1. TITLE PAGE

ADAPTATION CLINICAL TRIAL PROTOCOL AND STATISTICAL PLAN FOR CLINICALTRIALS.GOV:
Induction treatment with FOLFOX, with or without Aflibercept, followed by chemo-radiotherapy in locally advanced high-risk rectum adenocarcinoma. An open, phase II randomized trial (The RIA study)

Sponsor:	GEMCAD (Grupo Español Multidisciplinar en Cáncer Digestivo) [redacted]
Test Drug:	mFOLFOX6 with or without aflibercept
Indication Studied:	High risk locally advanced rectal carcinoma
Protocol Number:	GEMCAD-1402
EUDRACT Number	2014-002063-14
Study Phase:	II
Study Initiation Date:	FPI: 27-Jan-2015
Coordinating Investigator:	[redacted]
Clinical Research Organization:	[redacted]
Protocol version and date:	2.0; April 9th, 2015

This study was conducted in compliance with Good Clinical Practices, including the archiving of essential documents.

SIGNATURES

Sponsor

This clinical study protocol numbered GEMCAD-1402 entitled ***Induction treatment with FOLFOX, with or without Aflibercept, followed by chemoradiotherapy in locally advanced high-risk rectum adenocarcinoma. An open, phase II randomized trial (The RIA study)*** with EUDRACT No. 2014-002063-14 was subject to critical review and has been approved by the Sponsor.

Approved by:

_____ Signature	

_____ Name and title of Sponsor signatory On behalf of GEMCAD (Grupo Español Multidisciplinar en Cáncer Digestivo)	_____ Date

2. SYNOPSIS

Title of study

Induction treatment with FOLFOX, with or without Aflibercept, followed by chemo-radiotherapy in locally advanced high-risk rectum adenocarcinoma. An open, phase II randomized trial (The RIA study: GEMCAD-1402)

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Introduction and study rationale

Introduction

Different treatment strategies may be indicated for intermediate-risk (T1-T2N1, T3N0) versus moderately high (T1-2N2, T3N1, T4N0) or high-risk rectal cancer patients (T3N2, T4N1-2) based on differential survival rates and rates of relapse[1].
Neoadjuvant delivery of systemic therapy for patients with rectal cancer ensures that all patients obtain optimal systemic treatment which is feasible, safe, has improved toxicity and provides tumor downstaging [2]. This fact can be important in the higher risk population (>35% systemic failure at 5 years) group of patients.
Current guidelines to treat T3-4 and/or N+ rectal cancer continue to recommend preoperative strategy of combined chemo (fluoropyrimidine based) with radiation[6,7].

Aflibercept

Aflibercept is a monoclonal antibody inhibiting VEGF and placental growth factors (PIGF). Further details on preclinical, clinical safety and preliminary efficacy are provided in the Investigator Brochure (IB), which contains comprehensive information on aflibercept[17].
Aflibercept in combination therapy improves survival in advanced colorectal cancer when combined with FOLFIRI in the second line[18]. However, an understanding of responsive tumor characteristics is lacking, and it is crucial to identify early biomarkers that accurately predict patients responding to aflibercept.
Aflibercept is a specific antagonist that binds and inactivates circulating VEGF (A and B) and PIGF. Aflibercept was designed to prevent the growth of primary and metastatic tumors by blocking tumor angiogenesis and vascular permeability.

Rationale

With the background described above aflibercept is a highly attractive drug to be tested in locally advanced rectal cancer in a high-risk population as part of an induction therapy strategy.
Several authors have studied the relationship between the administration of antiangiogenic drugs and their effect on microvessel density (MVD) and interstitial fluid pressure (IFP) obtained directly from tumor biopsies in the appropriate timing, as the goal standard to analyze. Some preclinical and clinical data strongly affirm this effect[22–27].
The antiangiogenic treatment is effective by reducing microvessel density and interstitial fluid pressure in the vascular network of the tumor, leading to a more feasible penetration of chemotherapy into the tumoral microenvironment and lessening the degree of hypoxia inside the tumor. In these conditions, the tumor itself would reach the better setting for receiving radiotherapy treatment, thus increasing its effect in a synergistic way[28,29].
Otherwise, there are also publications finding relations between parameters of functional imaging and MVD and IFP in several tumors studied by functional MRI[30–36]. And although substantial efforts are being made to identify molecular biomarkers in tissue and blood that predict response to aflibercept, there are no validated biomarkers of response to aflibercept, so far. The candidate

biomarkers include proteins related to VEGF and VEGFR family members, angiogenic factors, cytokines or any other analyses with potential role in angiogenesis and inflammation, as previously suggested[12,37–43].

We proposed to conduct a phase II randomized trial comparing induction treatment with FOLFOX with or without aflibercept in a high risk population selected by MRI, prior to receiving standard chemoradiation (capecitabine combined with 50.4 Gy in 28 days) and surgery[31]. Moreover, since no molecular biomarkers of response to aflibercept have been validated in tissue/blood, we proposed to study an extensive panel of biomarkers at multiple time points during therapy.

Objectives

Primary objective: To evaluate the efficacy of induction therapy with mFOLFOX6 +/-aflibercept followed by CT/RT in terms of pathologic complete response (pCR).

Secondary objectives:

- To evaluate pathological parameters of efficacy: R0 resection, tumor regression grade (TRG), and positive or negative circumferential radial margin (CRM) rate.
- To evaluate the relationship between MRI changes and pathological tumor response. i.e. mrTRG.
- To further characterize the safety and tolerability of mFOLFOX6 +/- aflibercept followed by chemoradiation.
- To determine the rate of 30-day surgical complications.
- To evaluate the 3-year local recurrence and disease free survival (DFS).
- To determine the levels of tumor biomarkers expression at baseline and correlate them with response to treatment with mFOLFOX6 + aflibercept.

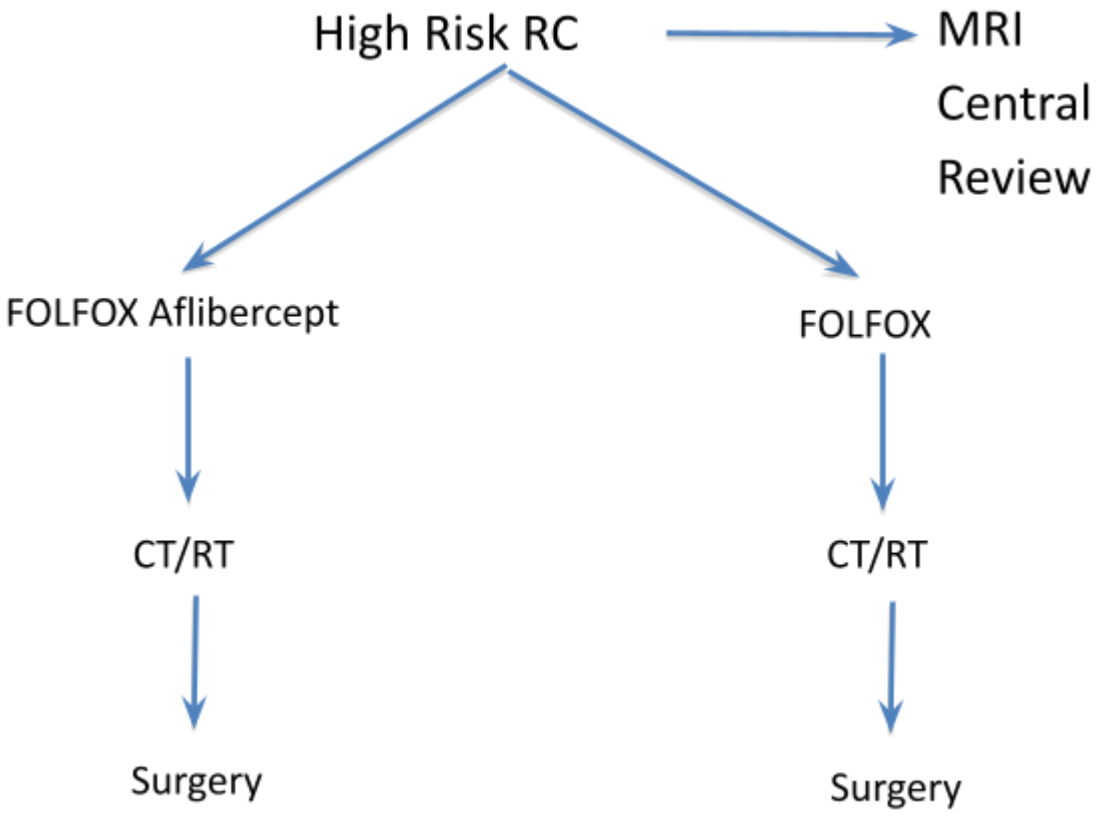
Methodology and Study design

This was an open-label, randomized, multicenter, prospective phase II study to evaluate the efficacy of aflibercept as part of an induction therapy strategy for locally advanced rectal carcinoma in a high-risk population (>35% systemic failure at 5 years) selected by MRI. 20 centers participated in the study. The study population consisted of adult patients with locally advanced high-risk rectal cancer (histological type: adenocarcinoma of the rectum), considered by the surgeon as feasible to perform a curative resection.

Once it was that the subjects fulfilled the eligibility criteria (MRI-defined high-risk RC), and had signed the informed consent, a central review was requested to confirm clinical stage, and then the patients were randomized to receive treatment.

All patients enrolled in the study had to receive one cycle of study medication every 14 days, for 6 cycles. After last cycle, patients received standard chemo-radiotherapy (CT/RT) (capecitabine 825 mg/m^2 twice daily combined with a total of 50.4 Gy in 28 days) followed by surgery, provided they had not progressed (Figure 1).

Figure 1. Study scheme



Patients with PD during the treatment phase were withdrawn from the study and received their treatment according to the investigator's judgement.

If a patient withdrew consent and refused to receive more treatment, the patient had to be followed up for DFS. If a patient withdrew consent and refused to continue in the study, the follow-up evaluations had to be discontinued.

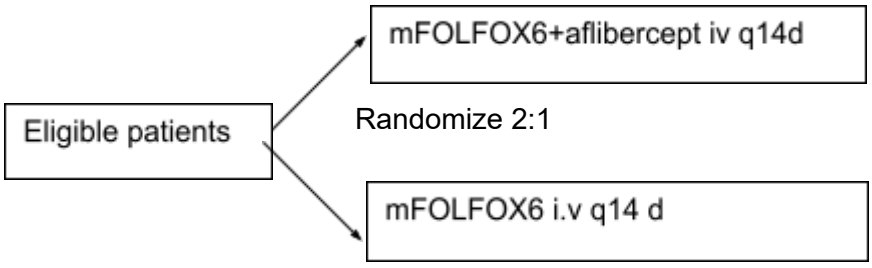
Table 1. STUDY TIMELINES:

Study times	Time
Screening (MRI central review)	4 weeks
Study therapy (mFOLFOX +/- aflibercept)	12 weeks
CT-RT	5 weeks
Second MRI (according to investigator´s judgement)	4 weeks +/-5 days after CT-RT
Surgery	6 +/-2 weeks
End of Treatment	4-6 weeks
Follow-up	Up to three years

Randomization

Once it was confirmed that the subjects fulfilled the eligibility criteria and had signed the informed consent, they were randomized 2:1 to receive treatment with or without aflibercept according to the study schema depicted in

Figure 2. Treatment assignment schema



Random assignment of treatment was stratified by EMVI+/EMVI- T3 versus T4 stage, and by study site.

Randomization (2:1) was centralized and done by Pivotal, the selected Contract Research Organization (CRO). The list of randomization codes was generated centrally by the CRO and the treatments were assigned centrally according to the list of randomization codes. The data control centre communicated to the investigator by fax or email the randomization number and treatment group to which each patient had been assigned.

Main inclusion criteria

- High-risk rectal cancer defined by MRI as with inferior border of the tumor distal to the peritoneal reflection or ≤ 12 cm from the anal margin and considered by the surgeon as feasible to perform a curative resection (including pelvic exenteration as curative resection). Presence of at least 1 of the following on high resolution, thin-slice MRI (3mm):

  Middle Third Tumors

  -mrT3

  a) Extramural vascular invasion (EMVI) positive
  b) Extramural extension > 5 mms into perirectal fat
  c) Mesorectal fascia (MRF) threatened or involved*

  -mrT4***

  Distal Third Tumors (≤ 5 cm from anal verge)

  -mrT3 tumor at or below levators

  -T4 as above

  N2**

  *tumor or lymph node < 1 mm from the mesorectal fascia

  **≥ 4 lymph nodes in the mesorectum showing morphological signs on MRI indicating metastatic disease. ≥ 4 nodes, whether enlarged or not, with a rounded, homogeneous appearance was thus not sufficient.

  ***T4a: tumor infiltrates peritoneal reflection. T4b: tumor infiltrates adjacent organs.

- Histologically confirmed adenocarcinoma of the rectum. All other histological types were excluded.

Main exclusion criteria

- Prior treatment with aflibercept
- History or evidence upon physical examination of metastasis
- Concomitant protocol unplanned antitumor therapy (e.g. chemotherapy, molecular targeted therapy, immunotherapy)
- Treatment with any other investigational medicinal product within 28 days prior to study entry

Statistical considerations

The primary objective of this study was to evaluate the efficacy of induction therapy with mFolfox6 +/- aflibercept followed by CT/RT in terms of pathologic complete response (pCR). The primary endpoint was to analyze the number of patients achieving pCR after induction therapy with mFolfox6 +/- aflibercept followed by CT/RT.

One hundred and sixty-two evaluable patients had to be recruited: 108 patients mFolfox6 + Aflibercept group and 54 patients for mFolfox6. Assuming 10% of drop-outs a total of 180 patients were recruited (120 patients for mFolfox6 + Aflibercept group and 60 patients for mFolfox6).

The assumptions were:

- 2 treatment arms with unequal 2:1 group allocation
- 0.20, two-sided, type-I error
- mFolfox6 efficacy: 15% pCR rate
- Aflibercept + mFolfox6 efficacy: 30% pCR rate
- 80% power to detect a 15% treatment difference
- 2 interim analyses:
    - At 33% of the sample size for safety, futility/efficacy
    - At 66% of the sample size for safety, futility/efficacy
- One final analysis
- Stopping rules:
    - Efficacy: Lan de Mets Alpha spending function (O'Brien-Fleming)
    - Futility: Lan de Mets Alpha spending function (O'Brien-Fleming), non-blinding

Study was a two-arm parallel randomized clinical trial with allocation ratio 2:1 (mFolfox6 + Aflibercept: mFolfox6).

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6. LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
AEMPS	Agencia Española del Medicamento y Productos Sanitarios/ Spanish Agency of Medicines and Medical Devices
ANC	Absolute Neutrophil Count
APE	Abdomino perineal excission
ASCO	American Association of Clinical Oncology
CKD-EPI	Chronic Kidney Disease Epidemiology Group
CNS	Central Nervous System
CR	Complete response
CRM	Circumferential radial margin
CRO	Contract Research Organization
CT	Computerized Tomography
CT/RT	Chemotherapy/radiotherapy
CTCAE	Common Terminology Criteria for Adverse Events
DBP	Diastolic blood pressure
DEHP	Dietilhexilftalato
DFS	Disease free survival
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group performance status scale
eCRF	Electronic case report form
EGFR	Epidermal growth factor receptor
EMVI	Extramural vascular invasion
ESMO	European Society for Medical Oncology
FMR	Fascia mesorectal

GCP	Good Clinical Practise
GGT	Gamma-glutamyl transpeptidase
Hb	Haemoglobin
IB	Investigator's brochure
ICH	International Conference on Harmonisation
IEC	Investigational Ethics Committee
IFP	Interstitial Fluid Pressure
ITT	Intent-to-treat population
LAR	Low Anterior Resection
LDH	Lactate Dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
MRF	Mesorectal Fascia
mrTRG	MRI Tumor Regresion Grade
MVD	Microvessel density
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
ORR	Objective response rate
OS	Overall Survival
pCR	Pathological complete response
PD	Progression disease
PFS	Progression-free survival
PIGF	Placental growth factors
PP	Per Protocol population
PR	Partial response
PS	Performance Status
PVC	Polyvinyl chloride

RC	Rectal carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RPLS	Reversible posterior leuko-encephalopathy
SAE	Serious Adverse Event
SBP	Systolic blood pressure
SGOT/AST	Aspartate aminotransferase
SGPT/ALT	Alanine aminotransferase
SUSAR	Suspected Unexpected Serious Adverse Reaction
TME	Total mesorectal excision
TKI	Tyrosine kinase inhibitor
TOTM	Tri Octyl Trimellitate
TRG	Tumor Regression Grade
ULN	Upper Limit of Normality
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor

7. ETHICS

7.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

The investigator submitted to the Independent Ethics Committee (IEC) the protocol and associated materials given to the patient (such as patient information sheets or descriptions of the study used to obtain informed consent, as well as documentation relative to advertising or compensation given to the patient). The approval of the IEC had to be obtained before initiating the study and had to be documented in a letter to the investigator specifying the date on which the Committee met and granted approval.

Any modification made to the protocol after its reception by the IEC had to be also submitted as a protocol amendment to the Committee in accordance with the procedures and local legislation.

Any modification made to the protocol after its reception by the IEC must also be submitted as a protocol amendment to the committee in accordance with the procedures and local legislation.

7.2 Ethical Conduct of the Study

This study was conducted in accordance with the ethical principles pronounced in the Declaration of Helsinki (Amendment 64th of the World Medical Association General Assembly, Fortaleza, Brazil, October 2013).

7.3 Patient Information and Consent

All subjects voluntarily consented prior to enrollment in the study. Each subject enrolled in the study received a copy of his or her signed and dated informed consent and a copy was kept on file at the institution. Significant new study developments were made known to the subjects and documented via a revised informed consent document.

8. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

COORDINATING INVESTIGATOR(S):

[redacted]

CONTRACT RESEARCH ORGANIZATION(S):

[redacted]

9. INTRODUCTION

Introduction

Different treatment strategies may be indicated for intermediate-risk (T1-T2N1, T3N0) versus moderately high (T1-2N2, T3N1, T4N0) or high-risk rectal cancer patients (T3N2, T4N1-2) based on differential survival rates and rates of relapse[1].

Neoadjuvant delivery of systemic therapy for patients with rectal cancer ensures that all patients obtain optimal systemic treatment which is feasible, safe, has improved toxicity and provides tumor downstaging [2]. This fact can be important in the higher risk population (>35% systemic failure at 5 years) group of patients. Attempts to intensify or modulate radiosensitization by adding additional cytotoxic agents have not been successful[3]. A systematic and rational approach to the development and testing of novel radiosensitizers is needed with platform serving a need to rapidly identify and test compounds with a moderately high bar of certainty prior to definitive randomized controlled studies. On the other hand, radiotherapy may not be needed in the lower risk population, because the strategy of chemotherapy alone before total mesorectal excision has shown 100% of R0 resection with high rates of pathologic complete response (pCR) (27-15%)[4,5]. This strategy is currently being tested versus the standard treatment (chemo-radiotherapy (CT/RT) for stages II and III) in an intermediate risk population, in a phase III cooperative trial in the USA. This strategy has never been tested in the higher population.

Nonetheless, current guidelines to treat T3-4 and/or N+ rectal cancer continue to recommend preoperative strategy of combined chemo (fluoropyrimidine based) with radiation[6,7].

Anti VEGF therapy in rectal cancer

A supra-additive effect of growth inhibition and cell death has been identified between ionizing radiation and either antibodies specific for vascular endothelial growth factor (VEGF) or tirosin kinase inhibitors (TKIs) of the vascular endothelial growth factor receptor (VEGF-R) [8–10]. Combining angiogenesis-targeting agents with cytotoxic chemotherapy not only provides the advantage of a non-overlapping toxicity and hence good tolerance, but also serves to enhance efficacy. The mechanism of increased efficacy with combination therapy has been explored. Experimental models have demonstrated that tumors have disorganized vasculature and lymphatics, leading to increased interstitial fluid pressure and poor delivery of cytotoxic chemotherapy and hypoxia, thereby providing resistance to chemotherapy and radiation [11]. In a phase I/II trial in locally advanced rectal cancer patients, Willet et al showed that bevacizumab decreased tumor interstitial fluid pressure and blood flow, suggesting normalization of the tumor vasculature [12].

More recently in a phase II trial, bevacizumab in combination with capecitabine and radiotherapy in locally advanced rectal cancer does not seem to improve pCR when compared with capecitabine-radiation[13]. However, one Spanish trial[14] showed impressive pCR rates (36%) with a strategy consisting in induction therapy with bevacizumab combined with capecitabine and oxaliplatin followed by bevacizumab/capecitabine/radiation and then surgery. Interestingly enough, this schema is the same used in the induction arm of the phase II randomized above[15] that obtained 14% of pCR in a very similar population and with the same strategy and schema except for the use

of bevacizumab and no oxaliplatin during radiation in the Nogue trial[14]. These observations suggest a positive effect of the anti-VEGF therapy when used as part of an induction therapy before CT/RT. Unfortunately, in the Nogue trial[14] a high rate of postsurgical complications were observed that led to 24% of reoperations. This fact can be linked to the use of anti-VEGF therapy during the radiation period as an unusual high rate of major postsurgical complications was observed in some trials with the bevacizumab, capecitabine and radiation combination[16].

Aflibercept

Aflibercept is a monoclonal antibody inhibiting VEGF and placental growth factors (PIGF). Further details on preclinical, clinical safety and preliminary efficacy are provided in the Investigator Brochure (IB), which contains comprehensive information on aflibercept[17].
Aflibercept in combination therapy improves survival in advanced colorectal cancer when combined with FOLFIRI in the second line[18]. However, an understanding of responsive tumor characteristics is lacking, and it is crucial to identify early biomarkers that accurately predict patients responding to aflibercept.
Aflibercept is a specific antagonist that binds and inactivates circulating VEGF (A and B) and PIGF. Aflibercept was designed to prevent the growth of primary and metastatic tumors by blocking tumor angiogenesis and vascular permeability.

Rationale

With the background described above aflibercept is a highly attractive drug to be tested in locally advanced rectal cancer in a high-risk population as part of an induction therapy strategy.
With antiangiogenic therapy if the goal is to deprive the tumor of its blood supply, therapy must continue until the vasculature no longer functions. If the goal is to improve vascular efficiency, as is the case in the neoadjuvant treatment of locally advanced rectal cancer, treatments should be fine-tuned accordingly. The delicate balance between too many and too few endothelial and perivascular cells warrants careful attention to the scheduling and dosing of combination therapies. Thus, measurements of vascular parameters is essential for assessing efficacy and understanding the mechanism of action of these agents[19]. Optimal scheduling may take advantage of a window of opportunity created by anti-angiogenic therapy wherein cytotoxic agents and oxygen will have maximal access to cancer cells.
As prolonged anti-VEGF treatment can destroy vessels and tumors may become resistant to anti-angiogenic drugs, tumor vessel normalization is limited to a transient window of time. Although the effects of vascular normalization have been extensively characterized in preclinical models, clinical evidence has not been fully appreciated yet.
Tumors exhibit interstitial hypertension while tumoral vessels become leaky and increase permeability and become poorly organized. This environment reduces blood supply to them, which impairs drug delivery[20,21].
Several authors have studied the relationship between the administration of antiangiogenic drugs and their effect on microvessel density (MVD) and interstitial fluid pressure (IFP) obtained directly from tumor biopsies in the appropriate timing, as the goal standard to analyze. Some preclinical and clinical data strongly affirm this effect[22–27].

The antiangiogenic treatment is effective by reducing microvessel density and interstitial fluid pressure in the vascular network of the tumor, leading to a more feasible penetration of chemotherapy into the tumoral microenvironment and lessening the degree of hypoxia inside the tumor. In these conditions, the tumor itself would reach the better setting for receiving radiotherapy treatment, thus increasing its effect in a synergistic way[28,29].

Otherwise, there are also publications finding relations between parameters of functional imaging and MVD and IFP in several tumors studied by functional MRI[30–36]. And although substantial efforts are being made to identify molecular biomarkers in tissue and blood that predict response to aflibercept, there are no validated biomarkers of response to aflibercept, so far. The candidate biomarkers include proteins related to VEGF and VEGFR family members, angiogenic factors, cytokines or any other analyses with potential role in angiogenesis and inflammation, as previously suggested[12,37–43].

In addition, single nucleotide polymorphisms (SNP) data from non-tumor tissue, have been suggested to correlate outcome to therapy[44–47]. Moreover, a “vascular normalization index” that combines changes in Ktrans, microvessel volume and circulating collagen IV significantly correlates with survival in patients with glioblastoma treated with cediranib, after a single dose of treatment[48].

Several issues remain unanswered and several hypotheses could be rised. On the first hand, an imaging biomarker would exhibit a decrease of blood flow volume and vascular permeability parameters (BF, BV and k-trans) if MVD and IFP decreases and would correlate with good response after treatment with Aflibercept. On the other hand, tissue and plasma molecular biomarkers in tumoral and blood samples would variate during treatment and correlate with response to treatment. Therefore, a “Vascular Normalization Index Biomarker” would significantly correlate with response to aflibercept.

We proposed to conduct a phase II randomized trial comparing induction treatment with FOLFOX with or without aflibercept in a high risk population selected by MRI, prior to receiving standard chemoradiation (capecitabine combined with 50.4 Gy in 28 days) and surgery[31]. Moreover, since no molecular biomarkers of response to aflibercept have been validated in tissue/blood, we proposed to study an extensive panel of biomarkers at multiple time points during therapy.

10. STUDY OBJECTIVES

10.1 Primary objective

The primary objective was to evaluate the efficacy of induction therapy with mFOLFOX6 +/- aflibercept followed by CT/RT in terms of pCR.

10.2 Secondary objectives

The secondary objectives were:

- To evaluate pathological parameters of efficacy: R0 resection, TRG, and positive or negative CRM rate.
- To evaluate the relationship between MRI changes with outcome.
- To further characterize the safety and tolerability of mFOLFOX6 +/- aflibercept followed by chemoradiation.
- To determine the rate of 30-day surgical complications.

- To evaluate the 3-year local recurrence and DFS.
- To determine the levels of tumor biomarkers expression at baseline and correlate them with response to treatment with mFOLFOX6 + aflibercept.

11. INVESTIGATIONAL PLAN

11.1 Overall Study Design and Plan: Description

This was a randomized, open and multicentric phase II trial comparing induction treatment with FOLFOX with or without aflibercept in a high risk population selected by MRI, prior to receiving standard chemoradiation (capecitabine combined with 50.4 Gy in 28 days) and surgery.
Once it was confirmed that the subjects fulfilled the eligibility criteria (MRI-defined high risk RC), and had signed the informed consent, a central review was requested to confirm clinical stage, and then they were randomized 2:1 to receive mFOLFOX6+aflibercept iv q 14d versus mFOLFOX iv q 14d.
Random assignment of treatment was stratified by EMVI+/EMVI-, T3 versus T4 stage, and by study site.
All the patients enrolled in the study received one cycle of study medication (mFOLFOX6 with or without aflibercept) every 14 days for 6 cycles, unless unacceptable toxicity or progression was detected. After this treatment, patients received standard chemo-radiotherapy (CT/RT)(capecitabine 825 mg/m^2 twice daily combined with a total dose of 50.4 Gy in 28 days) followed by surgery, provided they had not progressed.

Patients with progression disease (PD) during the treatment phase were withdrawn from the study and received their treatment according to the investigator's judgment.
If a patient withdrew consent and refused to receive further treatment, the patient had to be followed up for 3 years from randomization until progression, to evaluate disease-free survival.
If a patient withdrew consent and refused to continue in the study, the follow-up evaluations had to be discontinued.

CT and MRI were done at baseline. CTs were done prior to surgery (4weeks +/- 5 days after chemo-radiotherapy), between 1-3 months after surgery, and later every 6 months during follow-up. A second MRI was done, based on investigator's judgement, after CT-RT treatment (4 weeks +/- after chemo-radiotherapy).

11.2 Discussion of Study Design, Including the Choice of Control Groups

The primary objective of this study was to evaluate the efficacy of induction therapy with mFOLFOX6 +/- aflibercept followed by CT/RT in terms of pCR. The primary endpoint was to analyze the number of patients achieving pCR after induction therapy with mFOLFOX6 +/- aflibercept followed by CT/RT. pCR was defined as the absence of viable tumor cells in the primary tumor and in the lymph nodes (ypT0N0).

11.3 Study Population

The study population consisted of adult patients (≥18 and <75 years of age) with locally advanced high-risk rectal cancer (histological type: adenocarcinoma of the rectum), considered by the surgeon as feasible to perform a curative resection.

One hundred and sixty-two evaluable patients had to be recruited: 108 10% of drop-outs a total of 180 patients were recruited (120 patients for mFOLFOX6 + aflibercept group and 60 patients for mFOLFOX6).

11.3.1 Inclusion criteria

Only patients who fulfilled all the criteria listed below were enrolled in the study. Patients with:

1. Signed and dated informed consent, and willing and able to comply with protocol requirement;
2. Male or female subjects with rectal cancer ≥ 18 and < 75 years of age;
3. High-risk rectal cancer defined by MRI as that with inferior border of the tumor distal to the peritoneal reflection or ≤ 12 cm from the anal margin and considered by the surgeon as feasible to perform a curative resection (including pelvic exenteration as curative resection)

Presence of at least 1 of the following high-resolution, thin-slice MRI (3mm):

Middle Third Tumors

-mrT3
Extramural vascular invasion (EMVI) positive
Extramural extensión > 5 mms into perirectal fat
Mesorectal fascia (MRF) threatened or involved*
-mrT4***

Distal Third Tumors (≤ 5 cm from anal verge)

-mrT3 tumor at below levators
-T4 as above

N2**

*tumor or lymph node < 1mm from the mesorectal fascia.
**≥ 4 lymph nodes in the mesorectum showing morphological signs on MRI indicating metastatic disease. ≥ 4 nodes, whether enlarged or not, with a rounded, homogeneous appearance is thus not sufficient.
***T4a: tumor infiltrates peritoneal reflection. T4b: tumor infiltrates adjacent organs.

4. Histological or cytological documentation of adenocarcinoma of the rectum. All other histological types were excluded;
5. ECOG Performance Status of ≤ 1;

6. Hematological status: neutrophils (ANC) ≥ 1.5 x 10^9/L; platelets ≥ 100 x 10^9/L; hemoglobin ≥ 9 g/dL;
7. Adequate renal function: serum creatinine level < 1.5 x ULN;
8. Adequate liver function: serum bilirubin ≤ 1.5 x ULN, alkaline phosphatase < 5 x ULN, AST/ALT < 3 x ULN;
9. Proteinuria < 2+ (dipstick urinalysis) or ≤ 1 g/hour;
10. Regular follow-up feasible;
11. For female patients of childbearing potential, negative serum pregnancy test within 1-week (7 days) prior of starting study treatment;
12. Female patients must commit to using reliable and appropriate methods of contraception until at least three months after the end of study treatment (when applicable). Male patients with a partner of childbearing potential must agree to use contraception in addition to having their partner use another contraceptive method during the trial.

11.3.2 Exclusion criteria

Patients were excluded from the study if they present any of the criteria listed below**:**

1. Prior treatment with aflibercept;
2. History or evidence upon physical examination of metastasis;
3. Uncontrolled hypercalcemia;
4. Pre-existing permanent neuropathy (NCI grade ≥ 2);
5. Uncontrolled hypertension (defined as systolic blood pressure > 150 mmHg and/or diastolic blood pressure > 100 mmHg), or history of hypertensive crisis, or hypertensive encephalopathy;
6. Concomitant protocol unplanned antitumor therapy (e.g. chemotherapy, molecular targeted therapy, immunotherapy);
7. Treatment with any other investigational medicinal product within 28 days prior to study entry;
8. Other concomitant or previous malignancy, except: i/ adequately treated in-situ carcinoma of the uterine cervix, ii/ basal or squamous cell carcinoma of the skin, iii/ cancer in complete remission for > 5 years;
9. Any other serious and uncontrolled non-malignant disease, major surgery or traumatic injury within the last 28 days;
10. Pregnant or breastfeeding women;
11. Patients with known allergy to any excipient to study drugs;
12. Previous history of stable angina, uncontrolled arrhythmia, and acute coronary syndrome even if controlled with medication or with myocardial infarction or cerebrovascular accident within the last 12 months.
13. Bowel obstruction: Patients with intestinal occlusion, candidates to participate in the trial, may be included in the study after performing a derivative stoma;
14. Appearance of de novo deep vein thrombosis in the 4 weeks prior to randomization.

11.3.3 Removal of Patients from Therapy or Assessment

The clinical study ended when all the clinical study subjects had concluded the follow-up period.
In accordance with current revision of the Declaration of Helsinki (Appendix V) and other applicable regulations, any patient participating in the study had the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the physician or at the institution. If a subject (or a legally acceptable representative) requested or decided to withdraw, all efforts had to be made by the investigator to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information had to be reported on the applicable pages of the electronic Case Report Form (eCRF).
The investigator had the right to withdraw patients from the study if concomitant disease or AE occurred that, in the investigator's opinion, required the withdrawal of the patient from the study, if a protocol violation occurred, or for other reasons.

The reasons for premature interruption of treatment (early withdrawal) were the following:

- Voluntary discontinuation by the patient: **withdrawal of consent** and/or rejection of the treatment and/or uncooperativeness.
- **Safety reasons:** toxicity, AEs, or intercurrent disease that, in opinion of the investigator, justified withdrawal from oncologic treatment. If the toxicity required postponing treatment for more than 2 weeks, the patient ended the treatment due to toxicity and entered the study follow-up period. If the investigator thought therapy had to be continued in the best interest of the patient, this had to be discussed with the National Coordinating.
- **Pregnancy.**
- **Death.**
- **Disease progression.**
- Significant **protocol violation** or severe protocol non-compliance as judged by the investigator and/or the Sponsor.
- At the discretion of the **investigator or sponsor.**
- Request by **regulatory authorities.**

When early withdrawal occurred, the corresponding section of the eCRF had to be completed, indicating the reason for withdrawal, the last clinical and analytical data available, and the new treatment.
The subjects enrolled in the study who later withdrew from study participation were not replaced.
All the patients who terminated/discontinued the clinical study treatment had to remain in follow-up for 3 years from randomization until progression, except for patients who withdrew their informed consent, died or were lost to follow-up.

11.4 Treatments

Patients were randomized to receive treatment with mFOLFOX6 with or without aflibercept. All the patients enrolled in the study received one cycle of study medication every 14 days, for 6 cycles, unless unacceptable toxicity or progression was detected.
After they had completed these cycles of the study treatment, patients received standard chemoradiotherapy (CT/RT) followed by a second MRI and surgery, provided they had not progressed (a CT was performed prior to surgery).

11.4.1 Treatments Administered

Induction therapy
Each cycle of the study treatment consisted of:

- **mFOLFOX-6 scheme:** 5-Fluoruracil [5-FU], oxaliplatin and leucoverin were administered intravenously once every 14 days according to mFOLFOX-6 scheme:

  Day 1: Oxaliplatin 85 mg/m^2 IV infusion in 250-500 mL and leucovorin 400 mg/m^2 IV bolus and a 46h infusion of 5-FU 2400 mg/m^2.

  Aflibercept, was administered intravenously (I.V.) at doses of 4 mg/Kg on Day 1 every 14 days. Aflibercept was supplied to sites by the study Sponsor as 4 ml vials at a concentration of 25 mg/ml.

Treatment continued until six cycles were administered unless unacceptable toxicity or progression occurred. If it was necessary to interrupt or reduce the study drug dosage due to toxicity, dose adjustements were made according to the specific types of toxicities observed and the standard care at each site.

- **Chemo-radiotherapy (CT/RT)**

Standard CT/RT consisted of capecitabine 825mg/m^2 twice a day combined with a total dose of 50-4 Gy in 28 days, as neoadjuvant standard therapy.

Surgery
Surgery took place 6 +/- 2 weeks after the last CT/RT induction therapy dose. The following guidelines were followed:

- A complete exploration of the abdominal cavity had to be performed to rule out distant metastasis (M), whether liver or peritoneal. In case of doubt, suspicious tissue biopsy had to be performed. If the test was positive for malignancy, palliative surgery had to be performed and, preferentially, the primary tumor had to be resected if feasible (radical resection). Similarly, tumor stage T and N had to be assessed.
- Since tumor was in the middle or distal third of the rectum, a total mesorectal excision (TME) had to be performed. Low anterior resection (LAR) or Abdomino Perineal Excision (APE) were the techniques of choice.
- The preservation of the anal sphincter was in each case at the surgeon's discretion, depending on oncological safety criteria and characteristics of each patient (tumor distance from anal margin, degree of tumor differentiation, degree of continence, previous local tumor characteristics and the patient's pelvis).

The patients who discontinued treatment for any reason underwent safety observation that had to end 30 days after the last administration of the study treatment. It was the End-of-treatment/Early withdrawal visit.

Patients had a follow-up evaluation (follow-up visits) every 3 months ± 2 weeks.

11.4.2 Identity of Investigational Product(s)

Aflibercept presentation

Aflibercept was supplied to the sites by Sanofi, in 4mL vials at a concentration of 25mg/mL, and was packaged and labelled according to the applicable local legislation.

The clinical use and administration of aflibercept followed the instructions provided in the latest IB version.

Preparation and administration of aflibercept

Preparation

Multiple vials of aflibercept concentrate for solution for infusion could be required depending on the patient's weight and the intended dose. The necessary volume of aflibercept had to be withdrawn from the vials and injected directly into the infusion bag. Aflibercept concentrate had to be diluted for infusion with 0.9% sodium chloride solution or 5% dextrose by a healthcare professional. The dilution had to be carried out under aseptic conditions. Any unused portion left in a vial must be discarded, as the investigational drug does not contain any preservatives.

Infusion conditions

The infusion sets contained a 0.2 µm polyethersulfone inline filter. PDVF or Nylon filters couldn't be used. Infusion could be conducted by gravity, with an IV infusion pump, or with a syringe pump using administration sets made of the above materials. The aflibercept IV dose had to be infused over 1 hour. The infusion couldn't exceed 2 hours at room temperature (approximately 25ºC). Parenteral investigational drug products had to be inspected visually for particulate matter and discoloration prior to administration.

Storage period of premix and infusion solution

The aflibercept concentrate for solution for infusion in its original unopened container is stable for 36 months under refrigerated conditions (2 to 8ºC).

The Hospital Pharmacist was responsible for the appropriate storage of the investigational medicinal product (IMP) at the study centre, and they had immediately to inform the Monitor/Sponsor of non-respect of the required storage conditions. When closing the investigational centre, all unused IMP containers had to be destroyed on site. if an IMP batch is suspected to be defective, Sanofi had immediately to inform the Sponsor so that the Hospital Pharmacist could immediately got the appropppriate information. The Hospital

Pharmacist had to organize the destruction of the concerned batch(es) and new batch(es) had to be sent to the investigational centre when appropppriate.

mFOLFOX-6

mFOLFOX-6 was dispensed under the responsibility of the Pharmacy Service of each one of the participating sites according to the local legislation for dispensing marketed products for hospital use. Premedication was prescribed according to the sites procedures.

Oxaliplatin administration

Oxaliplatin had to be administered at the recommended dose in 2-hour intravenous infusion. Oxaliplatin infusion had to be administered by peripheral or central venous infusion over 2 hours. Pathways used to administer the infusion had to be washed with a solution of 5% dextrose (D5W) after finishing oxaliplatin infusion and before administering any other drug.

5-FU/Leucovorin administration
5-FU and leucovorin were administered according to the mFOLFOX6 schedule.

11.4.3 Method of Assigning Patients to Treatment Groups

Random assignment of treatment was stratified by EMVI+/EMVI-, T3 versus T4 stage and by study site.
All the patients enrolled in the study received one cycle of study medication (mFOLFOX6 with or without aflibercept) every 14 days for 6 cycles, unless unacceptable toxicity or progression was detected. After that treatment, patients received standard chemo-radiotherapy (CT/RT) (capecitabine 825 mg/m^2 twice daily combined with a total dose of 50.4 Gy in 28 days) followed by surgery, provided they had not progressed.

11.4.4 Selection of Doses in the Study

The clinical use and administration of aflibercept followed the instructions provided in the latest IB version.
mFOLFOX-6 was dispensed under the responsibility of the Pharmacy Service of each one of the participating sites according to the local legislation for dispensing marketed products for hospital use.
Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for AE, version 4.0.

Dose modifications for aflibercept

Dose adjustment and/or cycle delay were planned in case of toxicity. Dose adjustments were made according to the worst grade toxicity. Patient received the next cycle after recovery from the detected toxicity.
If a patient experienced several toxicities and there were conflicting recommendations, the conservative dose adjustment recommended (dose reduction appropriate to the most severe toxicity) had to be followed. Once a dose had been decreased, intra-patient re-escalation back to the previous dose level was not permitted.
In case of toxicity from treatment, administration had to be delayed until:

- neutrophil count was $\geq$ 1.5 x 10^9/L and platelet count was $\geq$ 75 x 10^9/L
- recovery to grade $\leq$ 1 for other toxicities (except alopecia and otherwise specified)

The maximum delay allowed was of 2 weeks. In case of treatment delay greater than 2 weeks, patient had to discontinue aflibercept, unless it was discussed with the National Coordinator and a decision was made to continue the treatment in the best interest of the patient.

Dose reduction
Aflibercept dose reduction is described in Table 2. Only one dose reduction was allowed.

Table 2. Aflibercept dose reduction level

	Initial dose (mg/kg)	Dose reduction, level – 1 (mg/kg)

Aflibercept	4	→	2

Dose modifications and dose delay for any component of mFOLFOX6

In addition to optimizing supportive care, oxaliplatin and 5-FU/LV dose adjustments based on the worst toxicity encountered during the previous cycle, were recommended. These were at the investigator's discretion considering the current local prescribing information and the standard clinical practice.

Table 3 sets out the recommended dose reductions for each component mFOLFOX-6. Dose adjustments for each agent had to be performed independently according to the specific types of toxicities observed. Dose leucovorin 400/m^2 couldn't be reduced and had to be given before 5-FU bolus administration and continuous infusion 5-FU.

Individuals who required a dose reduction for grade 2 toxicity and the same toxicity didn't reappear, could return to the original dose level for the next cycle, according to the investigator's discretion. If a dose reduction of any agent beyond the level -3 was required, the agent had to be discontinued and rest of the drugs could continue as per the protocol.

Table 3. Dose reductions for mFOLFOX6

Drug	First dose	Level -1	Level -2	Level -3
Oxaliplatin	85 mg/m^2	65 mg/m^2	50 mg/m^2	40 mg/m^2
5-FU bolus	400 mg/m^2	320 mg/m^2	270 mg/m^2	230 mg/m^2
5-FU continuous infusion	2400 mg/m^2 in 46-48 hours	1920 mg/m^2 in 46-48 hours	1600 mg/m^2 in 46-48 hours	1360 mg/m^2 in 46-48 hours

11.4.5 Prior and concomitant therapy

Permitted treatments

The use of any medication that, in the judgment of the investigator, was required by the patients for their correct clinical care was permitted, with the exception of the medications listed in section "Prohibited treatments", the use of which entailed withdrawal of the patient from the study. The data related to all concomitant medication used, as well as the diagnostic, therapeutic and surgical procedures performed during the study, had to be recorded in the eCRF. All patients in this study were offered the necessary palliative and support measures for the treatment of disease-related symptoms.

- In the case of nausea, vomiting or diarrhea, effective symptomatic treatment had to be given. Appropriate measures had to be taken to rehydrate patients.
- Low dose corticosteroids were allowed as an antiemetic therapy.
- Therapy with hematologic growth factors was allowed at the investigator's discretion. Transfusion with blood products was allowed.
- Vitamin / mineral supplements (not containing St. John's wort) were allowed at the investigator's discretion and clinical judgement, provided they didn't interfere with the study treatment. Supplements were not allowed unless all components are known and recorded.
- Prophylactic and therapeutic anticoagulant medication (i.e. in therapeutic range) was allowed as well as antiplatelet drugs.

Prohibited treatments

- The use of other antitumoral agents different from the study drugs was not permitted, whether or not they were experimental. Active or passive immunotherapy was not permitted.
- Cooling scalp systems to prevent alopecia, or ice mouthwashes to prevent stomatitis were not allowed because of the risk of causing cold-induced-oxaliplatin dysesthesia.
- The use of vitamin B6 (pyridoxine) for hand-foot syndrome prophylaxis/treatment was not allowed, because of loss of efficacy when administered with oxaliplatin.
- Subjects treated with oxaliplatin had not take cold drinks and ice on day 1 of each treatment cycle, since exposure to cold could exacerbate oral or pharyngeal dysestesia.
- Patients had to be monitored carefully for side effects, covering all concomitant medications regardless of the route of administration of the drug, especially those with narrow therapeutic indices, such as digoxin, warfarin (aldocumar) and quinidine. Blood levels had to be monitored and appropriate laboratory tests performed as clinically indicated.

11.4.6 Treatment Compliance

The study drug was administered by the nursing staff of the oncology-day care centre under the investigator's supervision.

Since the intravenous infusions were administered in a hospital or outpatient clinic setting, compliance with treatment could be easily monitored. The date and time of the beginning and end of the infusion and exact amount of study medication (aflibercept, oxaliplatin, 5FU and LV) administered in each infusion had to be documented in the patient's medical record and eCRF.

If the treatment had to be modified or interrupted during the infusion, the staff in charge of the procedure had to estimate the percentage of the dose received by the patient and document it in the eCRF. All the reasons for non-compliance with study treatment had to be recorded.

Insufficient compliance with the dosing regimen for aflibercept, oxaliplatin, 5FU or LV was defined as missing more than 2 consecutive infusions for reasons other than toxicity.

11.5 Efficacy and Safety Variables

11.5.1 Efficacy assessment

Efficacy variables:

- **Pathological Complete response (pCR):** pCR was defined as the percentage of patients with no tumor viable cells in the surgical sample after induction therapy.

- **Disease -Free Survival**: time from randomization to the appearance of any signs or symptoms of cancer relapse. Patients who withdrew from the study before reaching relapse and without completing the withdrawal consent had to be followed up to determine their status whenever possible.
- **"Vascular Normalization Index Biomarker":** correlation between permeability parameters (BF, BV and k-trans) and MVD and IFP were assessed, as well as the correlation between VEGFR, ANG/ANG2, and IL-2 with response to treatment.

11.5.2 Safety assessment

The safety profile was determined from the AEs reported by the subjects during the clinical study. Each patient was monitored regularly to detect possible AEs before beginning each cycle. The number and percentage of AEs observed, and their intensity were reported. The intensity of the AEs was classified according to the NCI-CTCAE v4.0.

The period of notification of AEs began when the informed consent was signed. Serious and non-serious AEs related to the study treatment that appeared up to 30 days after administration of the last dose had to be recorded.

Any AE or laboratory anomaly that was serious and occurred during the development of the study, independently of the treatment received by the patient, had to be reported immediately by the investigator (within 24 hours of first aware of the case).

A follow-up had to be made of the AEs, especially those whose relationship with the medication in investigation could not be classified as "non related", until the baseline situation had been restored or the AE was stabilized. If a clear explanation was stablished, it was recorded in the eCRF.

11.6 Statistical Methods Planned in the Protocol and Determination of Sample Size

11.6.1 Statistical and Analytical Plans

Study populations:
The primary efficacy analysis was based on the Intent to treat (ITT) Population, although a secondary analysis was also be performed based upon the Per Protocol (PP) Population to assess the sensitivity of the analysis to the choice of analysis population. All safety analyses were based upon the Safety Population.

- **ITT population:** formed by all randomized patients who received at least one dose of Trial Drug.
- **Per protocol population**: the PP Population included ITT patients who met both of the following criteria:
  - Received at least 80% of their intended Trial Drug.
  - Did not have any major protocol violations.

  The PP Population could be used in efficacy analyses as a sensitivity analysis.
- **Safety population (PP)**: all the subjects were evaluable for toxicity so long as they had received at least a first administration of the study drugs.

All primary analyses of the safety data were conducted using the safety population.

The ITT Population was used for all efficacy analyses. The rate of pCR was analyzed as a binary parameter. A z test was used for the differences between percentages (or Fisher's exact test if the assumptions were not met). A 90% confidence interval of the between-group difference was added.
Continuous demographic parameters, such as age at the time of enrollment, were summarized for the ITT Population using descriptive statistics (N, mean, median, SD, minimum, and maximum value).
Categorical demographic parameters, such as gender, were summarized as a frequency and proportion of the ITT Population.
R0 resection, TRG, and CRM rate were analyzed as binary parameters, like the main endpoint: A z test was used for the differences between percentages (or Fisher's exact test if the assumptions were not met).
The Kaplan-Meier plots were presented for the Time-to-Event. Log-rank test was applied to the groups' comparison. Hazard ratio through Cox model was calculated (if the assumptions were met).
Quantitative parameters, was analyzed through ANCOVA model, including basal value as covariate.
Any other continuous parameters: ANCOVA model on the changes from baseline with treatment, baseline value and any stratification factors as fixed effects. A 95% confidence interval of the between-group difference was added.

11.6.2 Determination of Sample Size

Assuming 10% of drop-outs a total of 180 patients were recruited (120 patients for mFolfox6 + Aflibercept group and 60 for mFolfox6).

Assumptions:

- 2 treatment arms with unequal 2:1 group allocation
- 0.20, two-sided, type-I error
- mFolfox6 efficacy: 15% pCR rate
- Aflibercept + mFolfox6 efficacy: 30% pCR rate
- 80% power to detect a 15% treatment difference
- 2 interim analyses:
    - At 33% of the sample size for safety, futility/efficacy
    - At 66% of the sample size for safety, futility/efficacy
- One final analysis
- Stopping rules:
    - Efficacy: Lan de Mets Alpha spending function (O'Brien-Fleming)
    - Futility: Lan de Mets Alpha spending function (O'Brien-Fleming), non-blinding

12. Study Subjects

Table 3. Distribution of patients by sites

Centres	Investigators	Patients enrolled
01-IVO	[redacted]	
02-H. Arnau de Vilanova Lleida	[redacted]	
03-C. Sanit. Parc Tauli	[redacted]	
04- H. del Mar	[redacted]	
05- H. Clinic i Prov Barcelona	[redacted]	
06- H. Miguel Servet	[redacted]	
08- H. de Navarra	[redacted]	
09- H. Gral. U. Elche	[redacted]	
10- H. La Paz	[redacted]	
11-H. Sta. Creu i Sant Pau	[redacted]	
12-C.I.O. Clara Campal	[redacted]	
13-H. Gral. Alicante	[redacted]	
14-H. de Granollers	[redacted]	
15-H. Sant Joan Despí-Moisés Broggi	[redacted]	
16-H. Vall d'Hebrón	[redacted]	
17-H. Prov. Castellón	[redacted]	
18-. Althaia Manresa	[redacted]	
20- H. GTiP	[redacted]	
21-H. Univ. 12 de Octubre	[redacted]	
22-H. Univ. Marqués Valdecilla	[redacted]	

12.1 Discontinuations

Table 4. Patients that did not complete the study treatment (Neoadjuvant CT or Neoadjuvant CT/RT) and end of treatment reasons

End of treatment				
		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	Total (N=)
Treatment withdrawal?				
Yes	n (%)			
No	n (%)			
Reasons of withdrawal				
Toxicity, AE	n (%)			
Withdrawal of inform consent and/or rejection of the treatment and/or uncooperativeness	n (%)			
Progression disease	n (%)			
Death	n (%)			
NA	n (%)			
At the discretion of the Investigator or Sponsor	n (%)			

Table 5. Reasons for withdrawal from the Study Treatment

Treatment arm	Patient ID	CT	CT/RT	SUR	Was the patient withdrawn for the study	Withdrawal causes	Toxicity description[1]	Progression description

12.2 Major protocol deviations

A description of all major deviations is shown in Table 6.

Table 6. Major Deviations

Subject code	Major deviation(s)

13. EFFICACY EVALUATION

13.1 Data Sets Analyzed

Table 7. Analysis populations

Populations in the study				
		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	Total (N=)
ITT population				
Yes	n (%)			
ITT population with curative surgery				
Yes	n (%)			
No	n (%)			
PP population				
Yes	n (%)			
No	n (%)			
PP population with curative surgery				
Yes	n (%)			
No	n (%)			
SAF population				
Yes	n (%)			

ITT population (ITT). It will include all randomized patients who received at least one dose of Trial Drug.

ITT population with curative surgery will include all ITT patients that received curative surgery.

PP population (PP) will include ITT patients who met both of the following criteria:

- Received at least 80% of their intended Trial Drug
- Did not have any major protocol violations

PP population with curative surgery will include all PP patients that received curative surgery.

Safety Population (SAF): It consists of all patients who received at least a first administration of Trial Drug.

All primary analyses of the efficacy data will be conducted using the ITT population and safety analyses will be performed for the SAF population. The PP population will be used for efficacy analyses as a sensitivity analysis.

13.2 Demographic and Other Baseline Characteristics

Demographics by treatment arm in ITT population are shown at the Table 8.

Table 8. Demographic Characteristics ITT

Demographic Characteristics ITT				
		AFLIBERCEPT + mFOLFOX-6 (N=))	mFOLFOX-6 (N=)	P Value Test
Age (years)				
	n			
	Mean (SD)			
	Median [Q1,Q3]			
	Min, Max			
Sex				
Female	n (%)			
Male	n (%)			
ECOG performance status				
0	n (%)			
1	n (%)			
Clinical T stage (middle and distal)				
Missing	n (%)			
mrT2	n (%)			
mrT3	n (%)			
mrT3B	n (%)			
mrT3A	n (%)			
mrT3C	n (%)			
mrT3D	n (%)			
mrT4	n (%)			
mrT4A	n (%)			

mrT4B	n (%)			
Clinical T stage (grouped)				
Missing	n (%)			
T2/T3	n (%)			
T4	n (%)			
FMR				
FMR + (distance <=1 mm)	n (%)			
NR	n (%)			
EMVI				
EMVI - (score 0/1/2)	n (%)			
EMVI + (score 3/4)	n (%)			
N2				
N2	n (%)			
NR	n (%)			
Location				
Distal	n (%)			
Middle	n (%)			
Missing	n (%)			
Histology				
Adenocarcinoma	n (%)			

Demographics by treatment arm in PP population are shown at the Table 9.

Table 9. Demographic Characteristics PP

Demographics PP				
		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	P Value Test
Age (years)				
	n			
	Mean (SD)			
	Median [Q1,Q3]			
	Min, Max			
Sex				
Female	n (%)			
Male	n (%)			
ECOG performance status				
0	n (%)			
1	n (%)			
Clinical T stage (middle and distal)				

Missing	n (%)			
mrT2	n (%)			
mrT3	n (%)			
mrT3B	n (%)			
mrT3A	n (%)			
mrT3C	n (%)			
mrT3D	n (%)			
mrT4	n (%)			
mrT4A	n (%)			
mrT4B	n (%)			
Clinical T stage (grouped)				
Missing	n (%)			
T2/T3	n (%)			
T4	n (%)			
FMR				
FMR + (distance <=1 mm)	n (%)			
NR	n (%)			

EMVI				
EMVI - (score 0/1/2)	n (%)			
EMVI + (score 3/4)	n (%)			
N2				
N2	n (%)			
NR	n (%)			
Location				
Distal	n (%)			
Middle	n (%)			
Missing	n (%)			
Histology				
Adenocarcinoma	n (%)			

13.3 Efficacy Results and Tabulations

13.3.1 Analysis of Efficacy

ITT population will be used for the efficacy analyses.

Primary Efficacy Analysis in the ITT Population

The primary objective of this study is to assess the efficacy of induction therapy with mFOLFOX +/- Aflibercept followed by CT/RT in terms of pathologic response (pCR) (Yes/No). pCR is defined as the absence of viable tumor cells in the primary tumor and in the lymph nodes (ypT0N0). The values from ypTNM stage are taken to create pCR categorical variable (Yes/No).

Table 10. pCR Response in the ITT Population

pCR response (ITT)				
		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	P Value Test
pCR (Yes/No)				
Yes	n (%)			
No	n (%)			

Secondary Efficacy Objectives in the ITT Population

The secondary efficacy objectives are the following:

- To evaluate pathological parameters of efficacy: R0 resection. **Endpoint**: To determine CRM negative (negative vs positive) and R0 resection rates (Yes/No)
- To evaluate the relationship between MRI changes and pathological tumor response. i.e mrTRG. **Endpoint**: TRG; residual tumor after preoperative therapy was evaluated according to the 5-point regression grading scale stablished by Mandard. Involvement of the histologic CRM was defined as tumor $\leq$ 2 mm from the resection margin.
- T Downstaging (Yes/No): defined as a lower pathologic T stage compared to pre-treatment mrT stage.
- To evaluate the 3 years local recurrence and disease-free survival. **Endpoint**: To determine the rate of local recurrence and disease-free survival (DFS) at 3-years.

In Table 11 are enclosed the results related to the first three first secondary efficacy variables.

Table 11. Secondary Efficacy Objectives in the ITT Population

Secondary efficacy objectives (ITT)				
		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	P Value Test
Resection type				

NA	n (%)			
R0	n (%)			
R1	n (%)			
R2	n (%)			
RX	n (%)			
Circumferential Resection Margin (CRM)				
<= 1 mm	n (%)			
> 1 mm	n (%)			
NA	n (%)			
NR	n (%)			
Tumor Regression Grade (TRG)				
TRG1	n (%)			
TRG2	n (%)			
TRG3	n (%)			
TRG4	n (%)			
NR	n (%)			
NA	n (%)			
TRG5	n (%)			
TRG1+TRG2 vs others?				
No	n (%)			
Yes	n (%)			
Local invasion (ypT)				
NA	n (%)			
T0	n (%)			
T1	n (%)			
T2	n (%)			
T3	n (%)			
T4A	n (%)			
T4B	n (%)			
TIS	n (%)			
T-Downstaging				
Yes	n (%)			
No	n (%)			
T-Downstaging (details)				
Yes	n (%)			
No	n (%)			
At least one value not recorded in CRF	n (%)			

In Table 12 it is shown the DFS of ITT population.

Table 12. Disease free survival ITT

	AFLIBERCEPT + mFOLFOX-6	mFOLFOX-6
Summary of events		
No of patients		
No of patients with event		
No of censored patients		
Progression free survival ITT		
Median (95% CI)		
25th-75th percentile		
Percent Survival (%, 95% CI)		
0 Months		
12 Months		
24 Months		
36 Months		
48 Months		
Kaplan_Meier Model		
P-value (Log-rank)		
Cox Model	**Hazard ratio (95% CI)**	**Cox Model P-value**
mFOLFOX-6 vs AFLIBERCEPT + mFOLFOX-6		

Figure 3. Disease free survival ITT

PP population will be used for the efficacy sensitivity analyses.

Primary Efficacy Analysis in the PP Population

Table 13. pCR Response in the PP population

pCR response (PP)			
	AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	P Value Test

pCR (Yes/No)				
Yes	n (%)			
No	n (%)			

Secondary Efficacy Objectives in the PP Population

The secondary efficacy objectives are the following:

- To determine CRM negative (negative vs positive) and R0 resection rates (Yes/No).
- TRG; residual tumor after preoperative therapy was evaluated according to the 5-point regression grading scale stablished by Mandard. Involvement of the histologic CRM was defined as tumor $\leq$ 2 mm from the resection margin.
- T Downstaging (Yes/No): defined as a lower pathologic T stage compared to pre-treatment mrTstage.
- To determine the rate of local recurrence and disease-free survival (DFS) at 3-years.

In Table 14 we can see the results related to the first three first efficacy variables.

Table 14. Secondary Efficacy Objectives in the PP Population

Secondary efficacy objectives (PP)				
		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	P Value Test
Resection type				
NA	n (%)			
R0	n (%)			
R1	n (%)			
R2	n (%)			
RX	n (%)			
Circumferential Resection Margin (CRM)				
<= 1 mm	n (%)			
> 1 mm	n (%)			
NA	n (%)			
NR	n (%)			
Tumor Regression Grade (TRG)				
TRG1	n (%)			
TRG2	n (%)			
TRG3	n (%)			
TRG4	n (%)			
NR	n (%)			

NA	n (%)			
TRG1+TRG2 vs others?				
No	n (%)			
Yes	n (%)			
Local invasion (ypT)				
NA	n (%)			
T0	n (%)			
T1	n (%)			
T2	n (%)			
T3	n (%)			
T4A	n (%)			
T4B	n (%)			
TIS	n (%)			
T-Downstaging				
Yes	n (%)			
No	n (%)			
T-Downstaging (details)				
Yes	n (%)			
No	n (%)			
At least one value not recorded in CRF	n (%)			

In Table 15 it is shown the DFS of PP population.

Table 15. Disease free survival in PP population

	AFLIBERCEPT + mFOLFOX-6	mFOLFOX-6
Summary of events		
No of patients		
No of patients with event		
No of censored patients		
Progression free survival PP		
Median (95% CI)		
25th-75th percentile		
Percent Survival (%, 95% CI)		
0 Months		
12 Months		

24 Months			
36 Months			
48 Months			
Kaplan_Meier Model			
P-value (Log-rank)			
Cox Model		**Hazard ratio (95% CI)**	**Cox Model P-value**
mFOLFOX-6	vs AFLIBERCEPT + mFOLFOX-6		

Figure 4. Disease free survival PP

14. SAFETY EVALUATION

Safety analyses will be performed on the SAF population and will be based mainly on the frequency and severity of the AEs.

The safety and tolerability of the study therapy will be assessed by means of AEs and changes in laboratory data that will be reported in the AEs page.

Treatment-Emergent Adverse Events (TEAEs) are defined as AEs that had occurred or worsened in severity and/or frequency after initiation of therapy. Any event with an onset on the day of the first dose of Trial Drug on which the time of onset will be missing was assumed to be a TEAE.

14.1 Adverse Events

For the statistical tables, adverse events will be coded according to the Medical Dictionary of Regulatory Activities (MedDRA 20.1) system. Their intensity will be coded by (NCI-CTCAE) v4.0 toxicity criteria.

14.1.1 Brief Summary of Adverse Events

Table 16. Summary of TEAEs during the Study Period

		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	Total (N=)	P-Value
Summary of adverse events (Study period)					
Patients with at least one adverse event	n (%)				
Patients with at least one grade 3/4 adverse event	n (%)				
Patients with at least one adverse event that led to permanently treatment discontinuation	n (%)				
Patients with at least one adverse event that led to death	n (%)				
Patients with at least one serious adverse event	n (%)				
Patients with at least one adverse event that the investigator considered related with study medication	n (%)				
Patients with at least one grade 3/4 adverse event that the investigator considered related with study medication	n (%)				
Patients with at least one adverse event that the investigator considered related with study medication and lead to death	n (%)				
Patients with at least one adverse event that the investigator considered related and led to permanently treatment discontinuation	n (%)				
Patients with at least one serious adverse event	n (%)				

14.1.2 Display of Adverse Events

Table 17. TEAEs During the Study Period: Worst Grade per patient (Most Frequent > 5%)

SOC MedDRA Term/Preferred MeDDRA Term	Treatment/Grade																		Total (N=)	
	AFLIBERCEPT + mFOLFOX-6 (N=)										mFOLFOX-6 (N=)									
	1		2		3		4		5[1]		1		2		3		4			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%

Table 18. Surgical procedures, toxicities and grading of TME in patients who undergo curative surgery

Surgical procedures, toxicities and grading of TME in patients who undergo curative surgery		
	AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)
	n (%)	n (%)
Type of curative surgery		
Low anterior resection		
Abdominoperineal resection		
Others		
Postoperative morbidity		
Overall registered AEs (any grade)		
Overall registered AEs Grade 3-4		
Overall complications (any grade)*		
Anastomotic fistula		
Wound infection (abdominal or perineal)		
Intraabdominal infection		
Stoma complications		
Reoperation		
Postoperative mortality		
Other morbidity		
Grading of operative specimen		
Mesorectal plane (good)		
Muscularis propria plane (poor)		
Intramesorectal plane (moderate)		
NR		

14.2 Deaths, Other SAEs, and Other Significant Adverse Events

14.2.1 Deaths

Table19. Deaths

		Deaths		
		AFLIBERCEPT + mFOLFOX-6 (N=)	mFOLFOX-6 (N=)	Total (N=)
Number of deaths				
No	n (%)			
Yes	n (%)			
Cause of death				
Progression disease	n (%)			
Intercurrent cause	n (%)			
Other Causes	n (%)			
NA	n (%)			

Narratives of Deaths, Other Serious AEs, and Certain Other Significant AEs

15. DISCUSSION AND OVERALL CONCLUSIONS

LIST MAIN CONCLUSIONS

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