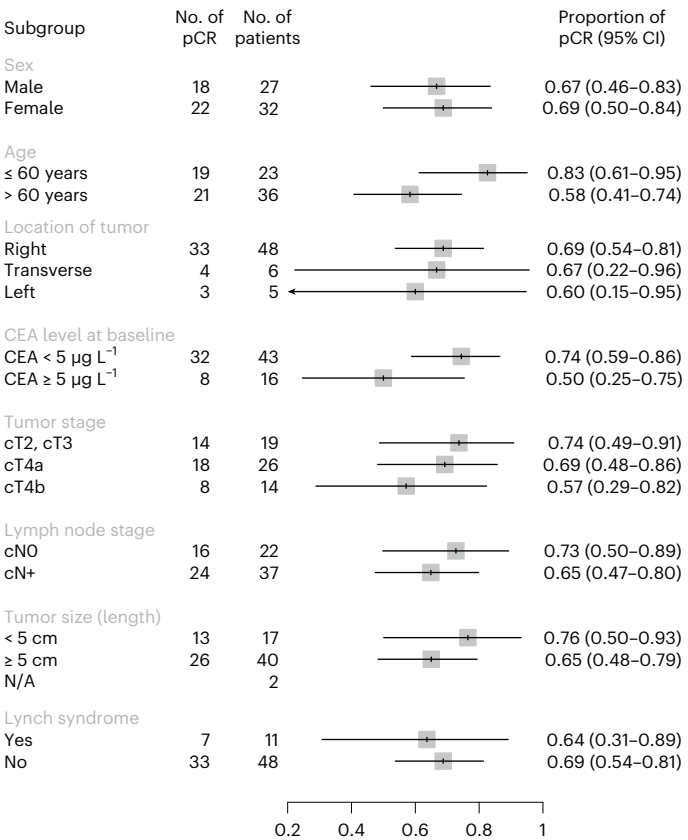

Fig. 3 | pCR according to subgroups. The pCR rates are stratified according to baseline patient and tumor characteristics of the 59 patients included in the efficacy analysis. Each gray square with a dash inside represents the proportion of pCR for a specific subgroup. The horizontal lines extending from the square depict the 95% CI. N/A, not available.

Discussion

Here we show that a short neoadjuvant regimen of nivolumab/relatlimab is highly effective in patients with locally advanced dMMR CC, resulting in a pathologic response rate of 97% and a pCR rate of 68%. To our knowledge, this is the first study to report on the efficacy of neoadjuvant PD-1 plus LAG-3 inhibition in CRC, demonstrating robust anti-tumor activity. These findings add to the compelling body of evidence in support of neoadjuvant ICB for this tumor type, with responses to mono and dual immunotherapy regimens all vastly exceeding the pathologic responses observed after neoadjuvant chemotherapy[8,14–16,22].

Although neoadjuvant chemotherapy is not commonly used in the treatment of patients with CC, data from the FOxTROT study showing a lower recurrence rate have leveraged its use. FOxTROT was also the first study to show a strong correlation between the depth of pathologic response and risk of recurrence in CC[16]. In our study, we observed an MPR rate of 92% in addition to a high pCR rate, both of which have been associated with an excellent outcome after neoadjuvant immunotherapy in several tumor types[23–25]. For CC specifically, the NICHE-2 study showed a similar association, with MPR and pCR rates of 95% and 68%, respectively, and no recurrences at a median follow-up of 26 months.

Interestingly, although pCR was observed across tumor stages in our study, patients with cT4 tumors and/or higher CEA levels at baseline had a numerically lower pCR rate, suggesting a possible relation between tumor burden and pathologic response. Although speculative, the high rates of MPR observed also hint toward a possible dynamic effect and that a longer time to surgery may have led to a higher pCR rate in more advanced tumors.

Despite the increasing number of studies using neoadjuvant immunotherapy in CRC, it remains unclear whether combination therapy improves outcome compared to monotherapy. Several small studies evaluated different durations of neoadjuvant single-agent anti-PD-1, with promising pCR rates ranging from 53% to 88%[14,15,22] but also a numerically higher proportion of patients with limited or no pathologic response compared to dual ICB[15,22].

Conversely, combination regimens are often associated with increased toxicity, but this may also differ based on the drugs, doses and duration. In the current study, the combination of nivolumab/relatlimab showed an acceptable safety profile with 10% grade 3–4 irAEs. The regimen in NICHE-3 was chosen based on preclinical data suggesting a dose–response relationship for dual PD-1 plus LAG-3 blockade[26,27], and the predefined maximum time from treatment initiation to surgery was increased to 8 weeks compared to 6 weeks in NICHE-2, with the hypothesis that a longer interval between treatment and surgery may increase pCR rates. Despite the limitations of cross-study comparisons, here, we observed a similar efficacy of nivolumab/relatlimab compared to nivolumab/ipilimumab in NICHE-2 while noting that patients received a higher dose of nivolumab and two doses of dual ICB and had a longer interval to surgery in the present study.

On the other hand, the immune-related toxicities in the current study suggest a higher rate of thyroid dysfunction, adrenal insufficiency and colitis compared to the nivolumab/ipilimumab regimen in NICHE-2 as well as in previous studies with nivolumab/relatlimab[18,28]. This may, at least in part, be related to the higher dose of relatlimab at 480 mg compared to previous studies using 80 mg or 160 mg. Future cohorts within the NICHE platform are currently underway and will include alternative treatment schedules of nivolumab/relatlimab with the aim of decreasing irAEs while maintaining similar efficacy. Data emerging from these studies will inform the design of a large international study testing nivolumab/relatlimab in patients with locally advanced dMMR CC.

The remarkable MPR and pCR rates in the current and previous studies have ignited interest in organ preservation for patients with dMMR CC. Although organ preservation has an established role in the treatment of rectal cancer, organ preservation for CC poses challenges that need to be addressed. Importantly, as shown in NICHE-2, radiographic assessment of response after neoadjuvant immunotherapy is grossly inaccurate[8]. Complementary diagnostic and monitoring modalities, including circulating tumor DNA, PET-CT scans and colonoscopies, are currently being explored and may help to increase the accuracy of response assessment with the aim of organ preservation.

Limitations of our study include the inaccuracy of radiographic tumor staging, particularly the limited sensitivity of diagnosing lymph node metastases, which is inherent to all neoadjuvant studies in CC[29,30]. On the other hand, the accuracy of cT4 staging is generally considered to be higher[31]. Both T4 and lymph node involvement are known to be associated with high recurrence risks of up to 35% in patients with dMMR CC, despite standard-of-care adjuvant chemotherapy[32,33]. In NICHE-3, we aimed to include patients with radiographically assessed locally advanced dMMR colon tumors at a high risk of recurrence, defined as at least cT3 or cN+[33,34]. After central review, cT4 and/or clinical lymph node involvement were observed in 68% and 63% of patients, respectively. Although overstaging may have led to overtreatment in a subgroup of patients in our study, in the FOxTROT study, which used similar inclusion criteria for T staging, recurrence rate at 2 years was approximately 20% for patients with dMMR tumors despite most patients receiving chemotherapy. These data contradict assumptions that patients with dMMR tumors included in neoadjuvant studies are generally cured by surgery alone[35,36].

At present, pathologic response after neoadjuvant treatment is not yet an accepted surrogate endpoint for CC, unlike the situation

in other tumor types, such as triple-negative breast cancer[37]. To our knowledge, NICHE-2 will be the first study in dMMR CC to provide correlative data on pathologic response to neoadjuvant immunotherapy and the widely accepted endpoint of 3-year disease-free survival (DFS). If an association between pathologic response and DFS is confirmed, this surrogate endpoint would allow more rapid evaluation of neoadjuvant immunotherapy studies in dMMR CC and, thereby, accelerate advances in treatments. Taken together, these and previous data suggest that patients with locally advanced dMMR CC treated with a short course of neoadjuvant ICB have a lower risk of recurrence compared to studies treating this patient population with (neo)adjuvant chemotherapy[8,16].

Based on the pathologic response data observed, randomized studies comparing neoadjuvant immunotherapy regimens to adjuvant chemotherapy may be considered unethical. On a more general note, our findings provide a strong argument for further investigation of this treatment combination in larger studies where efforts should be made to reduce toxicity and generate survival data.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-03250-w.

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Methods

Inclusion and ethics

The NICHE-3 study was reviewed and approved by an ethical review board (NedMec) and was conducted in accordance with the ICH Harmonized Tripartite Guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. NedMec is the joint ethical review board of the Netherlands Cancer Institute (NKI), University Medical Center Utrecht and the Princess Máxima Center for Children's Oncology. All patients provided written informed consent.

Patient population

Eligible patients were aged 18 years or older and were diagnosed with locally advanced (≥T3 and/or N+) dMMR resectable colon adenocarcinoma with no signs of distant metastases. MMR status was assessed using immunohistochemistry for MLH1, PMS2, MSH2 and MSH6, with deficiency defined as absent staining of one or more MMR proteins. Sporadic MMR deficiency was defined as dMMR due to *MLH1*-promoter hypermethylation or due to somatic MMR mutations. Patients had to have a World Health Organization (WHO) performance status of 0 or 1 and adequate renal, liver and hematologic organ function as assessed by screening laboratory tests. The key exclusion criteria included clinical or radiological signs of obstruction and/or perforation; prior treatment with chemotherapy and/or immune checkpoint inhibitors; other malignancies in the last 3 years unless a negligible recurrence risk (<10% in 5 years); history of or active immunodeficiency; autoimmune diseases; and conditions requiring >10-mg prednisone or equivalents daily. Complete eligibility criteria are described in the study protocol (Supplementary Protocol).

Study design

The NICHE-3 study (ClinicalTrials.gov identifier: NCT03026140) is an investigator-initiated, multi-center, non-randomized, open-label, phase 2 study. NICHE-3 is a study within the NICHE platform and was added as an amendment to the original protocol. Each cohort within the NICHE platform is considered separately, and the primary endpoints may differ per cohort. The NICHE platform includes cohorts of patients with pMMR and dMMR tumors, and data from other cohorts were published previously[8,40]. The study was carried out by the NKI in collaboration with five hospitals (Catharina Hospital Eindhoven, Haga Hospital, OLVG, Spaarne Gasthuis Hospital and Tergooi Medical Center). A Simon's two-stage design was used to calculate sample size[41]. The null hypothesis considered a pathologic response rate of 70%, and the one-sided alternative considered a response rate of 85%. In the first stage, 19 patients would be accrued. If more than 14 responses were observed in the first stage, 40 additional patients would be accrued, for a total of 59. The null hypothesis would be rejected if 47 or more responses were observed in 59 patients. Alpha was set at 0.05 and power at 80%. Sex and/or gender were not considered in the study design.

After screening for eligibility and subsequent enrollment, patients were treated with nivolumab (480 mg) and relatlimab (480 mg) on day 1 and day 29 (±3 d), followed by surgical resection 6–8 weeks after enrollment. Response was evaluated by central histopathologic assessment of the resected primary tumor bed and lymph nodes and quantified as percentage of RVT. In case of lymph node metastases in the resection specimen, patients were counseled for adjuvant chemotherapy, according to guidelines. CT scans of the chest, abdomen and pelvis were performed at baseline for radiographic staging. All CT scans were centrally reviewed by an independent radiologist in concurrence with international guidelines.

Blood draws were conducted at baseline, on treatment and during follow-up for monitoring of potential immune-related toxicity and for translational study purposes. Tissue for translational research was acquired at baseline by endoscopic biopsies, and post-treatment samples were acquired from the resection specimen. Long-term follow-up for disease recurrence was conducted at the NKI or at one of the participating hospitals. Follow-up consisted of 3-monthly visits in the first 2 years, followed by 6-monthly visits in year 3 and year 4 and yearly thereafter. Follow-up visits in the first 5 years included laboratory and radiographic assessments (at 6, 12, 18, 24, 36, 48 and 60 months) and endoscopic assessments according to national guidelines.

Endpoints

The primary endpoint of this study was pathologic response. Pathologic response was defined as ≤50% RVT in the primary tumor bed. If the pathologic response rate exceeded 85%, the treatment would be considered promising, whereas a pathologic response rate of ≤70% would be deemed unacceptable. Secondary endpoints included safety, MPR and pCR as well as disease-free and overall survival. All patients were closely monitored for 100 d after the last dose of nivolumab/relatlimab for the occurrence of AEs and serious AEs (SAEs). Safety was measured by number and severity of irAEs. AEs were assessed by the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 (ref. 39), reporting the highest grade for each individual AE. Treatment-related AEs leading to surgical delay of more than 2 weeks were considered unacceptable. Surgical delay due to reasons other than irAEs, including logistical reasons, pandemics or SAEs unrelated to treatment, were not considered treatment related. Postoperative AEs were graded according to Clavien–Dindo grading of surgical complications[42].

Statistical analysis

The safety population consisted of all patients receiving at least one dose of the study treatment. The efficacy population consisted of all patients who had no major protocol deviations and in whom pathologic response was evaluable. Binary endpoints were reported as proportions including 95% CIs calculated using the Clopper–Pearson method. Categorical variables were reported as frequencies. Continuous variables were reported as median including range. DFS was defined as time since surgery until disease recurrence or disease-related death. Survival endpoints were analyzed using the Kaplan–Meier method, and median follow-up was calculated using the reverse Kaplan–Meier method. Statistical analyses were conducted using R software, version 4.3.0, and SAS software, version 9.4. In R software, the following packages were used: Hmisc, xtable, arsenal, forestplot, survminer, survival, meta and DescTools.

Pathological assessment and immunohistochemistry

Formalin-fixed, paraffin-embedded (FFPE) blocks were obtained from baseline endoscopic biopsies and post-treatment surgical specimens. MMR status was assessed on baseline tumor biopsies by immunohistochemistry for MLH1, PMS2, MSH2 and MSH6, executed on a BenchMark ULTRA autostainer (Ventana Medical Systems) according to the manufacturer's instructions. In brief, 3-µm sections were cut from FFPE blocks and heated for 28 min at 75 °C and deparaffinized using EZ Prep solution (Ventana Medical Systems). Heat-induced antigen retrieval was performed for 32 min at 95 °C using Cell Conditioning Solution 1 (Ventana Medical Systems). The antibodies used for staining included MLH1, Ready-to-Use (=undiluted), M1 (6472966001, Roche); PMS2, 1:40 dilution, clone EP51 (M3647, Agilent Technologies); MSH2, Ready-to-Use (=undiluted), G219-1129 (5269270001, Roche); and MSH6, 1:50 dilution, EP49 (AC-0047, Abcam). An OptiView DAB Detection Kit was used to visualize bound antibody, and slides were counterstained with Hematoxylin and Bluing Reagent (Ventana Medical Systems).

All post-treatment resection specimens were centrally reviewed by an experienced gastrointestinal pathologist. Primary tumors were embedded in their entirety, including all resected lymph nodes, and FFPE blocks were cut and slides were counterstained with Hematoxylin and Bluing Reagent (Ventana Medical System). Pathologic response was determined by histopathological examination of all FFPE slides of the entire resected tumor, including all resected lymph nodes, and regression was determined by estimating the percentage of RVT. Staging was

performed according to the American Joint Committee on Cancer Staging Manual, 8th edition[38]. Pathologic response was defined as ≤50% RVT; MPR was defined as ≤10% RVT in the primary tumor bed; and pCR was defined as 0% RVT in the primary tumor bed and tumor-draining lymph nodes. Cases with a pCR of the primary tumor but with lymph node metastases were classified as an MPR.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Data supporting the findings of this study are available in the article and the supplementary materials.

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Author contributions

P.G.M.d.G. coordinated study procedures, analyzed and interpreted clinical data and wrote the first draft of the paper. Y.L.V. and L.D.W.v.d.D. coordinated study procedures and analyzed and interpreted clinical data. S.B. performed statistical analyses. C.G., M.C., M.H.G.F. and S.D. informed patients and were responsible for patient care. J.G.v.d.B. performed histopathologic scoring and central pathology review. R.G.H.B.-T. performed central review of CT scans. M.E.v.L. and W.H.M.V. performed endoscopies. J.J.v.d.B., K.W. and S.J.O. informed and referred patients. A.G.d.H., H.A.M., S.J.O. and K.F.D.K. performed surgeries. J.B.A.G.H. and T.N.S. provided advice on study design and procedures. M.C. was the principal investigator, designed the study, wrote the study protocol and supervised coordination of the study and the writing of the paper. All authors reviewed, edited and approved the paper and vouch for the accuracy of the data reported and adherence to the protocol.

Competing interests

J.B.A.G.H. is an advisor to Achilles Therapeutics, AstraZeneca, BioNTech, Bristol Myers Squibb, Curevac, Gadeta, Imcyse, Immunocore, Iovance Therapeutics, Ipsen, Merck Serono, Merck Sharp & Dohme, Molecular Partners, Neogene Therapeutics, Novartis, Pfizer, PokeAcel, Roche, Sanofi, Sastra Cell Therapy, Third Rock Ventures and T-Knife and has received research grants unrelated to this study from Amgen, Asher Bio, BioNTech US, Bristol Myers Squibb, Merck Sharp & Dohme, Novartis and Sastra Cell Therapy. J.B.A.G.H. owns stock in Neogene Therapeutics. All grants were paid to the institutions. T.N.S. is a venture partner at Third Rock Ventures. T.N.S. is founder of, advisor to and stockholder in Asher Bio, Cell Control and Neogene Therapeutics. T.N.S. is advisor to and stockholder in Allogene Therapeutics, Merus and Scenic Biotech. M.C. is advisor to Bristol Myers Squibb, Kineta, Merck Sharp & Dohme, NOUSCOM and Roche/Genentech and has received research grants unrelated to this study from Agenus, Merck Sharp & Dohme and Roche/Genentech. All grants were paid to the institutions. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-024-03250-w.

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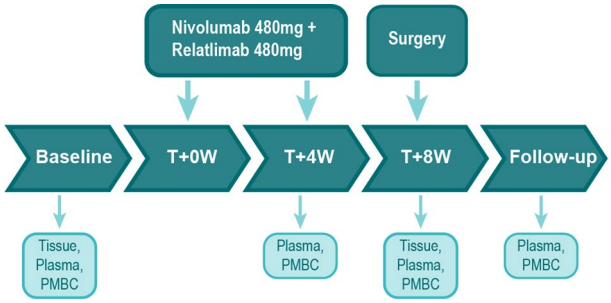

Extended Data Fig. 1 | Study design. All patients underwent screening through CT-scan, endoscopy and blood tests. After enrollment patients were treated with 480 mg nivolumab plus 480 mg relatlimab at day 1 and day 29 followed by surgical resection at a maximum of 8 weeks after enrollment.

https://doi.org/10.1038/s41591-024-03250-w

Extended Data Table 1 | Baseline characteristics of patients with Lynch syndrome and sporadic dMMR tumors

Characteristic	Sporadic* (*n*=48)	Lynch (*n*=11)
Tumor location		
Right	40 (83%)	8 (73%)
Transverse	4 (8%)	2 (18%)
Left	4 (8%)	1 (9%)
Tumor stage		
cT2	1 (2%)	0 (0%)
cT3 and cT3/T4a	13 (27%)	5 (45%)
cT4a	22 (46%)	4 (36%)
cT4b	12 (25%)	2 (18%)
Lymph node stage		
cN0	18 (38%)	4 (36%)
cN+	30 (63%)	7 (64%)
Age (years)		
Median (range)	68 (48-85)	48 (21-71)

*Sporadic MMR deficiency was defined as dMMR due to *MLH1*-promoter hypermethylation or due to somatic MMR mutations.

https://doi.org/10.1038/s41591-024-03250-w

Extended Data Table 2 | Pathologic response in two patients with synchronous colon tumors

MMR-status	Tumor location	Clinical stage	Pathologic response (RVT)	Pathologic stage
dMMR	Caecum*	cT4bN+	MPR (5%)	ypT1N0
pMMR	Caecum	N/A†	NR (90%)	ypT1N0
dMMR	Ascending colon*	cT4bN+	CR (0%)	ypT0N0
dMMR	Transverse colon	cT3N0	MPR (5%)	ypT3N0

CR, complete response; N/A, not available, NR, non-response. *Indicates the tumor used for assessment of the primary endpoint. In patients with synchronous tumors, the highest staged tumor was used. †Tumor first diagnosed during histopathological examination of resection specimen, not visible on baseline radiographic assessment.

https://doi.org/10.1038/s41591-024-03250-w

Extended Data Table 3 | Postoperative AEs

Surgery-related Adverse Events	Any grade	Grade 3-4
	No. of patients (%)	
Any adverse event	22 (37%)	4 (7%)
Gastroparesis and/or ileus	11 (17%)	2 (3%)
Surgical site infection	7 (12%)	1 (2%)
Post-operative hemorrhage	5 (8%)	1 (2%)
Congestive heart failure	2 (3%)	-
Acute kidney injury	1 (2%)	-
Anastomotic leak	1 (2%)	1 (2%)
Chylus leak	1 (2%)	-
Deep venous thrombosis	1 (2%)	-
Delayed wound healing	1 (2%)	-
Intra-abdominal abscess	1 (2%)	1 (2%)
Pneumonia	1 (2%)	-

AEs were graded according to the Clavien–Dindo classification[42]. Percentages of all-grade events may total more than 100% due to more than one AE per person.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	TENALEA clinical trial data management system version 18.6
Data analysis	- R version 4.3.0 with the following packages Hmisc (v5.1-3), xtable (v1.8-4), arsenal (v3.6.3), forestplot (v3.1.3), survminer (v0.4.9), survival (v3.7-0), meta (v7.0-0), DescTools (v0.99.55) - Statistical analysis system (SAS) version 9.4

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Data supporting the findings of this study are available in the article and the supplementary materials.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	The findings of this study do not apply to one sex or gender. Sex and/or gender was not considered in the study design. Patient sex was considered a demographic variable and reported as such and based on the assigned sex in the national personal records database (BRP). The study protocol does not include predetermined analyses of specific sex and/or genders. Pathologic complete response rates were assessed within subgroups including sex. Patients were included regardless of sex or gender.
Reporting on race, ethnicity, or other socially relevant groupings	Self reported data on race and ethnicity were included in reporting of the demographics of patients in this study, in case patient reported data was unavailable this was inferred on the basis of country of birth. Race and ethnicity classifications were based on the bias-free language guidelines from the American Psychology Association. No pre-specified analyses were included in the protocol nor conducted using these data.
Population characteristics	Patients 18 years or older with resectable, previously untreated mismatch repair deficient colon cancer of either clinical T3 stage or clinical N1 or more advanced were eligible for this trial. 59 patients were included between December 2022 and April 2024 with a median age of 65 years (range 21-85), 54% was female, 71% of patients had a world health organization performance status (WHO-PS) of 0, 29% had WHO-PS 1. The majority of patients (63%) had clinical stage III disease, 68% of patients had clinical T4 stage disease and 80% had right sided tumors. 19% of patients had confirmed Lynch syndrome. An overview of baseline patient characteristics is provided in table 1.
Recruitment	Patients that were diagnosed with MMR-deficient colon cancer at our hospital and potentially eligible patients from other hospitals were informed about this study, other potential studies and standard of care. Patients were informed about the objective of the study, study procedures, potential risks and adverse events of the study procedures and therapy. If patients desired to participate in the study, all screening procedures were conducted after written informed consent was provided followed by inclusion if eligible. Patients did not receive compensation for participating in the study.
Ethics oversight	The amended NICHE-3 protocol was reviewed and approved by the ethical review board (NedMec) and was conducted in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. NedMec is the joint ethical review board of the Netherlands Cancer Institute, University Medical Center Utrecht and the Princess Máxima Center for Childrens Oncology. All patients provided written informed consent. There was no independent data monitoring committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was calculated using a Simon's two stage study design. The null hypothesis considered a pathologic response rate of 70%, while the one-sided alternative a response rate of 85%. With an alpha set at 0.05 and power at 80%, 59 patients were needed in the full cohort. The null hypothesis would be rejected if 47 or more patients met the primary endpoint.
Data exclusions	No data were excluded from any of the analyses.
Replication	Replication was not applicable for clinical data and findings.
Randomization	Patients were not randomized in this study. After inclusion all patients were treated with 2 cycles of nivolumab (480mg) and relatlimab (480mg) at day 1 and day 29 followed by surgery.
Blinding	Neither investigators nor participants were blinded as all patients were allocated to be treated with the above stated study treatment.

Reporting for specific materials, systems and methods

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Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

MLH1 (= undiluted), Ready-to-Use, M1, 6472966001, LotNo: H22687, J11940, J2891, J21229, J18270, J03415, Roche Diagnostics, Tucson, AZ, United States of America
MSH2 (= undiluted), Ready-to-Use, G219-1129, 5269270001, LotNo: H33716, J22110, J29400, J17391, J12831, J09830, Roche Diagnostics, Tucson, AZ, United States of America
MSH6: 1:50 dilution, EP49, AC-0047, LotNo: 20021305, 20082502, 230101569, Abcam, Cambridge, United Kingdom
PMS2: 1:40 dilution, clone EP51, M3647, LotNo: 11394527, Agilent/DAKO, Santa Clara, CA, United States of America

Validation

IHC protocols have been developed and validated under standard operating procedures in a certified pathology lab (EN ISO15189, M258). Each new antibody lot is validated by testing multiple dilutions and evaluation by a pathologist using a standardized method, using positive control tissues suitable for the antibody (images and protocol details available upon request). In the case of ready-to-use antibodies each new lot was validated by comparing staining to previous lots and evaluated by qualified analysts. Validation of each primary antibody for the specific species and application was conducted according to guidelines available in online repositories on the manufacturers websites.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Clinicaltrials.gov: NCT03026140, EudraCT number: 2016-002940-17

Study protocol

The latest version of the study protocol, including a summary of changes per amendment is uploaded upon submission and may be made available upon request.

Data collection

Data was collected from time of signing informed consent. Clinical data on response was collected through the assessment of response in the resection speciment after surgery. Survival, safety and toxicity data was collected through patient consultation at the outpatient clinic or digital/telephone consultation. Consultation included regular laboratory, radiographic and endoscopic assessments. Long-term follow-up was conducted at the outpatient clinic of the Netherlands Cancer Institute and in participating hospitals (Catharina Hospital Eindhoven, Haga Hospital, OLVG, Spaarne Gasthuis Hospital and Tergooi Medical Center) and will be conducted until five years after date of surgery, while survival follow-up will continue after these five years. The first patient was enrolled on 15-12-2022, the last on 4-4-2024. The date of data-cut off used for the current analysis was on 16-07-2024.

Outcomes

The primary endpoint of the study was pathologic response. Pathologic response was defined as ≤50% residual viable tumor in the primary tumor bed and lymph nodes. If the pathologic response rate exceeds 85% the treatment would be considered promising, whereas a pathologic response rate of ≤70% would be deemed unacceptable. The null hypothesis would be reject if 47 or more of 59 patients displayed a pathologic response.

Secondary endpoints included:
- Safety. Patients were monitored for adverse event starting at date of informed consent until 100 days after last study treatment administration. Adverse events were classified according to the Common Terminology Criteria for Adverse events version 4.0 (CTCAE 4.0). Safety was measured by amount of immune-related adverse events and immune-related adverse events leading to surgical delay of 2 weeks past the last intended date.
- Major pathologic response rate (MPR; ≤10% residual viable tumor) and pathologic complete response rate (pCR; 0% residual viable tumor).
- Disease or relapse free survival, overall survival.
- Post-surgical outcome and infectious complications following neoadjuvant immunotherapy.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
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