STUDY SYNOPSIS

Title of Study	**Capecitabine-based chemoradiotherapy (CRT) in combination with the IL-1 receptor antagonist Anakinra for rectal cancer patients**
Sponsor	Dean of the Medical Faculty, University of Cologne
Study Chairman (LKP)	Prof. Dr. med. Dr. Emmanouil Fokas, Cologne, for the German Rectal Cancer Study Group (GRCSG)
Rationale	Preoperative fluoropyrimidine-based chemoradiotherapy (CRT) and total mesorectal excision (TME) surgery 6-10 weeks thereafter, followed by optional adjuvant chemotherapy, has been a standard multimodal treatment option for patients with intermediate to high risk rectal cancer (UICC II and III) during the last two decades. With this, pathological complete response rates (pCR) are in the range of 10-15%, 3 year-local failure rates in the range of 5%, distant recurrences occur in 25-30% of patients, and 3 years disease-free survival (DFS) amounts to 70%. More recently, total neoadjuvant treatment (TNT) with either 5x5 Gy or fluoropyrimidine-CRT, followed by consolidation chemotherapy with fluorouracil (or capecitabine) and oxaliplatin (FOLFOX/CAPOX), and TME, has significantly improved pCR and DFS compared to standard preoperative FU-CRT (+/- adjuvant chemotherapy) in recent phase 3 trials for patients with high-risk rectal cancer (cT4, mrCRM+, EMVI+, N2, lateral N+) and has recently been accepted as preferred treatment for this patient subgroup. In contrast, it remains unclear whether patients with intermediate risk rectal cancer benefit from TNT (currently under investigation in trials), whereas elderly and frail patients are not eligible for TNT and are rather be treated with 5x5 Gy or capecitabine-CRT alone. IL-1 is an inflammatory cytokine that plays a key role in tumor formation, progression and therapy resistance. Extensive preclinical and translational studies i employing tumor samples from GRCSG patient cohorts, patient-derived tumor organoids (PDO), and a novel orthotopic tumor organoid murine rectal cancer model, have showed that IL-1 mediates CRT resistance and disease progression, resulting in poor prognosis in rectal cancer patients. IL-1 inhibition using the IL-1 receptor antagonist (IL-1RA) Anakinra, a drug already approved for the treatment of patients with rheumatoid arthritis, significantly sensitized tumors to CRT. Thus, blockade of IL-1 signaling using Anakinra constitutes an attractive option to significantly improved response to CRT, organ preservation, and DFS in rectal cancer (Nicolas et al. Cancer Cell 2022).

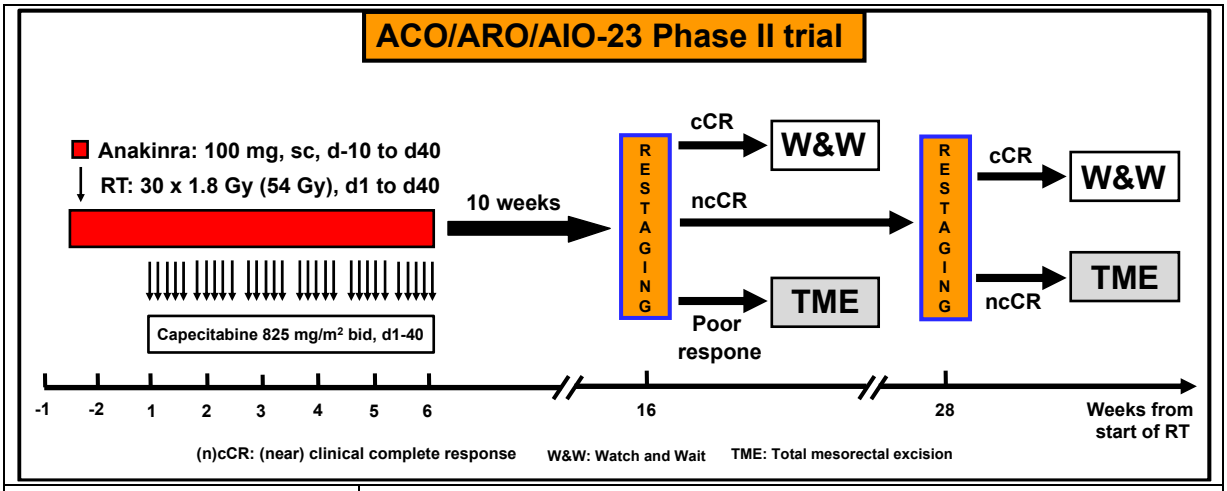
	Based on these data, we have recently completed the ACO/ARO/AIO-21 phase I drug re-purposing trial using a 3+3 capecitabine dose escalation design to examine whether the IL-1 receptor antagonist Anakinra can be safely combined with capecitabine-based CRT, followed by W&W for patients with clinical complete response (End of recruitment: 06/2023; ClinicalTrials.gov: NCT04942626; LKP: E. Fokas und C. Rödel; Protocol publication: Fleischmann et al. Clin Transl Radiat Oncol. 2022; doi: 10.1016/j.ctro.2022.04.003). We found that capecitabine at a standard dose of 825 mg/m^2 bid can be safely used as backbone CRT regimen when combined with Anakinra. Importantly, after a median follow-up of 12 months (range: 0-23 months), 6/12 (50%) still have a persistent cCR (or pCR), which compares favorably with the historical pCR rate of 10-15% after standard CRT and surgery. The hereby proposed **ACO/ARO/AIO-23** phase II trial will assess the efficacy of combining IL-1RA Anakinra with FU-CRT, followed by TME or selective organ preservation (Watch and Wait, W&W) for patients with clinical complete response (cCR), in rectal cancer. Concomitant chemotherapy with Capecitabine at a dose 825 mg/m^2 bid will be used during CRT based on our previous ACO/ARO/AIO-21 phase I trial. Potential benefits to patients with rectal cancer participating in this trial include access to the drug Anakinra that (a) is not available outside a clinical trial for rectal cancer but has been approved for standard use in rheumatoid arthritis, i.e. a different non-malignant disease, (b) has shown evidence of clinical activity when used in combination with chemotherapy in other tumor types including metastatic colorectal cancer, (c) has been used for over 15 years in approximately 150.000 patients with non-malignant diseases worldwide with a demonstrated safety record, and (d) based on preclinical studies, may augment the efficacy of FU-CRT that can result in increased tumor response and local disease control, but also can decrease distant metastases to improve long-term oncological outcome.
Study type and study design	Investigator-driven, open-labeled, phase II study
Primary objective and endpoint	The primary endpoint will be pathological or clinical complete response (pCR, cCR) after surgery or W&W, respectively, at re-staging/surgery 10 weeks after treatment completion. In patients with near clinical complete response (ncCR) at restaging, final response assessment will be performed 3 months thereafter, and, in case of cCR, this will count towards the primary endpoint.
Secondary objectives and endpoints	• Postoperative complications of (salvage) surgery • Acute and late toxicity assessment according to NCI CTCAE V.5.0

	• Rate of W&W with or without local regrowth • Cumulative incidence of locoregional regrowth after cCR • Rate of salvage surgery (LE/TME with or without APR/stoma) after locoregional regrowth • Cumulative incidence of local recurrence after (salvage) surgery • Cumulative incidence of distant recurrences • Disease-free survival • Overall survival • Pathological TNM-staging • R0 resection rate; negative circumferential resection rate • Tumor regression grading according to Dworak • Quality of TME according to MERCURY • Quality of life and functional outcome based on treatment arm and surgical procedures/organ preservation • Translational / biomarker studies
Inclusion criteria	• Male and female patients with histologically confirmed diagnosis of rectal adenocarcinoma localized 0 – 12 cm from the anocutaneous line as measured by rigid rectoscopy (i.e. lower and middle third of the rectum) • Staging requirements: High-resolution, thin-sliced (i.e. 3 mm) magnetic resonance imaging (MRI) of the pelvis is the mandatory local staging procedure. • Patients with MRI-defined low/intermdiate risk rectal cancer with the presence of at least one of the following conditions: - cT2N0 tumors of lower third of the rectum (≤6 cm) - cT3a-d N0/Nx of the lower or middle third of the rectum (≥ 6-12 cm from the anocutaneous line) - cT2-3$_{any}$ with clear criteria of lymph-node involvement in 1-3 lymph-nodes (cN1) based on strict MRI-criteria (see appendix) • Trans-rectal endoscopic ultrasound (EUS) is additionally used when MRI is not definitive to exclude early cT1 disease in the lower third or middle third of the rectum. • Spiral-CT of the abdomen and chest to exclude distant metastases. • Aged at least 18 years. No upper age limit • WHO/ECOG Performance Status ≤1 • Adequate hematological, hepatic, renal and metabolic function parameters: - Leukocytes ≥ 3.000/mm^3, ANC ≥ 1.500/mm^3, platelets ≥ 100.000/mm^3, Hb > 9 g/dl - Serum creatinine ≤ 1.5 x upper limit of normal - Bilirubin ≤ 2.0 mg/dl, SGOT-SGPT, and AP ≤ 3 x upper limit of normal

	• Informed consent of the patient
Exclusion criteria	• Rectal tumors in the upper third (>12-16 cm from the anocutaneous line) • cT4 tumors • cT_{any} middle/low third of rectum with clear MRI criteria for N2 • mrCRM+ (≤ 1mm) • Extramural venous invasion (EMVI+) • Distant metastases (to be excluded by CT scan of the thorax and abdomen) • Prior antineoplastic therapy for rectal cancer • Prior radiotherapy of the pelvic region • Major surgery within the last 4 weeks prior to inclusion • Subject pregnant or breast feeding, or planning to become pregnant within 6 months after the end of treatment. • Subject (male or female) is not willing to use highly effective methods of contraception during treatment and for 6 months after the end of treatment. • On-treatment participation in a clinical study in the period 30 days prior to inclusion • Previous or current drug abuse • Other concomitant antineoplastic therapy • Serious concurrent diseases, including neurologic or psychiatric disorders (incl. dementia and uncontrolled seizures), active, uncontrolled infections, active, disseminated coagulation disorder • Clinically significant cardiovascular disease in (incl. myocardial infarction, unstable angina, symptomatic congestive heart failure, serious uncontrolled cardiac arrhythmia) ≤ 6 months before enrolment • Prior or concurrent malignancy ≤ 3 years prior to enrolment in study (Exception: non-melanoma skin cancer or cervical carcinoma FIGO stage 0-1), if the patient is continuously disease-free • Known allergic reactions on study medication • Known dihydropyrimidine dehydrogenase deficiency • Psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule (these conditions should be discussed with the patient before registration in the trial). • History of severe hepatic impairment (e.g. Child-Pugh = Grade C) • Moderate (Creatinine Clearance 30 to 49 mL/minute), severe (Creatinine Clearance <30 mL/minute) renal impairment

	• Neutropenia (neutrophil count <1.5x10^9/l) • Known hypersensitivity to Anakinra or E. coli derived proteins, Anakinra or any of the components of the product • Asthma • Patients with clinically significant bacterial, fungal, parasitic or viral infection, which require acute therapy. Patients with acute bacterial infections requiring antibiotic use should delay screening/enrollment until the course of antibiotic therapy has been completed • Patients with known active hepatitis B, C or who are HIV-positive or who are at risk for HBV reactivation. At risk for HBV reactivation is defined as hepatitis B surface antigen positive or anti-hepatitis B core antibody positive. Prior test results obtained as part of standard of care that confirm a subject is immune and not at risk for reactivation (ie, hepatitis B surface antigen negative, surface antibody positive) may be used for purposes of eligibility and tests do not need to be repeated. Subjects with prior positive serology results must have negative polymerase chain reaction results. Subjects whose immune status is unknown or uncertain must have results confirming immune status before enrollment. • Subjects who are already using the following medications will not be allowed: - Tumor necrosis alpha inhibitors: Use on any of these biologics within 8 weeks of screening or baseline visit. - IL-6 inhibitors: Use of any IL-6 inhibitors within 8 weeks of screening or baseline visit - Janus Kinase inhibitors: Use of baricitinib, tofacinitib, upadacitinib, and ruxolitinib, oclacitinib, fedratinib, within 2 weeks from screening or baseline visit. - Bruton's tyrosine kinase inhibitors: Ibrutinib, acalabrutinib, zanubrutinib - CCR5 antagonist (CCR5 = C-C Chemokine Receptor Type 5; DMARD = Disease Modifying Anti-Rheumatic Drug): Leronlimab is also an immunomodulator. - DMARDs: cyclosporine, cyclophosphamide, mycophenolic acid, chlorambucil, penicillamine, azathioprine: Use within 6 months prior to screening or baseline visit. - Rituximab: Use of rituximab within 1 year of screening or baseline visit. - Abatacept: Use of abatacept within 8 weeks of screening or baseline visit. • Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their

	participation such as severe impaired lung functions as defined as spirometry and DLCO that is 50% of the normal predicted value and/or O_2 saturation that is 88% or less at rest on room air • Patients under ongoing treatment with another investigational medication or having been treated with an investigational medication within 30 days (incl. live attenuated vaccine) of screening or 5 half-lives (whichever is longer) prior to the first dose of investigational product • Patients receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent. Topical or inhaled corticosteroids are allowed • History of any other disease, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates use of an investigational drug, or that might affect interpretation of the results of this study, or render the subject at high risk for treatment complications.
Treatment	Patients receive standard preoperative CRT with oral capecitabine during RT (1.8 Gy to 45 Gy to the primary tumor and pelvic lymph nodes; followed by sequential boost of 9 Gy to the gross tumor volume) in combination with Anakinra 100 mg s.c. administered from d-10 (i.e. 10 days before initiation of RT) to the last day of RT. Capecitabine will be administered using the standard dose of 825 mg/m^2 bid po during CRT (as shown feasible by our previous ACO/ARO/AIO-21 phase I trial) from day 1 to day 40 of RT including weekends. Restaging to evaluate tumor response will be conducted 10 weeks after completion of CRT (week 16 after CRT start). For patients achieving a cCR, as strictly assessed by digital rectal examination, endoscopy and MRI, a watch and wait (W&W) option with close follow-up is scheduled. In case of poor response or even tumor progression, immediate total mesorectal excision (TME) surgery is recommended. In patients with ncCR at first restaging, final response assessment will be performed 3 months thereafter (week 28 after CRT start), and, in case of cCR, this will count towards the primary endpoint. According to the German S3-guidelines, adjuvant chemotherapy is optional (recommendations are given in the protocol, but are not mandatory).



Translational research	An extensive translational research program is implemented in order to further refine molecular prognostic and predictive profiling, and eventually identifying subgroups for treatment stratification and conservative surgical procedures.
Sample size	The treatment protocol in the present study is assumed to be non-promising if the cCR/pCR rate is 40% or less and it is assumed to be 55% or higher. Based on normal approximation, a sample size of 85 patients yields a power of 80% at the usual one-sided level of 2.5%. The sample size calculations were carried out using nQuery 9 (version 9.3.1; procedure POT0). Since the primary endpoint "clinical complete response" is assessed after completion of CRT + Anakinra, only a small dropout of about 5% is expected, which results in an expected **total number of 90 patients to be enrolled.**
Biostatistical methods and size justification	Summary statistics such as means, standard deviations and quantiles for continuous data and frequencies (proportions) for categorical data will be provided to describe the patients' baseline characteristics such as age, gender, clinical tumor category, clinical nodal category and distance of the tumor from the anal verge. Organ preservation, is defined as follows: survival with rectum intact, no major surgery, no stoma. Accordingly, the primary endpoint, organ preservation, will not be reached if any of the following occurs: (1) death, (2) any major surgery other than local excision (R0) performed after randomization, during treatment, at re-staging (initial or final) after completion of CRT+Anakinra due to clinical non-cCR, or for any locoregional regrowth after initial cCR requiring salvage-TME, (3) any locoregional regrowth not amenable to salvage surgery, or (4) any stoma (non-re-converted protective stoma within 6 months after completion of CRT+Anakinra, or any stoma needed for toxicity or poor function), whichever occurs first. Adverse events will be summarized by treatment arm, body system and preferred term, intensity, and causal relationship to

	study agent and their frequencies and percentages will be reported. The safety report is according to ICH E3 “Structure and content of Clinical Study Reports” (CPMP/ICH/137/95).
Follow-up analyses	Safety follow-up will be conducted by an independent data safety monitoring committee.
Estimated number of sites	In total, 10 centers of the GRCSG, including at least three NCT sites will participate to this trial.
Study duration	Submission of funding application: Q1 2024 Start of preparation: Q1 2025* Start of recruitment: Q3 2025 Planned termination of recruitment: Q3 2027 Planned termination of follow-up: Q3 2030 Final study report: Q1 2031 **In case of final funding approval*