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Original Article

AN0025, a novel antagonist of PGE2-receptor E-type 4 (EP4), in combination with total neoadjuvant treatment of advanced rectal cancer

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ABSTRACT

Purpose: To assess the safety and efficacy of AN0025 in combination with preoperative radiotherapy and chemotherapy in either short course (SCRT) or long course radiotherapy (LCRT) settings for those with locally advanced rectal cancer.

Patients and methods: Twenty-eight subjects with locally advanced rectal cancer participated in this multicenter, open-label, Phase Ib trial. Enrolled subjects received either 250 mg or 500 mg of AN0025 once daily for 10 weeks with either LCRT or SCRT with chemotherapy (7 subjects/group). Participants were assessed for safety/efficacy starting from the first dose of study drug administration and were followed for 2 years.

Results: No treatment-emergent adverse or serious adverse events meeting dose-limiting criteria were observed, with only 3 subjects discontinuing AN0025 treatment due to adverse events. Twenty-five of 28 subjects completed 10 weeks of AN0025 and adjuvant therapy and were evaluated for efficacy. Overall, 36.0% of subjects (9/25 subjects) achieved a pathological complete response or a complete clinical response, including 26.7% of subjects (4/15 subjects who underwent surgery) who achieved a pathological complete response. A total of 65.4% of subjects had magnetic resonance imaging-confirmed downstaging $\leq$ stage 3 following completion of treatment. With a median follow-up of 30 months. The 12-month disease-free survival and overall survival were 77.5% (95% confidence interval [CI]: 56.6, 89.2) and 96.3% (95% CI: 76.5, 99.5), respectively.

Conclusions: Treatment with AN0025 administered for 10 weeks along with preoperative SCRT or LCRT did not appear to worsen the toxicity in subjects with locally advanced rectal cancer, was well-tolerated and showed promise in inducing both a pathological and complete clinical response. These findings suggest its activity deserves further investigation in larger clinical trials.

During the past 20 years, the treatment of patients with locally advanced rectal cancer (LARC) has evolved. Surgery is the mainstay of treatment, but preoperative SCRT or LCRT is often used to prevent local recurrence or downstage the tumor and improve outcomes. Patients are selected for radiotherapy on the basis of more advanced TNM staging and/or magnetic resonance imaging (MRI) based adverse features [1,2]. Local recurrence is no longer the predominant problem, since many patients die of metastatic disease, which provides the rationale for total neoadjuvant (TNT) strategies. TNT is a popular strategy, which advances chemotherapy into the preoperative setting to increase compliance, expose micro-metastatic disease to cytotoxic agents early on, and provide additive activity in the primary tumour.

A spectrum of local response is observed after SCRT or LCCRT with or without TNT ranging from clinical complete response (cCR) or pathological complete response (pCR) to minimal/non-response. pCR is used as a surrogate surgical endpoint, which demonstrates an enhanced tumor regression in the primary. In general an excellent response heralds better oncological outcomes [3], and if CCR is achieved, patients have an enhanced opportunity for organ-sparing and non-operative management (NOM).

The strategies of dose-escalation of radiotherapy and concurrent chemotherapy in the preoperative setting, have not been particularly effective in high-risk patients. Therefore, novel strategies are required, which may be tailored to the molecular profile or provided by immunotherapy.

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Immune evasion is a hallmark of cancer (Hanahan, 2011 [4]. Disease progression of colorectal cancer appears influenced by the immune system and inflammatory mechanisms [5,6], There is evidence that the tumour microenvironment and systemic inflammation-based biomarkers may influence response to radiation (Anitei 2014, Kim 2017, Xu 2022) [7,8,9], and subsequent outcomes predominantly by the immune and inflammatory mechanisms (El Siisy 2020, Portale 2023) [10,11].

A key inflammatory mediator deregulated in many cancers is the COX enzyme, COX-2 [12]. COX-2 expression is inversely associated with patient survival [13,14,15]. Prostaglandin E2 (PGE2), a major cyclooxygenase-2-derived (C0X2) metabolite is important for mediating inflammatory responses [15]. Prostaglandin E2 causes and maintains an immunosuppressed tumor microenvironment. Targeting COX2/PGE2 signalling is therefore a rational therapeutic strategy [Bao 16].

AN0025 (formerly E7046) is a highly selective inhibitor of the PGE2 receptor EP4 [Albu 17]. AN0025 inhibits intratumoral monocytic myeloid cells by blocking EP4, one of the 4 known receptors for PGE2. These monocytic cells include tumor-associated macrophages (TAMs} and myeloid derived suppressor cells (MDSCs), which cooperate in the formation and maintenance of an immunosuppressive tumor microenvironment (Umansky, 2019 [19]_and facilitates tumor immune evasion, progression, and metastasis {De Cicco 2020) [20].

ANOO25 can enhance antitumor activity when combined with radiation [Bao 16]. Preclinical experiments support this concept [Albu, Sakiji 17,18]. A phase I trial of AN0025 as monotherapy, in patients with advanced cancers showed good tolerability with no maximum tolerated dose reached [Hong 15]. While no objective responses were observed, stable disease was reported in 23% of subjects. From this study, doses of 250 mg and 500 mg were proposed for future trials in combination with additional therapy modalities Gene-expression analysis revealed that 16 of a 92-immune-gene panel were modulated (upregulated or downregulated) by day 15 of cycle 1 compared with baseline [15].

The mechanism of action utilized by AN0025, and other PGE2 EP4 inhibitors, is distinct from immune checkpoint inhibitors (Anti-cytotoxic T lymphocyte–associated antigen 4 [CTLA-4] antibodies, and anti-programmed death 1 [PD 1]) antibodies) as demonstrated by synergistic therapeutic effects of AN0025 in combination with anti-PD-1 or anti-PD-L1 antibodies in a mouse colon cancer CT26 model. The combination showed greater efficacy compared with either alone (van Gulijk 2018) [21].

The aim of this present Phase Ib study was to determine the safety, tolerability, and efficacy of oral AN0025 (dose levels of 250 mg and 500 mg) in combination with either preoperative LCRT or SCRT and chemotherapy and /or to identify and the recommended Phase 2 dose (RP2D) of AN0025 in combination with preoperative radiotherapy and chemotherapy for individuals with LARC.

Exploratory objectives aimed to assess the immune cell populations in the tumor infiltrate and correlate with the anti-tumor activity of AN0025 and to explore the pharmacodynamic effect of AN0025 on selected immune cell populations and selected biomarkers in blood and tumor biopsies.

Materials and methods

Study design and subjects

This multicenter, open-label, Phase Ib trial involved 4 sites located in the United States (1), United Kingdom (2), and Poland (1) between October 2017 and September 2021.

The trial aimed to recruit a particularly high-risk population with a locally advanced rectal cancer where primary resection without chemoradiotherapy is unlikely to achieve clear margins as defined by MRI, with no metastatic disease. Eligibility also required subjects to be ≥18 years of age with the following inclusion criteria: newly diagnosed, histologically confirmed invasive primary rectal adenocarcinoma; an Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1;; a tumour encompassable within a radical radiotherapy treatment volume; and adequate renal, bone marrow, and liver function. Strict MRI-based criteria ensured only LARC patients were eligible (primary or malignant node at 1 mm or less from the mesorectal fascia or beyond the mesorectal fascia; orT3 equal to or more than T3c or T4a or T4b tumor; or extramural venous invasion (EMVI+); low rectal tumors (less than 6 cm from the anal verge) <1 mm to intersphincteric plane and anterior quadrant tumor lying <4 cm from anal verge; or cT2-T4 with EMVI+. Subjects were required to provide written informed consent and consent to repeated biopsy. The study was approved by the ethics boards at all 4 study sites.

Procedures

High-resolution, three-dimensional T2-weighted sequence MRI scans of the rectum were performed at baseline, within 14 days prior to surgery, approximately 4 weeks after surgery, and once every year during follow-up. Obtained images were used to assess T stage and MRI-based tumor regression grading (mrTRG). CT scans of the chest, abdomen, and pelvis were obtained at baseline and at 3, 6, 12, 18, and 24 months after surgery during follow-up.

Treatment

The neoadjuvant treatment period began with the first dose of study drug (AN0025; Day 1) and continued until surgery. The postoperative period started at the date of surgery for tumor removal and lasted until 4 weeks post-surgery, after which was the follow-up period.

Subjects were enrolled simultaneously to the LCRT and SCRT groups and subject allocation was balanced using a central allocation process when possible. The 250 mg AN0025 groups were enrolled first, followed by the 500 mg AN0025 groups, after a safety review was conducted.

Subjects in both study arms began AN0025 14 days before the first dose of radiotherapy, which was administered as 125 mg capsules orally for 10 weeks. Subjects in the low-dose cohorts received 250 mg AN0025 (2 capsules) daily and subjects in the high-dose cohorts received 500 mg (4 capsules) daily (Fig. 1). Dose interruptions were not allowed, unless due to an adverse event (AE).

Radiotherapy

Two weeks after starting AN0025, subjects started either LCRT (total of 45 Gy radiation administered in 1.8 Gy daily fractions) for 5 weeks or SCRT (total of 25 Gy radiation administered in 5 Gy daily fractions for 1 week [22,23].

For both schedules, radiotherapy was delivered with CT-based 3D-conformal treatment planning. The clinical target volume included the entire mesorectum with the primary tumour and relevant regional lymph nodes. Both intensity-modulated radiation therapy and 3D-conformal planning techniques were allowed at the discretion of the treating physician.

Chemotherapy

During LCRT, capecitabine was administered orally twice a day in equal doses of 825 mg/m^2 concomitantly with radiotherapy. Doses were limited to a maximum body surface area of 2.2 m^2.

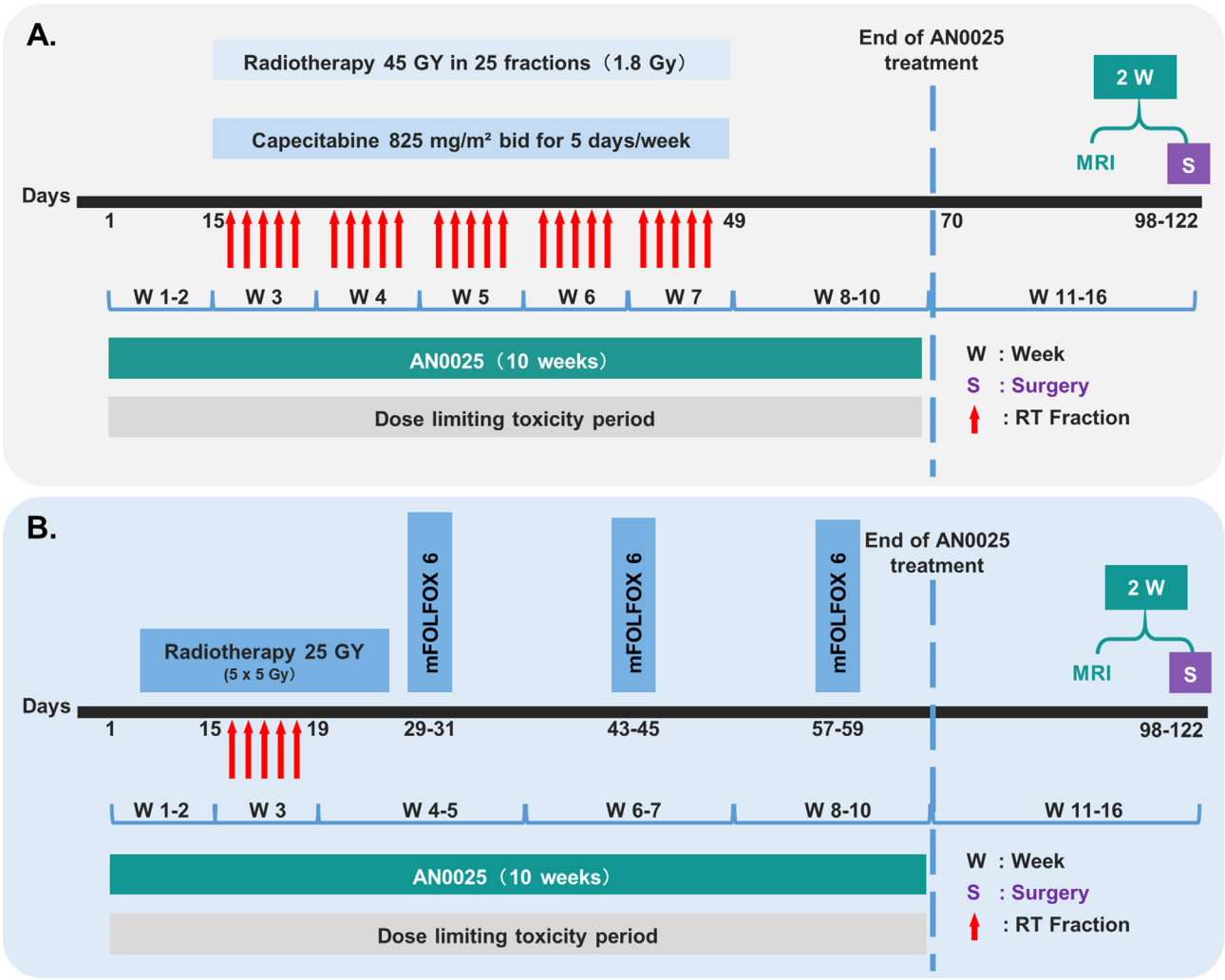

Fig. 1. Long Course Chemoradiotherapy (LCRT) and B. Short Course Radiotherapy (SCRT) study design diagram. Bid = twice a day; CT = chemotherapy; MRI = magnetic resonance imaging; RT = radiotherapy.

For SCRT, 10 days following radiotherapy completion, 3 cycles of a modified oxaliplatin + leucovorin + 5-fluorouracil (5-FU) 6 (mFOLFOX-6) regimen were administered on 2 consecutive days every 2 weeks. On Day 1, mFOLFOX-6 was administered as follows: 85 mg/m^2 oxaliplatin, 400 mg/m^2 leucovorin, and 400 mg/m^2 5-FU bolus intravenously over 2 hours. After Day 1, the dose of mFOLFOX-6 was 1200 mg/m^2/day. Doses of oxaliplatin was limited to a maximum body surface area of 2.2 m^2 (Fig. 1).

Surgery

Surgical treatment was performed according to total mesorectal excision principles; however, in tumors located in the proximal part of the rectum, partial mesorectal excision was permitted. Surgical procedures were performed 14 to 16 weeks after the first dose of AN0025, with an interval of 79 weeks between the end of radiotherapy and surgery. Surgery included anterior resection, abdominoperineal resection, or a low Hartmann's procedure. Potentially invaded structures were resected en bloc with the rectum. The circumferential resection margin (CRM) and completeness of resection were assessed pathologically [24]. Deferral of surgery ("watch and wait") in patients with complete response was not integrated into the original study design, but patient choice in the event of a CCR was accommodated.

Subjects who demonstrated a cCR at reassessment prior to surgery and chose to forgo surgery received more frequent follow-up involving 3 additional follow-up visits to diagnose regrowth in a timely fashion for intervention.

Safety

AEs were graded on a 5-point scale according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.03 The following occurrences were considered dose-limiting toxicities (DLTs): 1) non hematologic toxicity $\geq$ Grade 3 (except diarrhea, nausea and vomiting unless lasting > 5 days despite optimal supportive care); 2) hematologic toxicity: Grade 4 neutropenia $\geq$ 5 days, Grade 3 febrile neutropenia, Grade 4 thrombocytopenia, or Grade 3 thrombocytopenia with bleeding or lasting > 7 days; 3) any other toxicity assessed as study treatment related including increase in radiotherapy associated toxicity that in the opinion of the study investigator(s) and the sponsor physician, constituted a DLT; 4) missed > 7 days of consecutive dosing of AN0025 during DLT periods due to AN0025 related toxicity (where toxicity levels did not meet DLT criteria); and 5) missed > 5 days of radiotherapy due to AN0025 related toxicity (LCRT only). The maximum tolerated dose (MTD) was defined as 1 dose level below the dose level where $\geq$ 2

of 6 subjects experienced a DLT. If ≤ 1 of 6 subjects in all cohorts experienced a DLT, then an MTD was not reached.

Assessment

The primary endpoint was to determine the MTD of AN0025. Efficacy was assessed based on imaging and histopathologic findings. Secondary endpoints included pCR ie no viable cancer cells in the resected specimen; CRM-ve resection (microscopic tumor > 1 mm from the radial margin); pathological tumour regression grade (pTRG) using the Mandard system; MRI-confirmed tumor regression grade (mrTRG) [25]; MRI-confirmed T stage down-staging; Disease-free survival (DFS). A cCR was defined as having no viable tumor on palpation, endoscopy and/or MRI as per local guidelines for 'watch and wait'. To assess long-term efficacy, 12-month DFS and overall survival (OS) were also estimated.

Exploratory endpoints

Tumor and blood samples were collected for pharmacodynamic assessments of cytokines and biomarkers of immune infiltration to determine associations with therapeutic response – see Supplementary material for methodology.

Statistics

Demographic statistics and safety parameters were summarized using descriptive statistics (mean [standard deviation (SD)] and range for continuous variables; numbers and percentages for categorical measures where appropriate).

For the histopathology endpoints, pCR, cCR and CRM-ve rates were summarized using counts and percentages; pathological tumor regression grading (pTRG) and mrTRG were summarized using percentage of each category.

The median DFS and OS were calculated for each group and presented with 2-sided 95% confidence intervals (CIs). Kaplan-Meier estimates of DFS and OS for each treatment group were plotted over time. The Median and landmark (12-month) DFS and OS were calculated for each group and presented with 2-sided 95% confidence intervals (CIs).

Mean systemic exposure parameters by dose level (250 mg and 500 mg) including area under the plasma concentration versus time curves from time 0 to time of last measured concentration and from time 0 to infinity after first dose (AUC0-t and AUC0-inf, respectively) and maximum plasma concentration (Cmax) of AN0025 and ER 888188 were summarized descriptively for Day 1 and Day 8 of receiving study drug. In addition, dose proportionality was informally evaluated. Boxplots were used to provide visual representation of the cytokine data.

Results

Following screening, 28 subjects were enrolled in the study (7 per arm), with 25 (89.3%) subjects completing the study (Supplementary Fig. 1). A summary of subject baseline demographics can be found in Table 1. The mean (SD) age of participants was 58.6 (9.9) years (range: 39–74 years). The majority of subjects were male (20 [71.4%] subjects). Seventeen (60.7%) subjects had an ECOG performance status of 0 and 11 (39.3%) had an ECOG performance status of 1.

In total 25 of 28 subjects (89.3%) experienced at least one TEAE, with similar rates noted across each study arm (Table 2). The most commonly reported TEAEs across all study arms included those that would be expected in a population of subjects with rectal cancer or those receiving pelvic radiation: fatigue (14 [50.0%] subjects); diarrhea and nausea (9 [32.1%] subjects each); proctitis (7 [25.0%] subjects); constipation (6 [21.4%] subjects); and decreased

Table 1
Summary of demographics and baseline characteristics.

Characteristic	250 mg		500 mg		All Subjects (N = 28)
	LCRT (N = 7)	SCRT (N = 7)	LCRT (N = 7)	SCRT (N = 7)	
Age (years), Mean (SD)	57.0 (9.5)	63.3 (8.6)	61.7 (8.7)	52.4 (10.7)	58.6 (9.9)
Sex, n (% Male)	5 (71.4%)	5 (71.4%)	5 (71.4%)	5 (71.4%)	20 (71.4%)
Non-Hispanic or Latino Ethnicity, n (%)	7 (100%)	7 (100%)	7 (100%)	7 (100%)	28 (100%)
Race, n (% White)	6 (85.7%)[1]	7 (100%)	7 (100%)	7 (100%)	27 (96.4%)
Weight (kg), Mean (SD)	77.5 (16.3)	73.5 (8.7)	78.2 (17.9)	75.6 (14.3)	76.2 (14.0)
Height (cm), Mean (SD)	168.7 (12.3)[2]	171.2 (6.9)	168.6 (9.7)	178.4 (8.1)	171.8 (9.7)
ECOG Performance Status, n (%)					
ECOG 0	5 (71.4%)	7 (100%)	3 (42.9%)	2 (28.6%)	17 (60.7%)
ECOG 1	2 (28.6%)	0	4 (57.1%)	5 (71.4%)	11 (39.3%)
mrT stage assessment, n (%)					
cT3a	2 (28.6%)	2 (28.6%)	1 (14.3%)	0 1(14.3%)	5 (17.8%)
cT3b	1 (14.3%)	3 (42.9%)	1 (14.3%)	5 (71.4%)	6 (21.4%)
cT3c	0	2 (28.6%)	1 (14.3%)	0	8 (28.5%)
cT3d	1 (14.3%)	0	2 (28.6%)	1	3 (10.7%)
cT4a	1 (14.3%)	0	1 (14.3%)	(14.3%)	3 (10.7%)
cT4b	2 (28.6%)	0	1 (14.3%)	0	3 (10.7%)
mrN stage assessment, n (%)					
N0	1 (14.3%)	1 (14.3%)	2 (28.6%)	2 (28.6%)	6 (21.4%)
N1	3 (42.9%)	2 (28.6%)	1 (14.3%)	2 (28.6%)	8 (28.6%)
N1b	1 (14.3%)	1 (14.3%)	0	0	2 (7.1%)
N1c	2 (28.6%)	2 (28.6%)	1 (14.3%)	0	5 (17.9%)
N2	0	1 (14.3%)	2 (28.6%)	2 (28.6%)	5 (17.9%)
N2b	0	0	1 (14.3%)	1 (14.3%)	2 (7.1%)
mrEMVI+, n (%)	4 (57.1%)	4 (57.1%)	4 (57.1%)	5 (71.4%)	17 (60.7%)
Minimum tumor distance to mesorectal fascia was ≤1 mm, n (%)	5 (71.4%)	5 (71.4%)	6 (85.7%)	7 (100%)	23 (82.1%)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; EMVI+ = extramural venous invasion positive; LCRT = long course chemoradiotherapy; mr = magnetic resonance; SCRT = short course radiotherapy; SD = standard deviation.
[1] One subject's race was Asian.
[2] Height not recorded for 1 subject.

Table 2
Overview of treatment-emergent adverse events (>10% incidence).

	250 mg		500 mg		All Subjects (N = 28)
	LCRT (N = 7)	SCRT (N = 7)	LCRT (N = 7)	SCRT (N = 7)	
Patients with any TEAEs, n(%)	7 (100%)	7 (100%)	5 (71.4%)	6 (85.7%)	25 (89.3%)
At least 1 TEAE Grade ≥ 3	3 (42.9%)	1 (14.3%)	1 (14.3%)	3 (42.9%)	8 (28.6%)
At least 1 related TEAE, n (%)	6 (85.7%)	6 (85.7%)	4 (57.1%)	3 (42.9%)	19 (67.9%)
Patients with any SAEs, n (%)	3 (42.9%)	1 (14.3%)	3 (42.9%)	2 (28.6%)	9 (32.1%)
At least 1 related SAE, n (%)	0 (0%)	1 (14.3%)	1 (14.3%)	0 (0%)	2 (7.1%)
Patients with TEAE leading to:					
Death	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Study drug modification	0 (0%)	1 (14.3%)	2 (28.6%)	1 (14.3%)	4 (14.3%)
Study drug withdrawal	0 (0%)	1 (14.3%)	1 (14.3%)	1 (14.3%)	3 (10.7%)
Patients with a TEAE classified as a DLT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TEAE, System Organ Class, n (%)					
Preferred Term					
Blood and lymphatic system disorders, n (%)	1 (14.3%)	1 (14.3%)	2 (28.6%)	2 (28.6%)	6 (21.4%)
Anemia	1 (14.3%)	1 (14.3%)	0	1 (14.3%)	3 (10.7%)
Gastrointestinal disorders, n (%)	5 (71.4%)	7 (100%)	4 (57.1%)	5 (71.4%)	21 (75.0%)
Diarrhoea	3 (42.9%)	3 (42.9%)	2 (28.6%)	1 (14.3%)	9 (32.1%)
Nausea	2 (28.6%)	4 (57.1%)	1 (14.3%)	2 (28.6%)	9 (32.1%)
Proctitis	0	3 (42.9%)	0	4 (57.1%)	7 (25.0%)
Constipation	2 (28.6%)	2 (28.6%)	0	2 (28.6%)	6 (21.4%)
Abdominal pain	1 (14.3%)	1 (14.3%)	0	1 (14.3%)	3 (10.7%)
Vomiting	1 (14.3%)	1 (14.3%)	1 (14.3%)	0	3 (10.7%)
General disorders and administration site conditions, n (%)	5 (71.4%)	4 (57.1%)	3 (42.9%)	3 (42.9%)	15 (53.6%)
Fatigue	5 (71.4%)	4 (57.1%)	2 (28.6%)	3 (42.9%)	14 (50.0%)
Investigations, n(%)	2 (28.6%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	6 (21.4%)
Weight decreased	1 (14.3%)	2 (28.6%)	1 (14.3%)	0	4 (14.3%)
Metabolism and nutrition disorders, n (%)	0	3 (42.9%)	1 (14.3%)	2 (28.6%)	6 (21.4%)
Decreased appetite	0	3 (42.9%)	0	2 (28.6%)	5 (17.9%)
Hypokalaemia	0	1 (14.3%)	1 (14.3%)	1 (14.3%)	3 (10.7%)
Nervous system disorders, n (%)	3 (42.9%)	3 (42.9%)	3 (42.9%)	1 (14.3%)	10 (35.7%)
Dizziness	2 (28.6%)	1 (14.3%)	1 (14.3%)	0	4 (14.3%)
Headache	2 (28.6%)	0	1 (14.3%)	0	3 (10.7%)
Paresthesia	2 (28.6%)	0	1 (14.3%)	0	3 (10.7%)
Renal and urinary disorders, n (%)	2 (28.6%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	6 (21.4%)
Urinary tract pain	2 (28.6%)	1 (14.3%)	0	0	3 (10.7%)
Respiratory, thoracic, and mediastinal disorders, n (%)	4 (57.1%)	1 (14.3%)	1 (14.3%)	1 (14.3%)	7 (25.0%)
Cough	2 (28.6%)	1 (14.3%)	0	0	3 (10.7%)

Abbreviations: DLT = dose limiting toxicity; LCRT = long course chemoradiotherapy; SAE = serious adverse event; SCRT = short course radiotherapy; TEAE = treatment–emergent adverse event.

appetite (5 [17.9%] subjects). A summary of TEAEs can be found in Table 2.

The incidence of related TEAEs ≥ Grade 3 was low (2 [7.1%] subjects), and included fatigue, diarrhea, and lymphopenia. There were 9 (32.1%) subjects with at least 1 treatment-emergent SAE, but treatment-related SAEs (abdominal pain, vomiting, fatigue, anastomotic leak, and pelvic fluid collection) were only experienced by 2 (7.1%) subjects. Three subjects discontinued study treatment due to AEs, including the 2 subjects with treatment related SAEs. There were no TEAEs leading to death. There were no events meeting DLT criteria reported, and thus the MTD was not determined.

Five subjects died during follow-up, on average 450 days (range: 197–712 days) after their last dose of study drug, and all deaths were deemed not related to study drug.

Clinical and histopathological responses were observed. Following 10 weeks of AN0025 treatment in combination with either SCRT or LCRT, 5 of 25 subjects (20.0%) were determined to have achieved a cCR (Supplementary Table 1). Three of the subjects who achieved a cCR had loco-regional regrowth at 6.1, 4.6, and 2.3 months after achieving cCR; these 3 subjects underwent subsequent surgery during the 2-year follow-up period. The other 2 subjects remained disease-free at the end of the study. Twenty-six subjects had evaluable MRIs to assess mTRG. mrTRGs of Grade 3 or 4 were reported in 69.2% of subjects (18 of 26) and Grade 1 or 2 in 30.8% of subjects (8 of 26) (Table 3). The majority of subjects (17 of 26 [65.4%]) had MRI-confirmed down-staging of stage 3 or lower.

Of the 28 subjects who entered the study, 25 (89.3%) subjects completed 10 weeks of treatment with evaluable scans and 15 of 28 subjects (53.6%) underwent a surgical procedure to resect the primary tumor (Table 3). Four of 15 subjects (26.7%) who underwent surgery achieved a pCR, 1 each from the 250 mg AN0025 + SCRT group and 500 mg AN0025 + SCRT group, and 2 from the 500 mg AN0025 + LCRT group. For those who did not undergo surgery during the scheduled surgery period, the reasons were as follows: 5 subjects had inoperable tumors; 3 subjects did not agree to surgery due to mrTRG of 2 or 3; 3 subjects discontinued from study; and 2 subjects had a CCR. A summary of the Histopathology variables can be found in Table 3.

Subjects were followed for a median of 30.1 months from the time of their first study treatment. Of the 28 subjects enrolled, 21 (75.0%) were alive at study completion. Additionally, 3 subjects discontinued study treatment and 5 subjects died during the study (including 1 of the subjects who discontinued study treatment). Summary of OS and DFS events and censoring can be found in Table 4.

Overall, 13 of 28 subjects (46.4%) experienced disease progression or death. Five (17.9%) subjects had unequivocal metastatic lesions, 3 (10.7%) subjects had locoregional tumor regrowth, 2 (7.1%) subjects had locoregional recurrence, 2 (7.1%) subjects died during the DFS follow-up period (unrelated to the treatment), and

Table 3
Summary of histopathology variables (safety analysis set).

	250 mg		500 mg		All Subjects (N = 28)
	LCRT (N = 7)	SCRT (N = 7)	LCRT (N = 7)	SCRT (N = 7)	
Subjects who underwent a surgical procedure to resect the primary tumor, n	4[1]	1	4	6	15
Subjects with a pCR, n (%)[2]	0 (0%)	1 (100%)	2 (50%)	1 (16.7%)	4 (26.7%)
pTRG[3]					
TRG1, n (%)	0 (0%)	1 (14.3%)	2 (28.6%)	1 (14.3%)	4 (14.3%)
TRG2, n (%)	0 (0%)	0 (0%)	0 (0%)	1 (14.3%)	1 (3.6%)
TRG3, n (%)	4 (57.1%)	0 (0%)	2 (28.6%)	2 (28.6%)	8 (28.6%)
TRG4, n (%)	0 (0%)	2 (28.6%)	1 (14.3%)	2 (28.6%)	5 (17.9%)
TRG5, n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Subjects with a CRM-ve resection, n (%)[3]	2 (28.6%)	3 (42.9%)	5 (71.4%)	5 (71.4%)	15 (53.6%)

Table 4
Summary of events and censoring of overall survival and disease-free survival.

	250 mg		500 mg		All Subjects (N = 28)
	LCRT (N = 7)	SCRT (N = 7)	LCRT (N = 7)	SCRT (N = 7)	
Number of deaths	2 (28.6%)	2 (28.6%)	1 (14.3%)	0	5 (17.9%)
Subjects with OS censored	5 (71.4%)	5 (71.4%)	6 (85.7%)	7 (100%)	23 (82.1%)
Alive as of cut-off date	5 (71.4%)	4 (57.1%)	6 (85.7%)	6 (85.7%)	21 (75.0%)
Withdrawal of consent	0	1 (14.3%)	0	1 (14.3%)	2 (7.1%)
Subjects with DFS events	4 (57.1%)	4 (57.1%)	2 (28.6%)	3 (42.9%)	13 (46.4%)
Pelvic MRI demonstrate residual/recurrent tumor	1 (14.3%)	0	0	0	1 (3.6%)
Regrowth of the tumor compared to post-treatment MRI scans (non-surgical)	2 (28.6%)	0	1 (14.3%)	0	3 (10.7%)
Locoregional recurrence	0	2 (28.6%)	0	0	2 (7.1%)
Unequivocal metastatic lesion	0	1 (14.3%)	1 (14.3%)	3 (42.9%)	5 (17.9%)
Death	1 (14.3%)	1 (14.3%)	0	0	2 (7.1%)
Subjects with DFS censored	3 (42.9%)	3 (42.9%)	5 (71.4%)	4 (57.1%)	15 (53.6%)
Alive without disease as of cut-off date	3 (42.9%)	2 (28.6%)	5 (71.4%)	3 (42.9%)	13 (46.4%)
Withdrawal of consent	0	1 (14.3%)	0	1 (14.3%)	2 (7.1%)

Abbreviations: OS = overall survival; DFS = disease-free survival.

1 (3.6%) subject had a residual/recurrent tumor on MRI. Of the 13 subjects with disease progression or death, 4 subjects were in the 250 mg AN0025 + LCRT group, 4 subjects were in the 250 mg AN0025 + SCRT group, 2 subjects were in the 500 mg AN0025 + LCRT group, and 3 subjects were in the 500 mg AN0025 + SCRT group.

In terms of pharmacokinetics, all 28 subjects had evaluable plasma concentration–time data and were included in the PK analysis (Supplementary Table 2).

We performed biomarker analysis. Twenty-four of 25 (96.0%) subjects had cytokine values available at baseline. Subjects who achieved a pCR (4 subjects) or cCR (5 subjects) tended to have lower cytokine levels at baseline than the 15 non-CR subjects. Forty (89%) of the 45 cytokines measured had numerically higher levels in the non-CR subjects, with the following 11 cytokines in the panel having a GMR > 5 and a difference in medians with p-values < 0.1: interleukin 1(IL-1) alpha; IL-4; IL-18; IL-27; epidermal growth factor (EGF); hepatocyte growth factor (HGF); vascular endothelial growth factor D (VEGF-D); stem cell factor (SCF); interferon (IFN) gamma; monocyte chemoattractant protein 1 (MCP-1; C-C motif chemokine ligand [CCL2]); and Eotaxin (CCL11)Of these 11 cytokines identified as differentially expressed between responders and non-responders at baseline, 4 cytokines EGF, IL-4, IL-27, and MCP-1 also showed differential regulation after exposure to treatment. These 4 cytokines showed increased detection in the periphery after 15 days of treatment in responders and decreased expression in the periphery in non-responders (Fig. 2).

Immunohistochemistry data were available for 3 CR subjects and 5 non-CR subjects. The subjects who showed clinical response had a significantly higher level of activated (CD3 + CD8 + PD-1+) tumor infiltrating lymphocytes (TILs) within the tumor when compared to those subjects that did not show clinical response (Supplementary Fig. 2). Not only did the responders have higher baseline levels of activated CD8 TILs, the subjects demonstrating clinical response also showed increases in these activated TILs after 2 weeks of exposure to therapy. This observation was made in 2/3 CR subjects and 0/5 non-CR subjects (Supplementary Fig. 2).

Discussion

Overall, AN0025 using 250 mg or 500 mg AN0025 QD for 10 weeks in conjunction with radiotherapy and chemotherapy was well-tolerated. No subject experienced a DLT on AN0025 and chemoradiotherapy, and thus an MTD was not reached. In general, the proportion of subjects with TEAEs was comparable to those who previously received AN0025 alone [10] with the exception of fatigue, which was reported in 50% of subjects in the present study. Previous reports suggest 64% of patients with rectal cancer report fatigue with radiotherapy alone [26]. The extent of exposure (assessed by AUC) for AN0025 was dose proportional between the 250 mg and 500 dose levels. Furthermore, following 7 days of dosing, minimal accumulation was observed for AN0025 in both SCRT and LCRT treatment groups at the 250 mg and 500 mg dose levels.

AN0025 using 250 mg or 500 mg AN0025 QD for 10 weeks in conjunction with radiotherapy and chemotherapy and has been shown to achieved substantial downstaging with high rates of cCR and pCR, in comparison to many reported studies. The population was locally advanced and included no cT2 patients, 39.2% cT3a/b and 60.8% cT3c/d or cT4. Following surgery, 26.7% achieved a pCR, (Table 3). Achieving pCR following neoadjuvant CRT is associated with favourable long-term survival, with low rates of distant failure and local recurrence [Martin 2012] [27]. In addition, 5

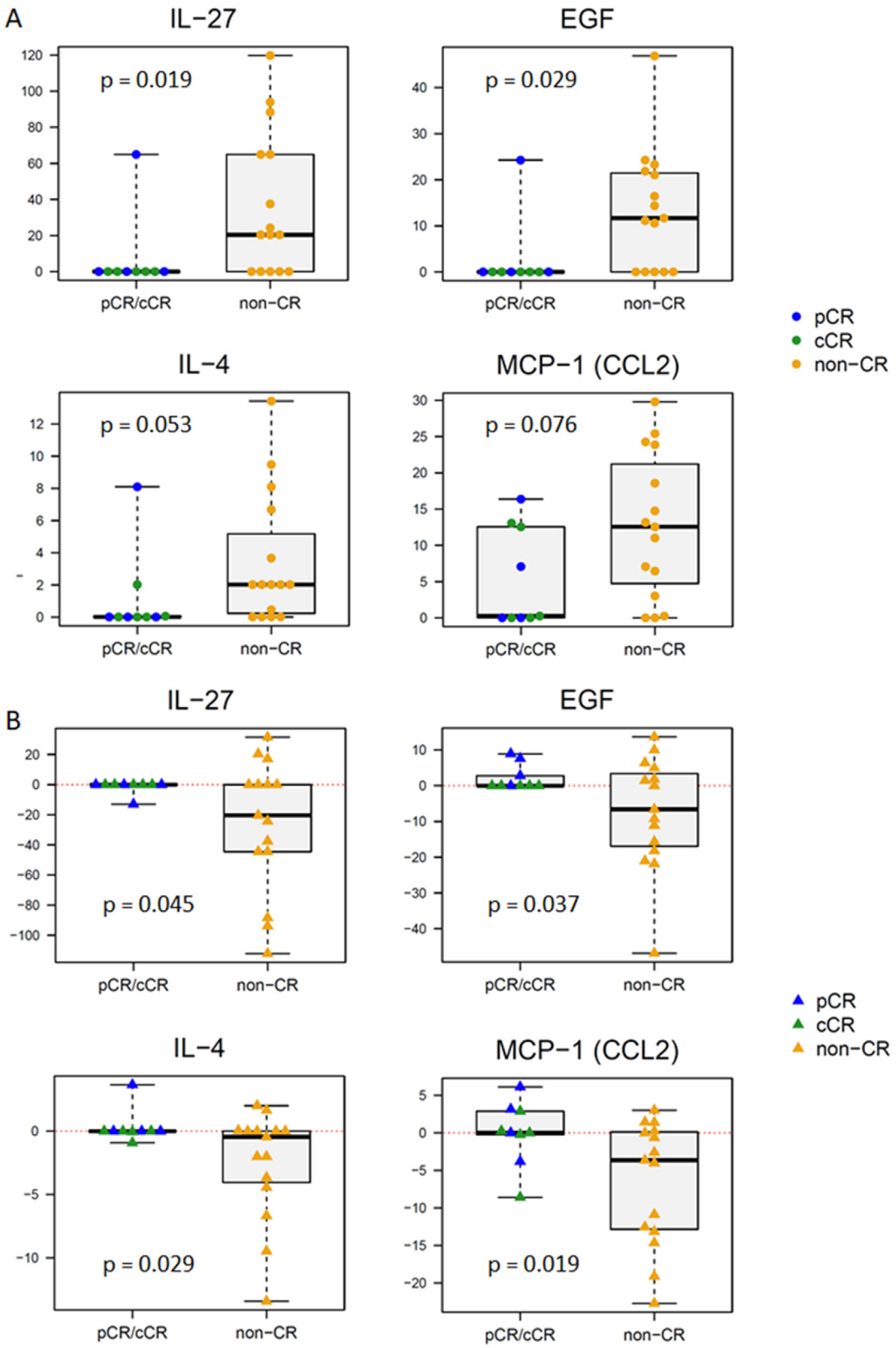

Fig. 2. Boxplots for cytokine levels, baseline and C1D15, in pCR/cCR and non-CR subjects. A. Differential expression between pCR/cCR and non-CR of select cytokines at baseline. B. Differential expression between pCR/cCR and non-CR of select cytokines in the change from C1D1 to C1D15. Values below lower limit of quantification (LLOQ) were noted in 4 (44%) of the 9 pCR/cCR subjects, while only 1 (7%) of the 15 non-CR subjects had such baseline cytokine measures. CCL = C–C motif chemokine ligand; cCR = complete clinical response; CR = complete response; EGF = epidermal growth factor; factor;; IL = interleukin; MCP-1 = monocyte chemoattractant protein; non-CR = non-complete response; pCR = pathological complete response;.

subjects had a cCR after the 10 weeks of treatment. A total of 65.4% of subjects had MRI-confirmed down-staging of stage 3 or lower following completion of treatment, with comparable numbers across all treatment groups.

Those patients who received 500 mg of AN0025 had an OS of 85.7%, whereas those who received the 250 mg dose had an OS of 64.3%. This finding may suggest potential increased efficacy with the 500 mg dose, but requires confirmation in larger Phase II/III trials.

This enhanced response offers an increased chance of achieving clear resection margins and the potential for organ-sparing. Many strategies have attempted to improve clinical outcomes of patients with LARC. Additional cytotoxic agents (oxaliplatin, irinotecan) and molecularly targeted agents have been added concurrently with fluoro-pyrimidine based LCCRT. With exception of a single trial, which reported a small but significant benefit in terms of pCR and disease-free survival (Rodel 2012, Rodel 2015) [28,29] these trials have not shown a substantial benefit and been associated with enhanced toxicity. Total neoadjuvant therapy using additional neoadjuvant chemotherapy has shown some benefit in terms of a doubling of pCR compared with LCCCRT alone, and a reduction in the risk of metastases by 7% (Bahadoer 2021, Conroy 2021) [30,31]. Yet, patients continue to relapse and die from LARC despite NACT (even with FOLFOXIRI). We may have reached a ceiling effect with currently available cytotoxic agents. Immunotherapy represent a potential alternative strategy. In addition, increasing interest and evidence for the safety of nonoperative treatment for patients achieving a CCR after neoadjuvant LCCRT has rekindled a drive for interventions to increase response.

AN0025 Is being further explored in combination with SCRT and LCCRT in the current ARTEMIS (Augmenting RadioTherapy in REctal Cancer to Minimise Invasive Surgery) study given orally at a dose of 500 mg once a day. This randomised phase II study (EUDRACT Number: 2021-005716-57) aims to recruit 140 patients with cCR at 6 months as the primary endpoint.

Proof of mechanism is demonstrated by a change in intratumoral immune cell populations consistent with inhibition of EP4 signaling such as a decrease in the ratio of M2/Total TAM or an increase in the number of CD8 + T cells. POP could also be evidenced by a decrease in the number or ratio of MDSC in circulation or change of immune gene expression in blood. Retrospective testing for potential subject selection/stratification (PS) markers may also be explored, for example: baseline CD8 + T cells or ratio of M2/Total TAM in tumor; baseline MDSC in circulation; tumor mutational status; immune gene signature.

In the exploratory biomarker analyses, clinical responders showed an increased immune response in both sample types, and non-responders a decreased immune response. Non-responders showed a decrease in pro-inflammatory peripheral cytokines, while responders showed an increase in pro-inflammatory cytokines (IL-4, IL-27, MCP-1) The responders also showed a greater potential to increase the number of activated T cells in the tumor microenvironment compared to non-responders. (Supplementary Fig. 3).

There are limitations in this study, including a small sample size, lack of a standard arm control group, the lack of long-term oncological outcomes and uncertain final pathology in patients, who chose watch & wait after neoadjuvant treatment, which dilutes the biomarker assessment. While the numbers in our study are small, results are encouraging and suggest an enhanced efficacy when using AN0025 and chemoradiotherapy compared to chemoradiotherapy alone.

This evidence for reprogramming of the immunosuppressive tumor microenvironment (TME make it appealing to combine AN0025 with currently available immunotherapies (e.g., immune checkpoint inhibitors, ICIs). to further enhance the efficacy of SCPRT and LCCRT.

Conclusions

In conclusion, preoperative treatment with 250 mg or 500 mg of AN0025 administered for 10 weeks with SCRT or LCRT and chemotherapy in subjects with LARC was well-tolerated and showed promise in inducing substantial macroscopic tumor regression with cCR, high rates of pCR and complete resection with clear margins. Translational research provided a rationale for future combinations of AN0025 and checkpoint inhibitors in this setting. Phase II trials will shed further light on the efficacy of AN0025 for the treatment of rectal cancer.

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Declaration of Competing Interest

LW has no conflicts of interest to report. MPS has received fees for meetings and lectures from Servier, Amgen, and Merck. MH has served on the advisory board and provided educational support for Clovis Oncology and GlaxoSmithKline and has received research funding from Bristol Myers Squibb, Clovis Oncology, and Merck Sharp & Dohme. JN has no conflicts of interest to report. TH has provided consulting for Synthetic Biologics, Novocure, Boston Scientific, Inivata, Merck, and GlaxoSmithKline; has served on scientific advisory boards for PanTher Therapeutics (Equity) and Lustgarten; and has received research funding for clinical trials from Taiho, AstraZeneca, *Bristol Myers Squibb, GlaxoSmithKline*, IntraOp, and Ipsen. SX, XL, and NL were employed by Adlai Nortye, USA at the time of this study. SF has provided consulting for Accuray, Boehringer Ingelheim, Roche, Genentech, AstraZeneca, and ViewRay; has received honoraria from Bayer, Bristol Myers Squibb, Varian, Elekta, Regeneron, Eisai, AstraZeneca, MedImmune, Merck US, and EMD Serono; and has received research grants from Merck, Varian, Bristol Myers Squibb, Regeneron, Eisai, and Eli-Lilly. RGJ has received fees for lectures from Servier, and consulting from Incyte.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2023.109669.

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