ABSTRACT

Background: The phase III multicenter randomized RAPIDO trial (Rectal Cancer And Preoperative Induction therapy followed by Dedicated Operation) has shown the advantage of Total Neoadjuvant treatment (TNT) with short course radiotherapy (RT). However, data on local control have not been confirmed at 5.6 years median follow-up in favor of the TNT arm because patients from the experimental (EXP) group received more often 3D-CRT (p=0.029). The primary end-point of this multicentric observational prospectic study is to assess the real life safety and efficacy of RAPIDO trial protocol using VMAT and IMRT treatment in all enrolled patients. Moreover FDG-PET and MRI images for predictive features compared with cancer phenotype (status of K-ras and EGFR) and study on tumor microenviroment response to short course RT will be also provided.

Methods: Locally advanced rectal cancer patients undergoing TNT as defined in RAPIDO trial will be enrolled and prospectively included in this study. MRI and FDG-PET will be performed to staging and restaging the disease. The tumor microenviroment changes assessment with immunophenotype and soluble PDL1 and on EVCs will be done. For sample size calculation, using OR 0.5, 100 patients will be enrolled into two years. Kaplan -model and proportional Cox method will be adapted for OS, DFS and predictive factors for local control.

Conclusions: The results of this study will be useful to assess prospectively the safety and effectiveness of VMAT and IMRT in short course TNT assuming predictive factors for response through radiomics, metabolomics and immunological features.

Trial Registration: COMITATO ETICO UNICO REGIONALE (CEUR) N.50/2022; San Carlo Hospital, Potenza, Italy.

Keywords
Rectal cancer, Rapido trial, Radiotherapy, Chemotherapy.

Introduction
The Rectal cancer And Preoperative Induction therapy followed by Dedicated Operation (RAPIDO) trial is a well known multicentric randomized phase III trial of TNT consisting of short course radiotherapy (5 Gy x 5 fractions) followed by chemotherapy (6 CAPOX or 9 FOLFOX) as EXP arm followed by surgery versus long courses standard (STD) chemoradiation [1]. At 3 years after randomisation, the cumulative probability of disease-related treatment failure was 23.7% (95% CI 19.8–27.6) in the EXP arm.
versus 30.4% in the STD group (hazard ratio 0.75, 95% CI 0.60–0.95; p = 0.019). The most common grade 3 or higher adverse event during preoperative therapy in both groups was diarrhoea (18% in the EXP arm vs 9% in the STD arm). Without chemotherapy, serious adverse events occurred in 38% for the EXP arm vs 34% of the STD arm [2]. However, at 5.6 years median follow up, loco-regional failure (LRF) was 12% in the EXP arm and 8% in the STD group (p = 0.07), taking into account that EXP patients were more often treated with 3D-CRT (p = 0.029). To this regard, patients from the EXP arm developed more often a LRR after 3D-CRT compared to the STD group (11.6% vs. 6.0%; p = 0.016). Futher, the LRR rate was comparable when considering intensity-modulated radiation therapy (IMRT)-volumetric modulated arc therapy (VMAT). Results were 6.3% in the EXP vs. 6.2% in the STD groups; p = 0.96. Overall survival after LRF was similar in both groups (HR 0.76 (95%CI 0.46-1.26); p = 0.29) [3]. Taking into account these results, our observational study aims to assess the safety of RAPIDO trial in terms of toxicity as primary endpoints focusing on the impact of VMAT-IMRT modalities in all enrolled patients. Data from MRI and FDG-PET will be analyzed for radiomics and radiometabolomics predictive features and compared to EGFR and K-Ras status. Moreover, blood samples will be collected to assess changes in adaptive and innate immunity cells and in PD-L1 expression in serum and tumoral extracellular vesicles (EVCs) before and after RT treatment short course. This study could prospectively add the correlation between clinical and pathological response with features from imaging and immunological information in the role of RT short course.

The impact on quality of life (QoL) will be also investigated and collected with QoL questionnaires in the same way of RAPIDO trial.

Design
The proposed study is a multicentric observational study. The flowchart is reported in Figure 1. All enrolled patients will receive TNT according RAPIDO trial. Patients will be staged with FDG-PET and pelvic MRI one week before RT short course, two weeks after RT and one week before surgery. Moreover a fresh blood sample and serum will be taken the day before RT start and two weeks after RT to assess immunophenotype and PD-L1 in sera and in EVCs. Data on acute and late toxicity, QoL, radiomics, immunologic findings will be collected and related to response. The study has been approved by the local ethical committee which is defined COMITATO ETICO UNICO REGIONALE - Unique Regional Ethical Committee - (CEUR 50/2022) in S. Carlo Hospital, Potenza, Italy.

Treatment Description
Patients selection
The inclusion and exclusion criteria are listed in Table 1 according to RAPIDO trial. Eligible criteria are as follows: age 18 years or older, biopsy-proven, newly diagnosed, primary, locally advanced rectal adenocarcinoma with distal extension less than 16 cm from the anal verge. On pelvic MRI in DWI sequences at least one of the following high-risk criteria will be described: clinical tumour (cT) stage cT4a or cT4b, extramural vascular invasion, clinical nodal (cN) stage cN2, involved mesorectal fascia (tumour or lymph node ≤1 mm from the mesorectal fascia), or enlarged lateral lymph nodes considered to be metastatic. Patients must be mentally and physically fit for chemotherapy, have an Eastern Cooperative Oncology Group (ECOG) performance score of 0–1 at the time of radiotherapy being available for follow-up, and provide written informed consent. Moreover are required: white blood cell count of 4.0×10⁹ cells per L or higher, platelet count of 100×10⁹ per L or higher, a clinically acceptable haemoglobin level, a creatinine level indicating renal clearance of 50 mL/min or higher, and bilirubin level below 35 µmol/L. Main exclusion criteria include extensive growth of the rectal tumour into the cranial part of the sacrum or the lumbosacral nerve roots indicating that surgery will never be possible even if substantial tumour downsizing is seen and presence of metastatic disease or recurrent rectal cancer; informed consent not provided as in the main trial [1].

Table 1: Main Inclusion Criteria according to RAPIDO trial.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>RAPIDO trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>≥ 18 yrs</td>
</tr>
<tr>
<td>Primary T and N</td>
<td>Extrap. Rectal cancer Locally advanced</td>
</tr>
<tr>
<td>Colonoscopy</td>
<td>done</td>
</tr>
<tr>
<td>Histology</td>
<td>Adenocarcinoma Kras-EGFR status</td>
</tr>
<tr>
<td>ECOG</td>
<td>≥ 0-1</td>
</tr>
<tr>
<td>Informed consent</td>
<td>Yes</td>
</tr>
</tbody>
</table>


**Clinical Evaluation**

Eligible patients with advanced extraperitoneal rectal cancer will be identified within a multidisciplinary board. Pelvic MRI and 18-FDG-PET will be programmed one week before radiotherapy (RT) and two weeks after RT. The day before RT (T0) a fresh blood sample for immunophenotype and serum for PD-L1 will be taken. One week before surgery pelvic, colonoscopy, pelvic MRI and FDG-PET will be performed to restage the disease.

**Radiation Treatment**

**Technique and Treatment Doses**

Image guided radiotherapy (IGRT) with VMAT or IMRT will be applied in all patients. A linear accelerator IGRT dedicated with energy of 6MV is required and image verification with digitally reconstructed radiography (DRR) or cone beam computed tomography (CBCT) should be done prior to treatment. The simulation CT scan will be performed in supine position. The dose will be reported according to the ICRU (International Commission on Radiation Units and Measurements) report 83 [4,5]. The dose to OARs will be assessed according to the dose constraints reported in EQD2 according QUANTEC report [6]. The prescribed dose is 25 Gy at the level of PTV with a daily fractionation of 5 Gy over 1 week.

**Treatment Volumes**

According the RAPIDO trial and on the basis of the international guidelines for rectal cancer, the clinical target volume (CTV) should include the entire mesorectum with the primary tumour and relevant regional lymph nodes. Planning target volume (PTV) will correspond to the CTV with a variable margin at the discretion of the center and image guided RT (IGRT) technique used. The organs at risk (OARs) will be bladder, small bowel (as bowel bag), single intestinal loops in particular clinical conditions (loops with diverticula or fixed loops in the Douglas cavity or posterior pelvis), anal canal and femoral heads, penile bulb, vagina (at least the lower 1/3), external genitalia will be considered.

**Sequential Chemotherapy**

DYPD evaluation is required. Two weeks after RT, chemotherapy (CT) defined by 6 CAPOX (capcitabine 1000 mg/m² orally twice daily on days 1–14, oxaliplatin 130 mg/m² intravenously on day 1, and a chemotherapy-free interval between days 15–21) or 9 FOLOFOX (oxaliplatin 85 mg/m² intravenously on day 1, leucovorin (folinic acid) 200 mg/m² intravenously on days 1 and 2, followed by bolus fluorouracil 400 mg/m² intravenously and fluorouracil 600 mg/m² intravenously for 22 h on days 1 and 2, and a chemotherapy-free interval between days 3–14) will be delivered. The choice is at the discretion of the individual center in relation to clinical and pathologic features. Before CT start at time T1, blood sample for immunophenotype and PD-L1 assessment will be drawn [8].

**Surgery**

After a restaging, surgery will follow 2-4 weeks after chemotherapy.

**Clinical Response Evaluation**

Clinical response will be defined with objective digital rectal examination (DRE) and endoscopy, RECIST criteria will be applied to define the clinical response [9]. MRI in DWI and in T2-weighted (T2w) sequences will be analysed [10]. P-RECIST criteria for FDG-PET will be applied analysing the value of SUV max, MTV and TLG [11-13].

**Immunophenotype and PD-L1 assessment**

Blood samples will be collected to assess as surrogate of tumor microenvironment (TME) changes, the impact on adaptive and innate immunity cells and PD-L1 expression. Immunophenotype characterization will be runned by cytophluorimetry assessing total T lymphocytes (CD3+), T helper (CD3+ CD4+), T cytotoxics (CD3+ CD8+), T regulators (Tregs: CD4+ CD25+ CD127low ), T double negative (DNT: CD3+ CD4- CD8- CD16- CD56-), T double positives (DPT: CD3+ CD4+ CD8+), T natural killer: CD3+ CD16+ CD56+, and B (CD19+) with fluorochromes monoclonal antibodies. The isolation of extracellular vesicles (cEVs), the analysis of size distribution and concentration by nano-particle tracking analysis will follow. Thereafter the quantification and the PD-L1 phenotyping of cEVs by flow cytometer will be performed togheter to the quantification of soluble form of PD-L1 by ELISA assay. A statistical analysis of results concerning the PDL-1 expression soluble and in the cEVs, immunophenotype correlation and clinical response will be evaluated. Thus cytofluorometry and soluble PD-L1 and ECVs will be analysed.

**Toxicity and Quality of life assessment**

Acute toxicity and chronic toxicity will be evaluated according CTCAE v.5 [14]. Quality of life will be assessed with QLQ-C30; QLQ-CR 29, QLQ-PR25 and QLQ-EN24. For peripheral neuropaty QLQ-CIPN20 while for RAR syndrome the Rockwood score will be adopted. QoL assessment will be done during follow up at 6-12-24-36-60 months from surgery [15-20].

**Follow Up**

The follow up surveillance will be conducted with clinical and instrumental assessments. Data on toxicity, and QoL will be evaluated during follow-up as reported in Table 2. Colonoscopy after 12 and 60 months and CT TB at 12-36 months will be scheduled, while FDG-PET and MRI in case of suspected relapsed or recurrent disease will be assessed.

**Endpoints**

The primary endpoints of this study is to evaluate the safety in acute and late toxicity of TNT with short course radiotherapy according
RAPIDO Trial delivered with VMAT-IMRT. The secondary endpoints are first of all the local recurrence free survival (LCFS) with the VMAT or IMRT technique. Moreover OS, DFS, colostomy-free survival and Metastases free survival (MFS) in real life will be analysed. Radiomics data from FDG-PET and MRI will be extracted in order to assess predictive features of response in agreement with EGFR and K-Ras status. Surrogate data on tumor microenviroment changes in with hypofractionated radiotherapy on large irradiated volumes will be assessed on blood samples.

Statistics

Sample size

Statistic significance is fixed for p= 0.05. A logistic regression model will be applied in order to achieve a correlation of prognostic variables. Considering the OR = 0.5 a sample size of 100 patients in 2 years is required. Data of OS, PFS will be collected and analysed by regression Cox model and Kaplan-Meyer. Data analysis will last 8 years.

Data Collection Procedure

Data from each center will be collected in electronic case report forms (CRFs) and transferred into a single cloud-based database. Subsequently, the aggregated data will be processed by the promoter center.

Planned timeline

It is scheduled as follows: 0–3 months: project organization; 18–24 months: patient enrolment; 36–48 months: statistical analysis and publication of data about primary end-point; 48–60 months: statistical analysis and publication of data on survival outcomes.

Ethics Committee Approval for Ongoing Research

The protocol has been written according to the principles of good clinical practice (GCP). This study is conducted in accordance with the most recent version of the Declaration of Helsinki and with the Italian laws and regulations. The study protocol was approved by the ethics committee of promoter center (ethics committee identifier code CEUR 20220037576, 50/2022 ). Approval by the respective ethics committee relevant to each site will be collected before opening new sites. Written informed consent, signed and personally dated is obtained from each patient before inclusion in the study.

References


Supplementary Data

List of participating centres

1) Madonna degli Angeli Hospital
Matera -Italy
Dr. Marina Susi
Medical Oncology Unit
Dr Beatrice Di Venere
Surgical Unit

2) San Giuseppe Moscati Hospital
Taranto-Italy
Dr Annarita Marsella
Radiation Oncology Unit

3) Ospedale San Carlo
Potenza-Italy
Dr Giuseppe Giovinazzo
Radiation Oncology Unit