I.R.C.C.S. Ospedale San Raffaele

CLINICAL STUDY PROTOCOL

Study Code: Adaptive Rectal Cancer Trial 02

Title: Preoperative, adaptive radiotherapy concomitant to chemotherapy for rectal adenocarcinoma (Adaptive Rectal Cancer Trial 02). An interventional study.

Principal Investigator: Dott. Paolo Passoni

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Study Code	***Adaptive Rectal Cancer Trial 02***
Other Protocol Identifiers	The study will be registered on clinicaltrials.gov
Version	1.2
Date	10.02.2026

Sponsor	IRCCS Ospedale San Raffaele
Co-Sponsor	NA
Authorized Sponsor Representative	Prof. Nadia Di Muzio Director of the Radiation Oncology Department IRCCS Ospedale San Raffaele
Funding Source(s)	*NA*
Principal Investigator	Dr. Paolo Passoni

VERSION HISTORY

Protocol version n.	Reason of changes	Date issued
1.2	First Version submitted to the Ethics Committee (EC)	10.02.2026

PROTOCOL SIGNATURE PAGE

Study Title: Preoperative, adaptive radiotherapy concomitant to chemotherapy for rectal adenocarcinoma (Adaptive Rectal Cancer Trial 02). An interventional study.

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Protocol Version and Date: version 1.2 of 10.02.2026

The undersigned has read and understood all the aspects of the protocol detailed within this document and agrees to supervise and conduct the study in accordance with the protocol, the Declaration of Helsinki, Guideline for Good Clinical Practice ICH E6 (R2), and all applicable regulatory requirements.

Nadia Di Muzio		**UO Radioterapia**	**10.02.2026**
Authorized Sponsor Representative Name	**Signature**	**Affiliation**	**Date**

Paolo Passoni		**UO Radioterapia**	**10.02.2026**
Principal Investigator Name	**Signature**	**Affiliation**	**Date**

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1. KEY STUDY CONTACTS

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Funding source(s)	NA
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2. SYNOPSIS

Study Code	Adaptive Rectal Cancer Trial 02
Study Title	Preoperative, adaptive radiotherapy concomitant to chemotherapy for rectal adenocarcinoma (Adaptive Rectal Cancer Trial 02). An interventional study.
Protocol Version and Date	1.2 10.02.2026
Sponsor	IRCCS Ospedale San Raffaele Via Olgettina 60 – 20132, Milan, Italy
Funding Source(s)	No profit study and no funding
Principal Investigator	Dr. Paolo Passoni
Study Description	Preoperative radiotherapy (RT), concomitant with chemotherapy, is the standard therapy for rectal adenocarcinoma in stage T3-4N0 or with positive lymph-nodes. For more than 20 years in our medical oncology department, concomitant chemotherapy has consisted of 3 cycles of oxaliplatin, the first administered alone 14 days before the start of RT, the second concomitantly with the start of RT and the third 14 days later, and capecitabine, tablets that the patient takes from the first day of oxaliplatin until the end of RT. Since 2009, in our Radiotherapy department, preoperative radiotherapy treatment has been based on the adaptive technique. Patients undergo a smc CT scan and a smc MRI for centering before radiotherapy. The two tests are used to prepare the radiotherapy treatment plan. A volume corresponding to the rectal disease, the mesorectum and the regional pelvic lymph nodes is identified as the target. A dose of 41.4 Gy in 18 fractions is prescribed on this volume, a dose biologically equivalent to the standard dose of 50.4 Gy in 28 fractions. Halfway through the RT treatment (so after 9 fractions of RT and two cycles of oxaliplatin) a smc MRI is repeated. In these images the residual rectal disease is identified which receives an increased dose (boost) of 3.1 Gy in the last 6 fractions of RT for a total dose of 46.2 Gy in 18 fractions. Patients are subsequently operated at least 7 weeks after the end of radiochemotherapy according to guidelines and the surgical specimen is examined by the pathologist. With this technique and doses, biologically equivalent to the standard, we treated 152 patients from 2009 to 2023. The percentage of complete pathological remissions was 26%, 29% in patients who received all three cycles of oxaliplatin (in line with the most recent phase III studies), 21% in patients who received two or less. Feasibility of RT 98%, diarrhea proctitis G3 respectively equal to 6.5% and 4%. No toxicity equal to or greater than G4. The primary endpoint of the present study is the increase in the percentage of pathological complete responses (pCR) from the current 41% (in patients selected by the radiobiological index ERI_TCP-see below-) to 70% obtained by an increase in the dose of radiotherapy on the tumor residue still visible in the images of an MRI performed halfway through the standard radiotherapy treatment, by a better choice of patients selected through the analysis of a radiobiological index called ERI_TCP, and by a better identification of the target on which to increase the dose through intermediate MRI as per clinical practice.

	pCR means the complete disappearance of the tumor in the histological examination of the surgical specimen. pCR is an important objective because it is a strong positive prognostic factor: patients who achieve a pCR have a prognosis comparable to that of patients in stage I. Furthermore, patients who achieve a pCR automatically have a clinical complete response (cCR) that is the complete disappearance of the tumor on pre-surgical restaging tests. These patients could even be offered a "watch and wait strategy" that is, resorting to surgery only in case of recurrence of the rectal disease. As mentioned, the increase in pCR could be achieved with 1) a use of higher doses of RT, 2) better patient selection, 3) better identification of the target on which to increase the dose. 1) Use of higher doses of RT. Based on a recent meta-analysis (1) and on a subsequent refinement of the analyses on ERI_TCP performed by our physicists, a dose increase of 10 Gy could double the number of complete responses. Translated into the hypofractionation used in our old one, the dose increase would be from the current 3.1 Gy/fraction to 4 Gy/fraction to be delivered as a boost in the last 6 fractions. 2) Better patient selection Image-based assessments of early response to neoadjuvant chemoradiotherapy (nCRT) are valuable, as rapid tumor regression has been well documented. The early regression index (ERI) was introduced by our group several years ago and was found to effectively predict pCR outcomes by combining initial tumor volume and regression measured through high-contrast MRI imaging before and at mid-Radiotherapy (2-6).This MRI-derived biomarker can potentially be used in other nCRT contexts, as seen in recent studies involving gynecological and esophageal cancers (7, 8). In short, It is a radiobiological index (ERI_TCP, early regression index_ tumor control probability) which is calculated with the following formula: $ERITCP=-\ln[(1-(Vmid/Vpre)]^{Vpre}$ where Vpre is the volume of the rectal tumor pre-therapy, Vmid is the volume of the residual tumor still visible in the images of the MR intermediate to RT. From this formula comes a number that is correlated to the complete pathological and clinical responses (total disappearance of the tumor at pre-surgical restaging, cCR):

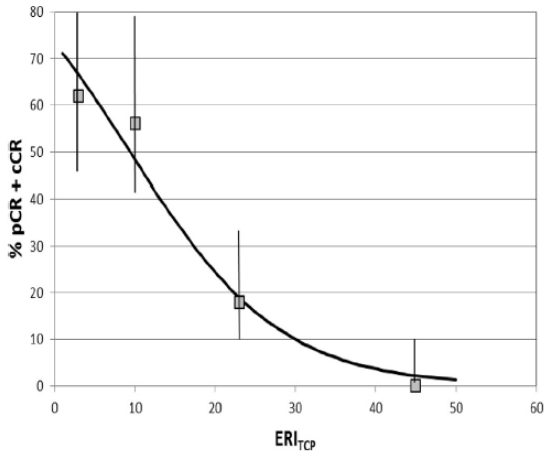

Fig. 1. The probability of pCR (or cCR followed by watch-and-wait) against the suggested radiobiological index (ERI$_{TCP}$) is plotted. The true rates (and their 95% CI) are also plotted, grouping the patients by quartiles.

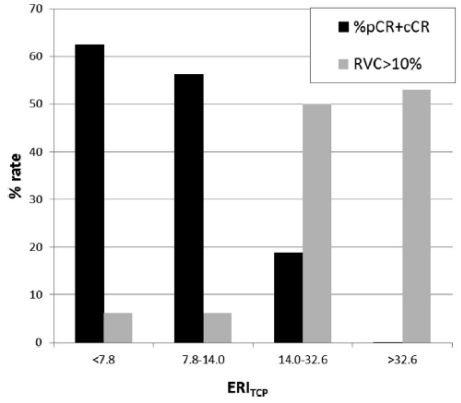

Fig. 2. The high discriminative power of ERI$_{TCP}$ is shown by grouping the patients by quartiles and plotting the rates of pCR or cCR (followed by watch-and-wait) and of patients with limited response (patients with a residual vital cells fraction, RVC >10%).

The probability of complete pathological and clinical response decreases as the ERI_TCP increases. Above the threshold value of 32.6, the probability is zero. By removing the quartile of patients with a value above this threshold from the denominator of our case study, the probability of complete response would increase from 29% (historical data cited above) to 41%.

3) Better identification of the target on which to increase the dose
In the current protocol, the final boost is delivered to the entire rectal tract included in the cranial and caudal limits of the residual tumor identified with an intermediate smc MRI. In the new protocol, the current dose will be maintained (3.1 Gy/fraction in the last 6 fractions) but the new increased dose (4 Gy in the last 6 fractions) will be delivered to the true residual tumor, also identified with the help of radiologists on an intermediate MRI with contrast medium acquired with diagnostic image quality.

Study Design	Prospective, single centre interventional study on procedure	
Primary Objective Increase in the rate of pathological complete responses (pCR)	**Primary Endpoint** The primary end-point is the demonstration of an increase of pCR from the present 41% to 70% in the population of patients with ERITCP<32.6.	**Time point(s)** 16 weeks, a time composed of 6 weeks of treatment duration, 7-12 weeks of waiting (standard waiting time) between the end of the radiochemotherapy and the surgical intervention to which must be added the time for the histological examination to be reported, approximately 3-4 weeks**.**
Secondary Objectives Tolerability and outcome	**Secondary endpoints** - Acute toxicity (graded according to CTCAE v 5.0-NCI) - Anal sphincter preservation rate - Incidence of surgical complications (graded according to Clavien-Dindo scale) - Outcome (time free from disease recurrence, local recurrence, distant recurrence, survival, calculated from the date of the first biopsy on rectal disease)	**Time point(s)** - Within 90 days from the end of radiochemotherapy - Act of surgery - Perioperative period - Periodic checks foreseen by the normal follow up for rectal adenocarcinoma
Study Population	Patients with resectable rectal adenocarcinoma	
Inclusion Criteria	<u>Initial phase (standard phase). These criteria represent the conditions for which preoperative chemoradiotherapy treatment for rectal cancer is clinically indicated</u> 1) Histologically confirmed rectal adenocarcinoma 2) Microsatellite status: stable 3) Stage T2N0 if lower rectal lesions are candidates for subsequent intersphincteric resection or abdominoperineal amputation with permanent colostomy 4) Stage T3-T4N0 or any T with positive lymph nodes 5) Lower margin of lesion no more than 12 cm from anal verge	

	Adaptive radiotherapy phase (experimental phase) 6) ERI_TCP < 32.6 calculated as follows: ERITCP=-ln[(1–(Vmid/Vpre)]Vpre where Vpre is the volume of the rectal tumor pre-therapy, Vmid is the volume of the residual tumor still visible in the images of the MR intermediate to RT) 7) Lower margin of rectal lesion at least 1 cm from surgical resection line on images of the intermediate smc MRI 8) ECOG (Eastern Cooperative Oncology Group) Performance Status ≤ 2 9) Age: 18-80 years 10) Written informed consent
Exclusion Criteria	Exclusion criteria for both standard and experimental phases 1) Distant metastases 2) Previous cancer excluding non-melanoma skin cancer diagnosed less than 5 years before rectal cancer appearance 3) Previous chemotherapy or radiotherapy to the pelvis 4) Contraindications to radiotherapy: active ulcerative colitis 5) Contraindications to chemotherapy: NE<1.5x10/L, Plt < 100x10/L), creatinine >1,5mg/dl, bilirubin > 2mg/dl, AST/ALT > 3x normal upper limit, significant cardiac disease, peripheral neuropathy. 6) Pregnancy 7) Breastfeeding
Intervention(s)	Increase the radiotherapy dose from 3.1 Gy/fraction to 4 Gy/fraction in the last 6 fractions of radiotherapy to the residual tumor still visible in the images of the MRI intermediate to RT (See study description)
Comparator	Historical case study on 152 patients having the same inclusion criteria as the standard phase of the present study over the period 2009-2023, mentioned in the study description.
OVER THE PERIOD	**Adverse events (data form our experience of adaptive RT, 152 pts treated from 2009 to 2023)**

Haematological toxicities	**Any grade**	**G1-2**	**G3-4**
Neutropenia	27 (14.5%)	24 (12.9%)	3 (1.6%)
Anemia	20 (10.7%)	19 (10.2%)	1 (0.5%)
Thrombocytopenia	26 (14%)	26 (14%)	0 (0%)

	Non-haematological toxicies	**Any grade**	**G1-2**	**G3-4**
	Fatigue	24 (12.9%)	22 (11.8%)	2 (1.1%)
	Diarrhea	99 (53.2%)	82 (44.1%)	17 (9.1%)
	Tenesmus	41 (22%)	39 (21%)	2 (1.1%)
	Rectal bleeding	18 (9.7%)	17 (9.1%)	1 (0.5%)
	Proctitis	95 (51%)	86 (46.2%)	9 (4.8%)
	Nausea	40 (21.5%)	39 (21%)	1 (0.5%)
	Vomiting	8 (4.3%)	7 (3.8%)	1 (0.5%)
	GU disorder	36 (19.3%)	34 (18.3%)	2 (1.1%)
	Skin disorder	38 (20.4%)	32 (17.2%)	6 (3.2%)
	Peripheral sensory neuropathy	30 (16.1%)	29 (15.6%)	1 (0.5%)
	Stomatitis	4 (2.1%)	4 (2.1%)	0 (0%)
	Postoperative complications			
	Any complication		79 (42.5%)	
	Anastomotic leakage		14 (7.5%)	
	Infection		20 (10.7%)	
	Fistula		19 (10.2%)	
	Bowel obstruction		12 (6.4%)	
	Vascular event		8 (4.3%)	
	Other		20 (10.7%)	
Efficacy Criteria	Percentage of complete pathological responses			
Sample Size	33 patients			

Statistical Design	Prospective single-center study. The primary endpoint is the demonstration of an increase in pCR from the current 41% to 70% in the patient population with ERITCP <32.6. Using Fisher's exact test to compare two proportions , a sample size of 30 patients was extimated to provide sufficient power to detect a difference from the hystorical value (alpha = 0.05 and beta = 0.80). Considering the actual rate of patient refusal and/or inability to undergo surgery (<10%), a total population of 33 patients can be considered sufficiently to complete the study. (9) Patients who refuse or are not fit for surgery will be considered not evaluable for the primary end point but evaluable for secondary endpoints
Duration of the Study	Duration of enrollment: two years from the inclusion of the first patient Duration of subject participation: 16 weeks Duration of treatment: 8 days Duration of total follow-up: 16 weeks for the primary endpoint Duration of total study period: 36 months from the date of recruitment of the first patient

3. ABBREVIATIONS AND DEFINITIONS

3.1. Abbreviations

ADL	Activity of Daily Living
AE	Adverse event
AESI	Adverse Event of Special Interest
CIOMS	Council for International Organization of Medical Science
CRF	Case Report Form
CRO	Contract Research Organization
DPIA	Data Protection Impact Assessment
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization
LVLP	Last Visit of Last Patient
MedDRA	Medical Dictionary for Regulatory Activities
PI	Principal Investigator
SAE	Serious Adverse Event

SOP	Standard Operating Procedure

3.2. Definitions: NA

4. BACKGROUND AND RATIONALE

Preoperative radiochemotherapy (RCT) has a beneficial impact on loco-regional control, improves pathological complete response (pCR) rate and has been considered a standard therapeutic option in advanced, resectable, rectal adenocarcinoma. In the last years, three phase III trials using total neoadjuvant therapy (TNT), that is radiotherapy and all cycles of chemotherapy before surgery, or intensified preoperative chemotherapy have changed this standard.

The RAPIDO trial included MRI-diagnosed LARC patients with either cT4a/b, extramural vascular invasion, cN2, involved mesorectal fascia or enlarged lateral lymph nodes considered to be metastatic. Patients were randomized to short course radiotherapy (SCRT), 5Gyx5 fractions, with subsequent six cycles of CAPOX or nine cycles of FOLFOX4 followed by total mesorectal excision (TME) (experimental arm) or capecitabine-based chemoradiotherapy (25-28 x 2.0-1.8 Gy) followed by TME and optional, predefined by hospital policy, postoperative eight cycles of CAPOX or twelve cycles of FOLFOX4 (standard arm). The main end point was to decrease Disease-related Treatment Failure (DrTF), defined as locoregional failure, distant metastasis, a new primary colorectal tumor or treatment-related death. 920 patients were enrolled. At three years, cumulative probability of DrTF was 23.7% in the experimental arm and 30.4% in the standard arm (HR 0.76 [0.60 – 0.96]; p = 0.02). ypT0N0 rate was 28% in TNT arm and 14% in standard arm (p<0.0001). The 3-years probability of distant metastasis was 20.0% in the experimental group and 26.8% in standard group (p=0.0048). The 3-years probability of locoregional failure was 8.3% and 6.0% in experimental and standard groups, respectively (P=0.12) (10).

The UNICANCER-PRODIGE 23 Trial randomized 461 patients staged cT3 (considered at risk of local recurrence and for which a multidisciplinary board recommended chemoradiotherapy) or cT4 to either neoadjuvant chemotherapy consisting of six cycles of folfirinox, chemoradiotherapy and surgery (experimental arm), or chemoradiotherapy followed by surgery (standard arm). Adjuvant chemotherapy consisted of modified Folfox6 for 6 cycle or 4 cycles of capecitabine months for experimental arm and 12 cycles of modified Folfox6 or 8 cycles of capecitabine for standard arm. Main end point was 3-years DFS. At a median follow up of 46.5 months, 3-years DFS were 76% in experimental arm and 69% in standard arm (p=0.034). ypT0N0 rates was significantly increased in experimental arm, 28% vs 12%, p<0.0001. 3-years metastasis free survival was 79% in the experimental group and 72% in the standard group (p=0.017). No difference in locoregional control was seen in the two groups (4% vs 6%) (11).

The STELLAR Trial enrolled cT3, cT4 or N positive stage and randomized them to short course RT (5x5Gy) followed by 4 cycles of Capox, the TNT arm (n=302 patients) or chemoradiotherapy, the standard arm, (n=297 patients). After total mesorectal excision, TNT arm receive 2 cycles of adjuvant Capox, the standard arm 6 cycles of Capox. Primary end point was 3-years DFS. At a median follow up of 35 months, 3-years DFS were 64.5% and 62.3 in TNT and standard arm, respectively (p<0.01). ypT0N0 or sustained cCR were 21.8 % in TNT arm and 12.3% in standard arm, p=0.002). There was no significant difference in metastasis free survival and locoregional relapse between the two groups (12).

Total neoadjuvant therapy or chemotherapy intensification in preoperative treatment has become a new standard included in international guidelines such as the NCCN guidelines (13) guidelines 2024). The above mentioned trials showed that distant control was still the main problem, however, TNT or induction chemotherapy intensification combined with radiotherapy provided significant improvement in distant disease free survival as well as in the rate of pathological complete remissions, indicating that this is the right path.
A new treatment option is now available for rectal cancer: the watch and wait strategy (WaW). After the pioneering publication by Habr-Gama (14), at least two large multicenter, retrospective, studies (15,16), two meta-analysis (17,18), a randomized phase II trial, the OPRA trial (19), and a phase III trial, the OPERA trial (20), show that watch and wait strategy is feasible without any negative effect on clinical outcome.
The achievement of a high pCR rate is still an important endpoint because it is strongly associated with good prognoses (21) and could maximize the probability of WaW strategy or conservative surgery in low rectal cancer.

In an exploratory study we tested feasibility and effectiveness of preoperative adaptive radiotherapy (RT) consisting of delivering a simultaneous integrated boost (SIB) to the residual tumor visible on the images of a simulation CT and MR acquired in the middle of chemoradiation treatment (22).
The rationale of the adaptive RT is based on the following considerations: 1) residual tumor may represent a more resistant component of disease for which it may be appropriate to deliver a higher dose; 2) due to the early reduction of the tumour during RCT, a boost to residual tumor in the final phase of treatment allows a reduction of volumes treated with the higher dose (23); 3) limiting the boost to the final phase of the treatment in “few” fractions is expected to reduce the risk of early treatment interruptions due to toxicity; 4) in a previous study we showed that rectal mobility is reduced in the second part of the treatment, permitting the application of reasonably small margins (24).
After our early report, we consolidated our clinical experience with adaptive RT concomitant to cisplatin and fluoropyrimidine and we applied a radiobiologic index based on the Early Regression Index_Tumor Control Probability (ERI_TCP) during neo-adjuvant radiochemotherapy to predict the pCR rate (25). In brief, ERI-TCP is a value calculated by the following formula:
ERITCP=-ln[(1–(Vmid/Vpre))Vpre
Vpre is the initial volume of the tumor
Vmid is the volume of residual tumor still visible on the images of a MRI performed in the middle of radiotherapy treatment.
This index proved to be a highly reliable predictor of pCR and complete clinical response as showed in the figure 1 and 2. In particular, for ERI_TCP value>32,6, the probability of pCR or cCR is zero.

Subsequently, this index was found to be an excellent predictor of outcome (26) and was used to investigate the impact of change of oxaliplatin (OXA) dose on the pCR and residual vital cell (RVC) fraction rate (27).

We more recently updated our experience with adaptive radiotherapy and sent the results to ESTRO meeting 2024 (28), summarized as follows. Patients with low T2N0 surely eligible for abdominal-perineal resection, T3/T4N0 or any T N+ rectal adenocarcinoma were considered. Concomitant chemotherapy consisted of Oxaliplatin 85 mg/m2 on days -14, 0, +14, and capecitabine 850 mg/m2 BID from day -14 to the end of radiotherapy (day 0 was the start of radiotherapy). From 2nd semester 2015 to December 2018 in the attempt to find a better balance between efficacy and toxicity, our medical oncologist team decided to prescribe only two cycles of oxaliplatin on day -14 and 0. Radiotherapy consisted in 41.4Gy in 18 fractions, 2.3 Gy/fraction, delivered with Tomotherapy or VMAT to the tumor and regional lymph-nodes expanded by 5 mm (PTV1) defined on simulation CT and MRI imaging. After 9 fractions (half treatment) CT and MRI were repeated for the planning of the adaptive phase: PTVadapt was generated by adding a 5mm margin to the residual tumour still visible on half treatment MRI. On the last 6 fractions, a boost of 3.1 Gy/fr delivering a total dose of 46.2 Gy in 18 fractions was delivered to PTVadapt while concomitantly delivering 2.3 Gy/fr to PTV1.
From September 2009 to March 2023, 152 patients (pts) were treated. Toxicity. Seventeen pts (11%) experienced G3 gastrointestinal toxicity: diarrhea 10 (6.5%), proctitis 6 (4%), rectal bleeding 1 (0.5%), nausea/vomiting 1 (0.5%). Five pts had G3 skin toxicity (3%), 1 pt (0.5%) genitourinary toxicity. Two thirds of pts experienced toxicity before the adaptive phase and the third cycle of oxaliplatin, when prescribed.
Feasibility. One hundred fourty-nine pts completed RT with full Radiotherapy dose. Three cycles of Oxaliplatin were prescribed in 90 pts (59%), < 2 cycles in 62 pts (41%). Eighty-five percent of pts received > 80% of capecitabine dose.
Efficacy. All 152 pts underwent surgery, 5 pts (3%) abdominal-perineal resection, 147 (97%) pts conservative resection. Overall, 39 pts (26%) achieved pathological complete response, 26 pts (17%) TGR 1-5%, 22 pts (14%) TRG 6-10%. Considering pts who received 3 cycles of oxaliplatin, 26 out of 90 (29%) achieved pCR against 13/62 (21%) pts who received 1-2 cycles
(not statistically significant). Ninety pts (60%) received adjuvant chemotherapy. Outcome. With a median FU of 50,3 months (5,9-149), relapse occurred in 45 pts (30%), locoregional in 5 pts (3%), distant in 43 pts (28%). Thirty-two pts (21%) died. Median DFS and OS were 105 and 133 months, respectively.

In the present protocol we aim at a further improvement of pCR rate by increasing the RT dose to residual tumor in patients selected be ERI_TCP and by better identification of the residual tumor.

5. RISK/BENEFIT ASSESSMENT

5.1. Known Potential Risks

Toxicity data from our previous experience in 152 patients treated with adaptive radiotherapy concomitant with chemotherapy have been updated after the publication (19) and are summarized in the table below.

Adverse events (data form our experience of adaptive RT, 152 pts trated from 2009 to 2023)			
Haematological toxicities	**Any grade**	**G1-2**	**G3-4**
Neutropenia	27 (14.5%)	24 (12.9%)	3 (1.6%)
Anemia	20 (10.7%)	19 (10.2%)	1 (0.5%)
Thrombocytopenia	26 (14%)	26 (14%)	0 (0%)
Non-haematological toxicies	**Any grade**	**G1-2**	**G3-4**
Fatigue	24 (12.9%)	22 (11.8%)	2 (1.1%)
Diarrhea	99 (53.2%)	82 (44.1%)	17 (9.1%)

Tenesmus	41 (22%)	39 (21%)	2 (1.1%)
Rectal bleeding	18 (9.7%)	17 (9.1%)	1 (0.5%)
Proctitis	95 (51%)	86 (46.2%)	9 (4.8%)
Nausea	40 (21.5%)	39 (21%)	1 (0.5%)
Vomiting	8 (4.3%)	7 (3.8%)	1 (0.5%)
GU disorder	36 (19.3%)	34 (18.3%)	2 (1.1%)
Skin disorder	38 (20.4%)	32 (17.2%)	6 (3.2%)
Peripheral sensory neuropathy	30 (16.1%)	29 (15.6%)	1 (0.5%)
Stomatitis	4 (2.1%)	4 (2.1%)	0 (0%)
Postoperative complic: tions			
Any complicatior		79 (42.5%)	
Anastomotic lea ‹age		14 (7.5%)	
Infection		20 (10.7%)	

Fistula	19 (10.2%)
Bowel obstruction	12 (6.4%)
Vascular event	8 (4.3%)
Other	20 (10.7%)

The increase in the dose delivered to the true tumor residue could lead to an increase in the frequency and severity of proctitis, whose main symptom is tenesmus, i.e. false evacuation urge, a symptom that is generally manageable with therapies and that resolves spontaneously within a couple of weeks.

5.2. Known Potential Benefits

The benefit will consist of a very high probability of obtaining a pathological complete response (pCR), a strong positive prognostic factor. Furthermore, the complete disappearance of the tumor, called clinical complete response (cCR), at restaging after radiochemotherapy could offer the possibility of postponing the surgical intervention, an option not foreseen in this study but admitted in the case of specific will of the patient. This could be particularly advantageous for patients who are definitely candidates for abdomino-perineal amputation with consequent definitive colostomy.

5.3. Assessment of Risks and Benefits ratio

The advantages deriving from a strong increase in the probability of pCR or cCR favorably counterbalance the risks of greater toxicity consisting of proctitis, an event that can be managed with effective medical therapy and, in any case, usually ends spontaneously within a couple of weeks after the end of radiochemotherapy.

6. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints	Time point(s)
Primary Objective Increase in the rate of pathological complete responses (pCR)	**Primary Endpoint** The primary end-point is the demonstration of an increase of pCR from the present 41% to 70% in the population of patients with ERITCP<32.6.	16 weeks, a time composed of 6 weeks of treatment duration, 7-12 weeks of waiting (standard waiting time) between the end of the radiochemotherapy and the surgical resection to which must be added the time for the histological examination to be reported, approximately 3-4 weeks.
Secondary Objectives Tolerability and outcome	**Secondary Endpoints** Acute toxicity (graded according to CTCAE v 5.0-NCI) Anal sphincter preservation rate	Within 90 days from the end of radiochemotherapy Act of surgery

	Incidence of surgical complications (graded according to Clavien-Dindo scale)	Perioperative period
	Outcome (time free from disease recurrence, local recurrence, distant recurrence, survival, calculated from the date of the first biopsy on rectal disease)	Periodic checks foreseen by the normal follow up for rectal adenocarcinoma

7. STUDY DESIGN

The study is prospective mono-centric trial, interventional study. The primary end-point is the demonstration of an increase of pCR from the present 41% to 70% in the population of patients with ERITCP<32.6. A power sampling sufficient to demonstrate this difference from the historical value was estimated to be equal to 30 patients (alpha=0.05 and beta=0.80). Given the actual rate of patient refusal and/or impossibility to perform surgery (<10%), a total population of 33 patients can be considered safely large enough to complete the study.

7.1. Study duration

Duration of enrollment: two years from the inclusion of the first patient
Duration of treatment/procedure: 16 weeks for primary endpoint
Duration of total follow-up: 5 years for secondary endpoints
Duration of total study period: 2.5 years

8. STUDY POPULATION

8.1. Study Participants

Patients with adenocarcinoma of rectum

8.2. Inclusion Criteria

Initial RT phase (standard phase)

These criteria represent the conditions for which preoperative chemoradiotherapy treatment for rectal cancer is clinically indicated

1) Histologically proven adenocarcinoma of rectum

2) pMSS (proficient microsatellite status)

3) Stage T2N0 in low rectal cancer candidates for intersphincteric resection or Abdomino-perineal resection.

4) Stage T3-T4N0 or any T with positive lymph nodes

5) Inferior margin of tumor no more than 12 cm above the anal verge

Adaptive RT phase (experimental phase)

6) ERI_TCP < 32.6

7) Inferior margin of true residual tumor at least 10 mm distant from the hypothetical resection line

8) ECOG (Eastern Cooperative Oncology Group) Performance Status PS ≤ 2

9) Age: 18-80 years

10) Written informed consent

8.3. Exclusion Criteria

1) Distant metastases

2) Previous cancer excluding non-melanoma skin cancer diagnosed less than 5 years before rectal cancer appearance

3) Previous chemotherapy or radiotherapy to the pelvis

4) Contraindications to radiotherapy: active ulcerative colitis

5) Contraindications to chemotherapy: NE<1.5x10/L, Plt < 100x10/L), creatinine <1,5mg/dl, bilirubin > 2mg/dl, AST/ALT > 3x normal upper limit, significant cardiac disease, peripheral neuropathy.

6) Pregnancy

7) Breastfeeding

8.4. Screening Failures

Eligible patients, who signed the informed consent, underwent the experimental treatment and achieved a complete clinical remission, could opt for the postponement of the surgical intervention up to local recurrence of the tumor. This option named “wait and see” is now included in the international guidelines. These patients will be considered screening failures. We estimate a 10% screening failures. We took this into account in the sample size

9. STUDY INTERVENTIONS

9.1 Intervention description

Radiotherapy

Initial RT phase (standard phase)
Patients are immobilized on Comby-Fix® in supine position.
Simulation CT and T2 weighted RM of the pelvis without contrast media are performed and matched.
Clinical Target Volume (CTV) definition follows international guidelines and encompass primary tumor, mesorectum, obturator lymph-nodes, internal iliac and common iliac chains as well as the whole anterior surface of sacrum, coccyx and piriformis muscle. Anal canal and perineum are included in case of lesser limit of tumor located less than 6 cm from the anal verge. External iliac lymph-nodes are included in case of T4 disease infiltrating pelvic organs or wall. Inguinal lymph-nodes are treated in case of massive infiltration of the anal canal.
Planning target volume (PTV) is defined as CTV expanded by 0.5 cm in all directions.
The prescribed dose to PTV is 27.6 Gy in the first 12 out of 18 fractions (2.3 Gy/fr).

Adaptive RT phase (experimental phase)
After 8-10 fractions of RT a new CT (CTadapt) and T2 weighted RM (MRadapt) of the pelvis without contrast media are performed following the same set-up as the original planning scans and matched.
Adaptive Gross Tumor Volume (GTV) 1 (GTV1adapt) is defined as the portion of rectum included between the cranium-caudal limits of residual tumor. GTV2adapt is defined as the true residual tumor (T) still detectable on the high quality MR images with the help of an expert radiologist.
PTV1adapt and PTV2adapt are obtained by expanding GTV1adapt and GTV2adapt of 0.5 cm in all directions.

ERI_TCP calculation
In order to confirm the enrollment of the patient into the study, ERITCP at half-RT needs to be assessed. For this reason, tumor volumes are contoured on MRI T2 images by the same radiation oncologist (or radiologist) expert in rectal tumor MR imaging on axial images of initial MR (Vpre corresponding to GTVpre) and MRadapt (Vmid corresponding to GTV2adapt), and ERITCP is calculated as follows:

ERITCP=-ln[(1–(Vmid/Vpre))Vpre

The “hyperboost” to PTV2adapt will be delivered only in two cases:

1) the inferior margin of PTV2adapt, examined by multidisciplinary team composed of radiologists, surgeons and radiation oncologists, is located more than 10 mm from the hypothetical resection line
2) ERITCP < 32.6, defining responsive patients

Dose prescription for adaptive phase

PTV: 2.3 Gy/F x 6 fractions (total dose 41.4 Gy in 18F)
PTV1adapt : 3.1 Gy/F x 6 fractions (total dose 46.2 Gy in 18 fractions).
PTV2adapt : 4.0 Gy/F x 6 fractions (total dose 51.6 Gy in 18 fractions)

Treatment Planning and image-guidance; preliminary planning study
Patients are treated using Volumetric Modulated Arc Therapy or Tomotherapy. Concerning planning optimisation, PTVS coverage and dose homogeneity have the highest priority: the fraction of PTV/PTV1-2adapt receiving 95% of the prescribed dose (V95%) is set ≥98% and maximum PTV/PTVadapt dose (Dmax) <105% respectively, both in the initial and in the adaptive plan; the homogeneity of the dose distribution within PTV is minimized even in the adaptive plan. Dmax of femoral heads is set <42Gy. Bladder and bowel are spared “as much as possible” in the portion outside PTV. The dose to external genitalia are minimized by blocking any beam passage through them.
In order to better define proper templates for plan optimization with the hyperboost (delivering up to 4Gy/F to the true residual tumor), an in-silico plan comparison study on at least 10 previously treated patients showing ERITCP<32.6 will be accomplished. Plans with hyperboost and without (i.e.: the clinically delivered plans) will be compared and evaluated in terms of potential worsening of dose-volume parameters of the different organks at risk, primarily bowel and bladder.

Concerning image-guidance, before delivering each fraction, a ConeBeam CT (CBCT) or Megavoltage CT (MVCT) scan is performed: first, an automatic bone match between planning kVCT and CBCT or MVCT is carried out; the physician then apply fine manual adjustments, if necessary, in order to minimize the residual error after bone matching due to rotations and/or deformations occurring during therapy.

9.2 Method for Assigning Subjects to Treatment/Intervention Groups

Not applicable

9.3 Blinding of treatment/procedure

Not applicable

9.4 Preparation, Administration and Accountability of the study Treatments

See intervention description

9.5 Compliance with study treatment

We estimate that 100% of patients will be compliant with the treatment and 90% with the primary endpoint.

9.6 Concomitant Medication

Concomitant chemotherapy

Concomitant chemotherapy consists of Oxaliplatin 85 mg/m2 delivered on days -14, 0, +14, and Capecitabine 825 mg/m2 twice daily or less according to DPYD status, assumed from day -14 until the end of RT. Radiotherapy starts on day 0.

Day -14°	Day 0	Day +14°
1st OXA	2nd OXA	3rd OXA
Capecitabine----------		
	RT (18 fractions)----------	

Dose reduction

In case of toxic effects, chemotherapy cycles will be delayed until the neutrophil count will be 1·5 × 109 cells per L or higher and the platelet count was 100 × 109 per L or higher. Briefly, dose reductions will be recommended in patients who develop febrile neutropenia, grade 4 neutropenia lasting more than a week, grade 3–4 thrombocytopenia, or any toxicity worse than grade 2. The oxaliplatin dose will be reduced when grade 2 peripheral neuropathy will be painful or persistent between cycles and oxaliplatin will be stopped in case of motor impairment. Capecitabine dose will be adapted to blood counts and renal clearance. Dose reduction or discontinuation of capecitabine will be considered in case of grade 2 toxicity.

9.7 Other Interventions

Not applicable

10. EFFICACY AND SAFETY CRITERIA

10.1 Efficacy Criteria

Pathologic complete response (pCR) in the pathologic report of histologic exam on the surgical speciment

10.2 Safety Criteria

Patients are visited weekly by radiation oncologists and before each cycle of chemotherapy by medical oncologists.

Acute toxicity is graded on the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) v 5.0-NCI

11. STUDY PROCEDURES

C= clinical standard of care activities; R: research activities

	Enrollment	Simulation	Start of chemotherapy	Start of radiotherapy	Restaging	Study Visit	Restaging	Surgery	Final Study Visit	Follow up visits
Day **Procedures**	Date of first visit	Day 14-21 after the first visit	Day 22-30	14 days after the start of chemotherapy	9-12 days after the start of radiotherapy	Weekly during radiotherapy by radiation oncologist; befor each of the 3 cycle of chemotherapy by medical oncologists	7-12 weeks after the end of radiochemotherapy	7-12 weeks after the end of radiochemotherapy	Approximately 3-4 weeks after surgery (time for the histological examination to be reported)	Every 4 months for the first year, every 6 months subequently up to the 5th year
Informed consent	R									
Demographics	C									
Medical history	C									
Eligibility assessment	C									
Physical examination (including height and weight)	C									C
ECOG Performance status	C									
Laboratory test: blood count and complete liver and kidney function tests; CEA		C	C							C
dihydropyrimidine dehydrogenase (DPYD)		C								
Compliance evaluation	C									
Radiation oncology vsit	C									
Medical oncology visit	C		C	C		C				C
Cardiologic evaluation		C								
Simulation pelvic CT and MRI		C			C					
RT Boost to residual tumor					R					
Contrast enhanced thorax and abdomen CT							C			C
Contrast enhanced Pelvic MRI							C			
Radiation oncology visit	C									C
Linear accellerator				C						
Surgical resection								C		
Histological examination								C		
Adverse event assessments						C			C	C

11.1 Informed Consent

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be EC-approved, and the participant will be asked to read and review the document. The investigator (according to applicable regulatory requirements) or a person designated by the investigator, and under the investigator's responsibility, will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant MUST sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.2 Subject Recruitment and Screening

Patients with histologically proven adenocarcinoma of rectum, pMSS (proficient microsatellite status), stage T2N0 in low rectal cancer candidates for intersphincteric resection or Abdomino-perineal resection, stage T3-T4N0 or any T with positive lymph nodes inferior margin of tumor no more than 12 cm above the anal verge will be included for the initial, standard phase.

Thirty-three patients with ERI_TCP < 32.6, inferior margin of true residual tumor at least 10 mm distant from the hypothetical resection line, ECOG PS ≤ 2, age: 18-80 years after signing the written informed consent will be included in the Adaptive RT phase (experimental phase).
The expected accrual rate is around 20 patients/year.

11.3 Subject Identification

From the signing of the informed consent by the patient, the subject is considered enrolled in the clinical study. An identification code composed of the acronym of the trial code Adaptive Rectal CancerTrial 02, ARCT02, followed by consecutive two-digit number 01, 02 .
An identification list of patients will be kept.

11.4 Randomization and Blinding

NA

11.5 Baseline Assessments

During the first radiotherapy visit, patients will be evaluated to verify if they meet the inclusion and exclusion criteria.

11.6 Visits and Follow Up

A standardised, minimal follow-up schedule will be defined, according to national guidelines, with clinical examination, CEA and chest-abdomen CT scan with contrast medium every 4 months for the first year, every 6 months subsequently up for 4 years. Abdominal ultrasound and chest x-ray can represent an alternative option to CT but considering the minor one sensitivity. Total colonoscopy is mandatory within the first year after surgery unless done preoperatively and then every 2 years until 5 years. On indication, other diagnostics (eg, PET CT scan) is allowed, to confirm or detect recurrent disease.

11.7 Definition of End of Study

According to the study design, the end of the study is defined as the date on which the histologic exam of the last patient is reported.

11.8 Premature termination or suspension of a study

The only possible reasons for termination or temporary suspension of the study is an unexpected high G≥3 toxicity rate or other AEs. In case of early termination the PI will inform study participants, the EC, and the authorized sponsor representative and provide the reason(s) for the termination or temporary suspension.

12. DISCONTINUATION AND WITHDRAWAL

The only reason for discontinuation by PI or patients would consist of the appearance of intolerable toxicity during the experimental “adaptive RT phase”

13. SAMPLE HANDLING

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NA

14. PATIENT SAFETY

AEs will be reported and managed with standard medical care. The only toxicity expected to be increasing is proctitis that can be cured with usual topic or systemic steroid or non-steroid anti-inflammatory drugs.

14.1 Safety profile of the procedure

The known side effects for the treatment with the relative frequencies have been reported above.

14.2 Adverse Event Definitions

Adverse Event Definitions

Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical investigation subject treated with the investigational treatment and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the treatment, whether or not related to the tratment. Reference: adapted from GCP ICH E6(R2)
Serious Adverse Event (SAE)	Any untoward medical occurrence that at any dose: – results in death, – is life-threatening, – requires inpatient hospitalization or prolongation of existing hospitalization, – results in persistent or significant disability/incapacity, or – is a congenital anomaly/birth defect. Reference: GCP ICH E6(R2)
Adverse Event of Special Interest AESI	An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them. Depending on the nature of the event, rapid communication by the study sponsor to other parties may also be needed (e.g., regulators). Reference: CIOMS VI
The AEs will be coded using the current version of Medical Dictionary for Regulatory Activities (MedDRA; refer to https://www.meddra.org).	

14.3 Adverse Event Severity Grading Scale

Grade 1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; no intervention indicated
Grade 2	Moderate	Minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4	Life-threatening	Life-threatening consequences; urgent intervention indicated
Grade 5	Death	Death related to AE
Reference: Common Terminology Criteria for Adverse Events (CTCAE) version 5.0		

14.4 AE Attribution Scale

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated to investigational agent/intervention	Unrelated	The AE is ***clearly NOT*** related to the intervention.
	Unlikely	The AE is ***doubtfully related*** to the intervention.
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention.
	Probable	The AE is ***likely related*** to the intervention.
	Definite	The AE is ***clearly related*** to the intervention.
Reference: NCI Guidelines: Adverse Event Reporting Requirements		

14.5 Reporting procedures for AE

All related, any grade, AEs occurring during the safety window for the study as defined above that are observed by the Investigator or reported by the participant will be reported in the study CRF whether or not attributed to study intervention.
The following information will be reported in the CRF: description, date of onset and end date, severity, assessment of relatedness to study tratment and action taken. Follow-up information should be provided as necessary.
The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.
Non-serious AEs considered related to the study medication as judged by a medically qualified investigator will be followed up either until resolution or until the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from the study. A participant may also voluntarily withdraw from the study due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must <Insert statement of requirements/conditions here (e.g., undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable).

14.6 Reporting Procedures for SAE/ AESI

All Serious Adverse Events (SAEs) will be reported using MedDRA (Medical Dictionary for Regulatory Activities) terms. Any deaths occurring during the course of the study must always be reported to the ethics committee.

14.7 Follow-up of AE / SAE / AESI

The Investigator should take all appropriate measures to ensure the safety of the patients. Notably, they should follow up on the outcome of any event (clinical signs, laboratory values, etc.) until resolution or until the event is considered stable.
In the case of any Serious Adverse Event, the patient must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. This may imply that follow-up will continue after the patient has left the study and that the Ethics Committee may request additional investigations.

14.8 Unblinding Procedures

NA

15. DATA MANAGEMENT

15.1 Definition of source data and source documents

Source Data: All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies). Any data recorded directly on the CRFs (i.e., no prior written or electronic record of data), is considered to be source data.
Source Documents: Original documents, data, and records (e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subjects' diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, or records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study).

All parameters asked for in the case report form (CRF) should be documented in the source documents.

A record of patient screen failures will be maintained for patients who do not qualify for enrollment, including the reason for the screen failure.

15.2 Documentation of data in Case Report Forms (CRFs)

All relevant data collected during the study for all patients enrolled will be entered into the eCRF (RedCap) by the PI or their delegated responsible researcher or by someone authorized by the researcher (as soon as possible after data collection) to ensure that the information is clear and legible. The physician must confirm the completeness, accuracy, plausibility, and compliance with ICH guidelines and institutional SOPs by signing and dating the records. An explanation must be provided for any missing data.

15.3 Data Recording and Record Keeping

The investigator shall arrange for the retention of Essential Documents for the Conduct of a Clinical study (according to Chapter 8 of GCP ICH E6(R2) (e.g., patient files, other source data, and the Trial Master File/Investigator Site File) after the completion or discontinuation of the study according to institutional procedures and applicable laws.

15.4 Data Protection

The Investigator undertakes to:
- use the data only for the purposes of the foreseen analyses and within the limits established by the study and approved by the competent EC;
- store data in a secure network system
- prohibit unauthorised third parties from accessing, even partially, data.
- guarantees to limit access to and processing of data only to its employees and collaborators who, upon appointment as an authorized person:
    1. need to process the data in order to carry out their work in relation to the study;
    2. have undertaken to maintain the confidentiality of the Data and of any information deriving from it or that is communicated to them.

If, within the context of the Study, the Investigator (or the Center that will receive the data) needs to make use of its own suppliers, the latter undertakes to appoint these subjects as Data Processors /Responsabili dei Trattamenti with a specific agreement or other legal act suitable for this, before the start of any data processing by them, according to Regulation (EU) n. 2016/679, art. 28.
The Investigator (or the Center that will receive the data) also undertakes to adopt suitable measures to facilitate the exercise of the rights of the subject provided for by Regulation (EU) n.

2016/679, art. 15 – 22, including the rights of access, rectification, cancellation, limitation, opposition and portability, within 30 days of receiving the relative request.

In case the Investigator need to communicate the data outside OSR (the Data Controller /Titolare) in pseudonymized form, the Investigator (or the Center that will receive the data) will refrain from carrying out any activity aimed at identifying the identity of the subjects to whom such data refer. In the event, however, that the data could not be communicated in pseudonymized form by the Data Controller, the Investigator (or the Center that will receive the data) undertakes to adopt all the security and organizational measures aimed at protecting the confidentiality of the Data Subject).
Within [indicate a suitable term - for example 30 days] following the end of the study, the Investigator (or the Center that will receive the data) undertakes to cancel the data communicated by the Data Controller or to make them irreversibly anonymous and to promptly communicate it in writing.

16. STATISTICS

16.1 Description of Statistical Methods

Sample size is calculate with the Fisher test for a single proportion. The primary endpoint will be the proportion of patients achieving pCR among those with ERITCP <32.6. If the 70% target is not reached, statistical significance will be assessed using an exact binomial test comparing the observed proportion to the expected proportion, with calculation of the p-value and 95% confidence interval.

Secondary endpoints will be described as frequencies and percentages (acute toxicity, anal sphincter preservation, surgical complications). Survival outcomes (DFS, LRFS, DRFS, OS) will be analyzed using the Kaplan-Meier method, compared with the log-rank test, and, when appropriate, evaluated in multivariable analysis using Cox proportional hazards regression to estimate hazard ratios with 95% confidence intervals.

16.2 Sample Size Determination

This is a prospective single-center study. The historical pCR rate is 41% in patients with ERI_TCP < 32.6 as reported from our previous data [2]. Infact in that study, the overall pCR rate reported among 63 patients was 31.7%. When excluding the quartile of patients with ERI_TCP >32.6—who are known to have an extremely low likelihood of achieving pCR—the rate increased to 41%, which we therefore adopted as the reference value for this subgroup. The primary endpoint is to demonstrate of an increase in the pCR from the current 41% to 70% in the patient with ERITCP <32.6. Using Fisher's exact test for a single proportion, a sample size of 22 patients was estimated to provide 80% power to detect a difference from the historical value ($\alpha = 0.05$, $\beta = 0.20$). Considering that approximately 25% of enrolled patients are expected to have

an ERI_TCP > 32.6, we estimate that enrolling 30 patients in total will be sufficient to demonstrate the primary endpoint. Accounting for a potential <10% rate of patient refusal and/or inability to undergo surgery, a total sample size of 33 patients is considered adequate to complete the study (9).

Patients who refuse or are not fit for surgery will be considered not evaluable for the primary end point but evaluable for secondary endpoints

16.3 Analysis Populations

All participants as enrolled (intention to treat).

16.4 Interim Analysis

NA

16.5 Stopping Rules

When the first 5 proctitis G3 will be observed (≥ 15% on the total enrolled subjects), the study will be temporarily suspended.. A standard treatment for proctitis will be decided jointly with the gastroenterologists' input. The opportunity to continue the radiation doses set out in the current protocol or to reduce them will be discussed. In any case, the protocol will be amended.

17. ETHICAL AND REGULATORY CONSIDERATIONS

This clinical study will be conducted in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments established by the World Medical Assemblies, and the ICH guidelines for Good Clinical Practice.
This clinical study will be conducted in compliance with all international laws and regulations; national laws and regulations of the country in which the clinical study is performed; as well as any other applicable guidelines.

17.1 Responsibilities of the Investigator(s)

The Investigator(s) undertake(s) the responsibility to perform the study in accordance with this Protocol, Good Clinical Practice, and the applicable regulatory requirements. The Investigator is required to ensure compliance with the investigational product schedule, visits schedule, and procedures required by the protocol. The Investigator agrees to provide all information requested in the Case Report Form (CRF) in an accurate and legible manner. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to study subjects without prior EC approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted. The investigator must have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the study to conduct the study properly and safely.

17.2 Ethics Committee (EC) Approvals

This clinical study protocol as well as the Informed Consent are to be submitted to the appropriate Ethics Committee, and it is mandatory to obtain the written and dated approval, signed by the chairman with Ethics Committee(s) composition.
The clinical study the documents reviewed, the list of voting members and their qualifications, and the date of the review should be clearly stated on the written Ethics Committee approval.

17.3 Other Ethical Considerations

NA

18. GENDER MEDICINE IN RESEARCH PROTOCOL

NA

19. QUALITY ASSURANCE AND QUALITY CONTROL

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations, and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the study to reflect significant changes to the protocol or outcomes of monitoring activities. Investigators involved in the study will permit study-related monitoring, audits, EC review, and regulatory inspections by providing direct access to all study records.
The Investigator should notify the CTC promptly of any inspection scheduled by any regulatory authorities and will promptly forward copies of any inspection reports received.

19.1 Monitoring

Regular monitoring will be performed by our Clinical Trial Center, according to the Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

19.2 Deviation from study protocol

A deviation from the protocol is an unintended departure from the procedures or processes approved by the Sponsor and the EC.
The Investigator or designee must document and explain in the patient's source documentation any deviation from the approved protocol. The Investigator may implement a deviation from the protocol to eliminate an immediate hazard to study patients without prior IEC approval. As soon as possible after such an occurrence, the implemented deviation, the reasons for it, and any

proposed protocol amendments should be submitted to the EC for review and approval, and to the regulatory authorities, if required.
Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Investigators will be notified in writing by the monitor of deviations. As required by local regulatory authorities, the Investigator will notify the EC of any applicable protocol deviations in a timely manner.
Every deviation from the study protocol must be specified and documented separately for each patient. The investigator must consult with the monitor and discuss the type and extent of deviation as well as the possible consequences for further participation of the patient in the study. If the evaluability of a patient is questionable, the coordinating investigator will be consulted.

19.3 Data and Safety Monitoring Board

NA

19.4 DSMB roles and responsibilities

NA

20. FINANCE AND INSURANCE

20.1 Funding

The study has a non-profit nature, having no purpose of economic exploitation of the data and the results of the experiment itself and does not receive any funding (or indicate the funding).

20.2 Patient Insurance

A specific policy will be taken out, the cost of which will be covered by funds from the Radiotherapy Unit.

21. END OF CLINICAL STUDY

In accordance with applicable regulation, ICH GCP and SOPs, the PI shall notify the end of the clinical study within 15 days from the end of the clinical study.
The PI shall notify a temporary halt of a clinical study for reasons not affecting the benefit-risk balance within 15 days from the temporary halt of the clinical study and shall include the reasons for such action. When a temporarily halted clinical study is resumed the PI shall notify within 15 days from the restart of the temporarily halted clinical study. If a temporarily halted clinical study is not resumed within two years, the expiry date of this period or the date of the decision of the PI

not to resume the clinical study shall be deemed to be the date of the end of the clinical study. In the case of early termination of the clinical study, the date of the early termination shall be deemed to be the date of the end of the clinical study. In the case of early termination of the clinical study for reasons not affecting the benefit-risk balance, the PI shall notify the reasons for such action and, when appropriate, follow-up measures for the subjects.

Upon completion or termination of the study, the study monitor will conduct site closure activities with the Investigator or site staff (as appropriate).

21.1 Summary of the results of the clinical study

Irrespective of the outcome of a clinical study, within six months from the end of a clinical study, the PI shall submit a summary of the results of the clinical study to Ethical Committee and Clinical Trial Center.

22. INTELLECTUAL PROPERTY

The ownership of the data and results related to the trial, as well as any decisions regarding their publication, is exclusively held by the sponsor (IRCCS Ospedale San Raffaele).

The principal investigator/sponsor is responsible for preparing an annual report on the clinical study to be sent to the Ethics Committee, as well as preparing a final report on the clinical study.

23. PUBLICATION POLICY

The publication policy should cover authorship, acknowledgements, and review procedures for scientific publications. If there is a department or institution policy, or agreement, the protocol can refer to it. Consider describing how study results may be disseminated to study participants.
Ensure that the publication policy stated here is consistent with any contract applicable to the study.

24. REFERENCES

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25. APPENDIX A: AMENDMENT HISTORY

List details of all protocol amendments here whenever a new version of the protocol is produced.

Amendment No.	Protocol Version No.	Date issued	Rationale	Study status	Details of Changes

26. APPENDIX B: LIST OF CLINICAL SERVICES / LABORATORIES

List details of all services and Laboratories involved in the study.

Name	Institution	Responsible person	Activity/service	Notes