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Title: Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicentre, open-label, phase 3, randomised controlled trial by GONO

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Corresponding Author: Professor Alfredo Falcone,

Corresponding Author's Institution: Unit of Medical Oncology 2

First Author: Chiara Cremolini, MD, PhD

Order of Authors: Chiara Cremolini, MD, PhD; Carlotta Antoniotti, MD; Daniele Rossini, MD; Sara Lonardi, MD; Fotios Loupakis, MD, PhD; Filippo Pietrantonio, MD; Roberto Bordonaro, MD; Tiziana Pia Latiano, MD; Emiliano Tamburini, MD; Daniele Santini, MD; Alessandro Passardi, MD; Federica Marmorino, MD; Roberta Grande, MD; Giuseppe Aprile, MD; Alberto Zaniboni, MD; Sabina Murgioni, MD; Cristina Granetto, MD; Angela Buonadonna, MD; Roberto Moretto, MD; Salvatore Corallo, MD; Stefano Cordio, MD; Lorenzo Antonuzzo, MD; Gianluca Tomasello, MD; Gianluca Masi, MD; Monica Ronzoni, MD; Samantha Di Donato, MD; Chiara Carlomagno, MD; Matteo Clavarezza, MD; Giuliana Ritorto, MD; Andrea Mambrini, MD; Mario Roselli, MD; Samanta Cupini, MD; Serafina Mammoliti, MD; Elisabetta Fenocchio, MD; Enrichetta Corgna, MD; Vittorina Zagonel, MD; Gabriella Fontanini, MD, PhD; Clara Ugolini, MD; Luca Boni, MD; Alfredo Falcone

Abstract: Background
The triplet FOLFOXIRI (fluorouracil, L-leucovorin, oxaliplatin, and irinotecan) plus bevacizumab showed improved outcomes of patients with metastatic colorectal cancer, when compared to FOLFIRI (fluorouracil, L-leucovorin, and irinotecan) plus bevacizumab. However, the actual benefit of the upfront exposure to the three cytotoxics when compared with a pre-planned sequential strategy of doublets was not clear, as well as the feasibility and efficacy of therapies after progression. To this purpose, we aimed at comparing a pre-planned strategy of upfront FOLFOXIRI followed by the reintroduction of the same regimen after disease progression to a sequence of mFOLFOX6 (fluorouracil, L-leucovorin, and oxaliplatin) and FOLFIRI doublets, in combination with bevacizumab.
Methods
TRIBE2 was an open-label, prospective, phase 3 randomised study of patients (aged 18-70 years with Eastern Cooperative Oncology Group [ECOG] performance status of 2 or less and aged 71-75 years with an ECOG performance status of 0), with unresectable, previously untreated metastatic colorectal cancer, who were recruited from 58 Italian Oncology Units. Patients were stratified according to center, ECOG performance status, primary tumour location and previous adjuvant chemotherapy, and randomly assigned (1:1) via a web-based procedure to two different strategies: first-line mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab after disease progression (control group) or FOLFOXIRI plus bevacizumab followed by the reintroduction of the same regimen after

disease progression (experimental group). Combination treatments were administered up to 8 cycles followed by fluorouracil/L-leucovorin plus bevacizumab maintenance until disease progression, unacceptable adverse events, or consent withdrawal. Both patients and investigators were aware of treatment assignment. The primary endpoint was progression-free survival 2, defined as the time from randomization to disease progression on any treatment given after first disease progression or death, analysed by intention to treat. Safety was assessed in the population of patients who received at least one dose of their assigned treatment. The study recruitment was completed, and follow-up of participants is still ongoing. The trial is registered at Clinicaltrials.gov: NCT02339116.

Findings

Between February 26, 2015, and May 15, 2017, 679 patients were randomly assigned and received treatment (340 in the control group and 339 in the experimental group). 81% of enrolled patients had a right-sided and/or RAS or BRAF mutated tumour. At data cut-off (July 30, 2019) the median follow-up was 35·9 months (IQR 30·1-41·4). Median progression-free survival 2 was 19·2 months (95% CI 17·3-21·4) in the experimental group and 16·4 months (95% CI 15·1-17·5) in the control group (hazard ratio [HR] 0·74, 95% CI 0·63-0·88; p<0·001). Median 1st progression-free survival was 12·0 months (95% CI 11·1-12·9) with FOLFOXIRI plus bevacizumab and 9·8 months (95% CI 9·0-10·5) with mFOLFOX6 plus bevacizumab (HR 0·74, 95% CI 0·63-0·86, p<0·001). Higher incidences of grade 3 or 4 diarrhoea (17% vs 5%, p<0·001), neutropenia (50% vs 21%, p<0·001) and febrile neutropenia (7% vs 3%, p=0·045) were reported in the experimental group. Out of 570 patients alive at the time of disease progression, 82% and 88% received a treatment after progression in the experimental and in the control group, respectively. Median 2nd progression-free survival was 6·2 months (95% CI 5·6-6·6) in the experimental group and 5·6 months (95% CI 4·9-6·4) in the control group, (HR 0·87, 95% CI 0·73-1·04; p=0·116). Median overall survival was 27·4 months (95% CI 23·7-30·0) in the experimental group and 22·5 months (95% CI 20·7-24·8) in the control group (HR 0·82, 95% CI 0·68-0·98; p=0·032).

Interpretation

Upfront FOLFOXIRI plus bevacizumab followed by the reintroduction of the same regimen in case of disease progression is the best therapeutic strategy for patients with metastatic colorectal cancer selected according to the study criteria and particularly for those with right-sided and/or a RAS or BRAF mutated tumours.

Funding

Supported by the GONO and the ARCO Foundations. A research grant was provided by F. Hoffmann-La Roche.

Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicentre, open-label, phase 3, randomised controlled trial by GONO

Authors

Chiara Cremolini, MD, PhD; Carlotta Antoniotti, MD; Daniele Rossini, MD; Sara Lonardi, MD; Fotios Loupakis, MD PhD; Filippo Pietrantonio, MD; Roberto Bordonaro, MD; Tiziana Pia Latiano, MD; Emiliano Tamburini[§], MD; Daniele Santini, MD; Alessandro Passardi, MD; Federica Marmorino, MD; Roberta Grande, MD; Giuseppe Aprile[#], MD; Alberto Zaniboni, MD; Sabina Murgioni, MD; Cristina Granetto, MD; Angela Buonadonna, MD; Roberto Moretto, MD; Salvatore Corallo, MD; Stefano Cordio, MD; Lorenzo Antonuzzo, MD; Gianluca Tomasello, MD; Gianluca Masi, MD; Monica Ronzoni, MD; Samantha Di Donato, MD; Chiara Carlomagno, MD; Matteo Clavarezza, MD; Giuliana Ritorto, MD; Andrea Mambrini; Mario Roselli, MD; Samanta Cupini, MD; Serafina Mammoliti, MD; Elisabetta Fenocchio, MD; Enrichetta Corgna, MD; Vittorina Zagonel, MD; Gabriella Fontanini, MD, PhD; Clara Ugolini, MD; Luca Boni, MD; Alfredo Falcone, MD, on behalf of the GONO Foundation Investigators.

Affiliations

Department of Oncology, University Hospital of Pisa, Pisa, Italy (Chiara Cremolini, MD, PhD; Carlotta Antoniotti, MD; Daniele Rossini, MD; Federica Marmorino, MD; Roberto Moretto, MD; Prof Gianluca Masi, MD; Prof Alfredo Falcone, MD) **and Department of Translational Research and New Technologies in Medicine, University of Pisa, Pisa, Italy** (Chiara Cremolini, MD, PhD; Carlotta Antoniotti, MD; Daniele Rossini, MD; Federica Marmorino, MD; Prof Gianluca Masi, MD; Prof Alfredo Falcone, MD).

Medical Oncology Unit 1, Department of Clinical and Experimental Oncology, Istituto Oncologico Veneto – IRCCS, Padova, Italy (Sara Lonardi, MD; Fotios Loupakis, MD, PhD; Sabina Murgioni; Vittorina Zagonel, MD).

Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy (Filippo Pietrantonio, MD; Salvatore Corallo, MD) **and Oncology and Hemato-oncology Department, Università degli Studi di Milano, Milan, Italy** (Filippo Pietrantonio, MD).

Medical Oncology Unit, ARNAS Garibaldi Catania, Catania, Italy (Roberto Bordonaro, MD; Stefano Cordio, MD).

Oncology Unit, Foundation IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy (Tiziana Pia Latiano, MD).

Oncology Unit, Ospedale degli Infermi, Rimini, Italy (Emiliano Tamburini, MD).

Department of Medical Oncology, University Campus Biomedico, Roma, Italy (Daniele Santini, MD).

Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy (Alessandro Passardi, MD)**.**

Department of Medical Oncology, F. Spaziani Hospital, Frosinone, Italy (Roberta Grande, MD).

Department of Oncology, University and General Hospital, Udine, Italy (Giuseppe Aprile, MD).

Medical Oncology Unit, Poliambulanza Foundation, Brescia, Italy (Alberto Zaniboni, MD).

Department of Oncology, S. Croce and Carle Teaching Hospital, Cuneo, Italy (Cristina Granetto, MD).

Department of Medical Oncology, IRCCS CRO National Cancer Institute, Aviano, Italy (Angela Buonadonna, MD).

Medical Oncology Unit, Careggi University Hospital, Firenze, Italy (Lorenzo Antonuzzo, MD).

Medical Oncology Unit, ASST of Cremona, Cremona Hospital, Cremona, Italy (Gianluca Tomasello, MD).

Department of Oncology, Hospital San Raffaele IRCSS, Milan, Italy (Monica Ronzoni, MD).

Department of Medical Oncology, General Hospital, Prato, Italy (Samantha Di Donato, MD).

Department of Clinical Medicine and Surgery, University Federico II, Napoli, Italy (Chiara Carlomagno, MD).

Medical Oncology Unit, Ente Ospedaliero Ospedali Galliera, Genoa, Italy (Matteo Clavarezza, MD).

SSD ColoRectal Cancer Unit, Department of Oncology, AOU Città della Salute e della Scienza di Torino, Torino, Italy (Giuliana Ritorto, MD).

Oncological Department, Azienda USL Toscana Nord Ovest, Oncological Unit of Massa Carrara, Carrara, Italy (Andrea Mambrini, MD).

Department of Systems Medicine, Medical Oncology Unit, Tor Vergata University, Rome, Italy (Mario Roselli, MD).

Department of Oncology, Division of Medical Oncology, Azienda Toscana Nord Ovest, Livorno, Italy (Samanta Cupini, MD).

Medical Oncology, IRCCS Ospedale San Martino IST, Genova, Italy (Serafina Mammoliti, MD).

Department of Medical Oncology, Candiolo Cancer Institute, FPO, IRCCS, Candiolo, Italy (Elisabetta Fenocchio, MD).

Unit of Medical Oncology, Ospedale Santa Maria della Misericordia, Perugia, Italy (Enrichetta Corgna, MD).

Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy (Gabriella Fontanini, MD, PhD).

Department of Laboratory, Pathology section, University Hospital of Pisa, Pisa, Italy (Clara Ugolini, MD).

Clinical Trials Coordinating Center, Toscano Cancer Institute, University Hospital Careggi, Firenze, Italy (Luca Boni, MD).

[§]Current affiliation: Department of Oncology and Palliative Care, Cardinale G. Panico, Tricase City Hospital, Tricase, Italy.

[#] Current affiliation: Department of Oncology, San Bortolo General Hospital, ULSS8 Berica, East District, Vicenza, Italy.

Correspondence to

Prof. Alfredo Falcone

Unit of Medical Oncology 2, Azienda Ospedaliera-Universitaria Pisana, Pisa, Italy

Via Roma, 67 56126 PISA, Italy

Phone: +39 050 993064; Fax: +39 050 992069

e-mail: alfredo.falcone@med.unipi.it

Summary

Background

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Methods

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Upfront FOLFOXIRI plus bevacizumab followed by the reintroduction of the same regimen in case of disease progression is the best therapeutic strategy for patients with metastatic colorectal cancer selected according to the study criteria and particularly for those with right-sided and/or a *RAS* or *BRAF* mutated tumours.

Funding

Supported by the GONO and the ARCO Foundations. A research grant was provided by F. Hoffmann–La Roche.

Introduction

Several options are currently available for the upfront treatment of metastatic colorectal cancer patients. Based on the results of the phase III TRIBE study[1,2] and of other phase II randomized trials conducted worldwide,[3-6] the combination of the three-drugs regimen FOLFOXIRI (fluorouracil, L-leucovorin, oxaliplatin, and irinotecan) with the antiangiogenic bevacizumab is now regarded as a valuable first-line option by major guidelines.[7,8]

In fact, the previous TRIBE study by GONO demonstrated significantly better progression-free survival (hazard ratio [HR] for progression: 0·77 (95% CI: 0·65-0·93); p=0·003), primary endpoint of the study, response rate (odds ratio [OR] for response: 1·59 [95% CI: 1·10-2·28]; p=0·006) and overall survival (HR for death: 0·80 (95% CI: 0·65-0·98); p=0·030) with the triplet FOLFOXIRI plus bevacizumab when compared with the doublet FOLFIRI (fluorouracil, L-leucovorin, irinotecan) plus bevacizumab, at the price of an increased incidence of specific grade 3 and 4 adverse events (diarrhoea, stomatitis, neutropenia).[1,2]

However, since in the TRIBE study treatments after progression were left at investigators' choice and collected as post-study treatments, the efficacy of the triplet when compared with the exposure to the same agents in a sequential strategy of less toxic doublets was not demonstrated. Furthermore, in spite of the significant benefit achieved in terms of overall survival with the intensified chemotherapy backbone, some concerns raised with regard to the feasibility and efficacy of treatments after progression following the upfront exposure to the three cytotoxics.

In the last years the role of the inhibition of angiogenesis as a therapeutic strategy in metastatic colorectal cancer was strengthened by important achievements in the field of maintenance and treatments after progression: following a 4-6 months first-line treatment with a combination chemotherapy regimen plus bevacizumab, maintenance with a

fluoropyrimidine plus bevacizumab until disease progression is recommended,[9-12] and the continuation of angiogenesis inhibition also beyond disease progression is a valuable option supported by evidence from phase III trials.[13,14]

From these considerations, the TRIBE2 study was conceived in order to verify whether the upfront exposure to the three cytotoxics in the FOLFOXIRI regimen was superior to a pre-planned sequence of doublets (first-line mFOLFOX6 [fluorouracil, L-leucovorin, oxaliplatin], followed by FOLFIRI after disease progression), in the frame of a sustained inhibition of angiogenesis with bevacizumab in both groups.

Methods

Study design and participants

TRIBE2 (First-line FOLFOXIRI plus bevacizumab followed by reintroduction of FOLFOXIRI plus bevacizumab at progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab at progression in first- and second-line treatment of unresectable metastatic colorectal cancer) was a prospective, open-label, multicentre, randomized phase III study that included patients with metastatic colorectal cancer recruited from 58 Italian Oncology Units. Main inclusion criteria were the following: histologically confirmed colorectal adenocarcinoma; age between 18 and 75 years; Eastern Cooperative Oncology Group performance status 0–2 if age ≤70 years, or 0 if age 71–75 years; unresectable and measurable metastatic disease according to Response Evaluation Criteria In Solid Tumours (RECIST) version 1.1;[15] adequate bone marrow, hepatic and renal function (neutrophils ≥1·5 × 10^9 cells per L, platelets ≥100 × 10^9 cells per L, and haemoglobin ≥90 g/L; serum bilirubin ≤1·5 times the upper limit of normal [ULN]; alanine aminotransferase and aspartate aminotransferase ≤2·5 × ULN or ≤5 × ULN in the presence of liver metastases; alkaline

phosphatase ≤2·5 × ULN or ≤5 × ULN in the presence of liver metastases; serum creatinine ≤1·5 × ULN or creatinine clearance >50 mL/min). Main exclusion criteria were: previous palliative chemotherapy or biologic therapy for metastatic disease; adjuvant treatment with oxaliplatin; adjuvant treatment with fluoropyrimidine monotherapy completed less than 6 months before relapse; peripheral neuropathy of grade 2 or higher according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0.[16] The study was conducted in accordance with the Declaration of Helsinki and adhered to Good Clinical Practice guidelines. Approval for the protocol was obtained from local ethics committees of participating sites. All patients provided written informed consent to study procedures before enrolment.

Randomisation and masking

Patients were randomly assigned in a 1:1 ratio to receive either first-line mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab after disease progression (control group) or first-line FOLFOXIRI plus bevacizumab followed by reintroduction of FOLFOXIRI plus bevacizumab after disease progression (experimental group). All combination treatments were administrated up to 8 cycles followed by fluorouracil/L-leucovorin plus bevacizumab maintenance until disease progression, unacceptable adverse events, or consent withdrawal.

Eligible patients were randomized using a centralized web-based system and stratified according to centre, ECOG performance status (0 *versus* 1–2), primary tumour location (right-sided *versus* left-sided or rectum) and previous exposure to an adjuvant treatment (yes *versus* no). The random allocation sequence was generated at the Clinical Trials Coordinating Center, Istituto Toscano Tumori (Florence, Italy). Treatment allocation was not masked.

Study treatments and procedures

Patients received first-line induction with mFOLFOX6 plus bevacizumab (control group), consisting of an intravenous infusion of 5 mg/kg of bevacizumab over 30 min, followed by a 85 mg/m² intravenous infusion of oxaliplatin given concurrently with L-leucovorin at a dose of 200 mg/m² over 120 min, followed by a 400 mg/m² intravenous bolus of fluorouracil, and a 2400 mg/m² continuous infusion of fluorouracil for 48 hours, starting on day 1; or FOLFOXIRI plus bevacizumab (experimental group), consisting of an intravenous infusion of 5 mg/kg of bevacizumab over 30 min, followed by a 165 mg/m² intravenous infusion of irinotecan over 60 min, followed by an 85 mg/m² intravenous infusion of oxaliplatin given concurrently with L-leucovorin at a dose of 200 mg/m² for 120 min, followed by a 3200 mg/m² continuous infusion of fluorouracil for 48 h, starting on day 1. Treatment cycles were repeated every 14 days for up to 8 cycles.

The use of granulocyte colony-stimulating factor was not recommended as primary prophylaxis.

In the case of pre-specified adverse events, treatment modifications were allowed according to study protocol.

Thereafter, maintenance treatment with fluorouracil/L-leucovorin and bevacizumab was planned in both groups at same dose used at the last cycle of the induction treatment, every 14 days, until progressive disease, patient's refusal, unacceptable adverse events or consent withdrawal.

At the first evidence of disease progression, patients enrolled in the control group received FOLFIRI plus bevacizumab (5 mg/kg intravenous infusion of bevacizumab for 30 minutes, followed by 180 mg/m² intravenous infusion of irinotecan for 120 min given concomitantly with a 200 mg/m² intravenous infusion of L-leucovorin, followed by a 400 mg/m² intravenous

bolus of fluorouracil, and a 2400 mg/m² continuous infusion of fluorouracil for 48 hours, starting on day 1), repeated every 14 days for a maximum of 8 cycles, then followed by fluorouracil/L-leucovorin and bevacizumab maintenance. Patients enrolled in the experimental group received the re-induction of FOLFOXIRI plus bevacizumab (according to the above described schedule) up to 8 cycles, followed by fluorouracil/L-leucovorin and bevacizumab as maintenance. If disease progression occurred during the first-line induction with FOLFOXIRI plus bevacizumab, a second-line treatment at investigator' s choice was allowed.

In the case of surgical radical resection of residual metastases, post-operative therapy with the same pre-operative regimen was planned up to an overall duration of 6 months (12 cycles), then followed by fluorouracil/L-leucovorin with bevacizumab up to 6 months after resection.

The assessment of response and progression was based on investigator-reported measurements, subsequently confirmed by a central review, and was performed according to RECIST 1.1 criteria with CT scans repeated every 8 weeks. [15]

At the start of every cycle, the patients' medical history, ECOG performance status, results of physical examination, and adverse events were recorded and graded according to the NCI-CTCAE version 4.0.[16]

RAS and BRAF status and microsatellite instability analyses

Data about *RAS* (codons 12, 13, 59, 61, 117 and 146 of *KRAS* and *NRAS*) and *BRAF* (V600E mutation) mutational status were collected based on the local assessment. Microsatellite instability was centrally analysed by means of immunohistochemistry as previously reported.[17-19]

Outcomes

To properly assess the efficacy of the whole first- and second-line strategy, the primary endpoint was progression-free survival 2, defined as the time from randomization to disease progression, according to RECIST version 1.1,[15] on any treatment given after first disease progression, or death from any cause. For patients who did not receive any treatment within 3 months after first disease progression, progression-free survival 2 was equal to 1st progression-free survival, defined as the time from randomization to the first evidence of disease progression, or death from any cause. Secondary endpoints included 1st progression-free survival, 2nd progression-free survival, defined as the time between the first and the second evidence of disease progression or death from any cause, safety, response rate, radical resection rate of metastases and overall survival.

Statistical analyses

To detect a hazard ratio (HR) for progression-free survival 2 of 0·77 (corresponding to an increase in the progression-free survival 2 rate at 15 months from 50% to 60%) in favour of the experimental group with an overall two-sided alpha error of 5% and an estimated power of 80%, we planned to enrol 654 patients in order to observe 466 events of progression-free survival 2 or death from any cause.

An interim analysis was planned to assess the superiority of the experimental group versus the control group for the primary endpoint when 2/3 of the expected progression-free survival 2 events had occurred (303 out of 466 events). According to the O'Brien Fleming spending rule, two-sided alpha levels of significance were set at 0·0131 and 0·0455 for the interim and final analysis, respectively.

All efficacy analyses were performed on an intention-to-treat basis. Safety, including summary of adverse events, was assessed in all enrolled patients who received at least one dose of study treatment (safety population). 2nd progression-free survival was assessed also in the per protocol population, including patients that received the treatment after progression planned according to the random assignment. The rate of adverse events was evaluated in the safety population, including patients who received at least one cycle of the study treatment. The median period of follow-up was calculated for the entire study cohort according to the reverse Kaplan-Meier method. Distributions of time-to-event variables for progression-free survival 2, 1st and 2nd progression-free survival, and overall survival were estimated with the use of the Kaplan-Meier product-limit method. Cox proportional-hazards modelling was also performed as supportive analyses. Subgroup analyses of progression-free survival 2 and 1st progression-free survival were performed by means of an interaction test to determine the consistency of the treatment effect according to key baseline characteristics. The objective response rate, the resection rate for metastases, and the rate of adverse events in the two groups were compared with the use of the chi-square test for heterogeneity or with Fisher's exact test when appropriate. All statistical tests were two-sided, and p values of 0·05 or less were deemed significant. No adjustments for multiple comparisons were performed.

Statistical analyses were done using SAS version 9.2.

The trial is registered with ClinicalTrials.gov, number NCT02339116.

Role of funding source

The Italian GONO Foundation sponsored the trial and GONO investigators were responsible for study design, data collection, data analysis, and data interpretation. The writing of the report and the decision to submit for publication was the responsibility of the GONO

Foundation. The no-profit ARCO Foundation supported molecular analyses, but had no role in study design, data collection, data analysis, data interpretation, or writing of the report. F. Hoffman-La Roche partially supported the trial with a research grant and providing bevacizumab for the whole study treatment of the experimental group and for the treatment beyond progression of the control group, but had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From February 26th, 2015 to May 15th, 2017, 679 patients with metastatic colorectal cancer were randomly assigned to the control (n=340) or the experimental group (n=339) (figure 1). Six hundred and seventy-two patients (336 per group) received at least one dose of study treatment and were included in the safety population. The cut-off date for the present analysis was July 30th, 2019.

Patients' demographic, clinical and molecular baseline characteristics were well balanced in the two groups (table 1). The median (interquartile range [IQR]) age of the study population was 61 (53-67) years and most patients (86%) had an ECOG Performance Status of 0. The 38% of patients had a right-sided primary tumour, 59% had multiple sites of metastases and 30% had liver-limited disease. *RAS* and *BRAF* mutations were found in the 64% and 10% of cases, respectively, and the 5% of patients had microsatellite instable (MSI-high) tumours. Overall, the 81% of enrolled patients had a right-sided and/or a *RAS* or *BRAF* mutated tumour.

At a median follow-up of 35·9 months (IQR 30·1-41·4), 546 (80%) events of progression-free survival 2 [286 (84%) in the control group and 260 (77%) in the experimental group] were

observed. Median progression-free survival 2 was 19·2 months (95% CI 17·3–21·4) in the experimental group and 16·4 months (95% CI 15·1–17·5) in the control group (HR 0·74, 95% CI 0·63–0·88; p<0·001; figure 2A). Treatment effect was consistent across all analysed clinical and molecular subgroups (figure 2B).

First-line disease progression occurred in 605 (89%) patients: 310 in the control group and 295 in the experimental group. Median 1st progression-free survival was 12·0 months (95% CI 11·1-12·9) in the experimental group receiving FOLFOXIRI plus bevacizumab, and 9·8 months (95% CI 9·0-10·5) in the control group receiving mFOLFOX6 plus bevacizumab (HR 0·74, 95% CI 0·63-0·86; p<0·001; figure 3A). Treatment effect was consistent across all analysed clinical and molecular subgroups (figure 3B). The response rate according to RECIST 1.1 was 62% (95% CI 57-67) in the experimental group as compared with 50% (95% CI 45-56) in the control group (odds ratio 1·61, 95% CI 1·19–2·18; p=0·002). The rate of R0 resection of metastases (*i.e.*, no macroscopic or microscopic residual tumour) was 17% in the experimental group and 12% in the control group (odds ratio 1·55, 95% CI 1·00-2·39; p=0·047). The incidence of grade 3 or 4 neutropenia, febrile neutropenia and diarrhoea was significantly higher in the experimental than in the control group (table 2).

Out of 570 patients still alive at the time of first disease progression (296 in the control group and 274 in the experimental group), 259 (88%) in the control group and 224 (82%) in the experimental group received a further treatment (figure 1; table S1, appendix). The 2nd progression-free survival analysis was based on 511 events (90%) – 272 (92%) in the control group and 239 (87%) in the experimental group. Median 2nd progression-free survival was 6·2 months (95% CI 5·6-6·6) in the experimental group and 5·6 months (95% CI 4·9–6·4) in the control group (HR 0·87, 95% CI 0·73–1·04; p=0·116; figure 4A).

Two-hundred and one patients (78%) in the control group and 132 (59%) in the experimental group received the treatment after progression planned according to the random assignment (FOLFIRI plus bevacizumab and FOLFOXIRI plus bevacizumab, respectively) and were included in the per protocol population (figure 1; table S1, appendix).

In the per protocol population, the 2nd progression-free survival was based on 186 (93%) events in the control group and 115 (87%) in the experimental group. Median 2nd progression-free survival was 6·5 months (95% CI 6·2–7·5) in the experimental group and 5·8 months (95% CI 4·9–6·5) in the control group (HR 0·79, 95% CI 0·63–1·00; p=0·049; figure 4B).

No significant differences in the incidence of grade 3 or 4 adverse events between FOLFIRI plus bevacizumab and FOLFOXIRI plus bevacizumab, given after disease progression, were observed, with the only exception of neurotoxicity, whose incidence was significantly higher in the experimental than in the control group (table 2).

The overall survival analysis was based on 459 events (68%) – 241 (71%) in the control group and 218 (64%) in the experimental group. Median overall survival was 27·4 months (95% CI 23·7-30·0) in the experimental group and 22·5 months (95% CI 20·7-24·8) in the control group (HR 0·82, 95% CI 0·68-0·98; p=0·032; figure 5).

Discussion

Our findings demonstrate the superiority of the upfront exposure to FOLFOXIRI plus bevacizumab followed by the re-induction with the same agents when compared with a pre-planned sequential strategy of administration of the three cytotoxics across two subsequent lines of therapy (mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab after disease progression) in the treatment of patients with metastatic colorectal cancer. Of note, the percentage of patients enrolled in the control group and actually exposed to the three

cytotoxics was as high as 88%, thus further strengthening the clinical significance of the advantage reported by the experimental group.

We provide a meaningful demonstration of the efficacy of FOLFOXIRI plus bevacizumab administered up to 8 cycles as first-line option for metastatic colorectal cancer patients, by corroborating results previously achieved in the TRIBE trial, where the treatment was planned up to 12 cycles.[1,2] Indeed, FOLFOXIRI plus bevacizumab was associated with statistically significant and clinically relevant improvements in terms of response rate, progression-free and overall survival in a population with initial poor prognostic features, thus showing the impact of the first-line regimen on the therapeutic route of patients with metastatic colorectal cancer, and particularly the high magnitude of the effect of the upfront intensified treatment on patients' long-term outcome. In fact, the 89% of patients included in the TRIBE2 study presented with synchronous metastases, the 38% had a right-sided primary tumour, the 59% had more than one metastatic site, and the 64% and 10% bore a *RAS* or *BRAF* mutated tumour, respectively. These poor prognostic features may explain the shorter duration of overall survival reported in both groups, when compared with results in the *RAS* wild-type population of other recent randomized trials.[20-24]

In terms of safety, the toxicity profiles of study regimens were consistent with the known adverse events of the individual drugs, and highly coherent with results from previous studies investigating the triplet plus bevacizumab.[1,3-6,25-29] The TRIBE2 study was conducted in 58 Italian sites, highlighting the large scale feasibility of the experimental strategy.

We also showed that treatments after progression to first-line FOLFOXIRI plus bevacizumab, then followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab, were feasible in the 82% of patients, and their efficacy was not affected by the upfront exposure to the three cytotoxics, as demonstrated by the absence of difference in terms of 2nd progression-

free survival between the two study groups. FOLFOXIRI plus bevacizumab was reintroduced after disease progression in the 59% of patients in the experimental group, and a per protocol analysis reported a significant advantage in terms of 2nd progression-free survival in these patients when compared with those who received FOLFIRI plus bevacizumab after progression to first-line mFOLFOX6 plus bevacizumab, with no increase in grade 3 or 4 adverse events except for an expected higher incidence of neuropathy. The relatively good tolerability is probably explained by a careful clinical selection of those patients able to receive FOLFOXIRI plus bevacizumab after progression, made by treating physicians on the basis of their previous tolerance to this regimen and of the health status of patients.

With regard to treatments after progression, a potential limitation of our study is the choice to switch to FOLFIRI after first-line mFOLFOX6 instead of re-introducing an oxaliplatin-based regimen. Even if this strategy was previously evaluated in clinical trials,[30,31] our choice was driven by the objective of exposing the highest percentage of patients to the three cytotoxics also in the control group. Moreover, by a pragmatic point of view, the switch to the alternate doublet is the most common approach in the daily clinical practice.

As shown by the subgroup analyses, no interaction was observed between treatment effect and *RAS* and *BRAF* mutational status, as in the previous TRIBE study. Nonetheless, based on the high magnitude of benefit reported in the small subgroup of patients with *BRAF* mutated tumours in the previous TRIBE,[1,2] FOLFOXIRI plus bevacizumab was identified as a preferable option in this subgroup. The evidence of no increased benefit from the intensified approach reported here may be explained by the molecular and clinical heterogeneity of *BRAF* mutated tumours, and the different comparator group (oxaliplatin- instead of irinotecan-based doublet).

Based on previous findings of the TRIBE study, a higher benefit from the experimental treatment could be expected among patients with a *BRAF* mutated tumour. However, also in the TRIBE study no significant interaction effect between treatment group and *RAS* or *BRAF* mutational status was described, thus dictating a cautious interpretation of results achieved in small subgroups.

In order to properly translate our study in the current landscape of the first-line treatment of metastatic colorectal cancer, it should be acknowledged that the vast majority (81%) of enrolled patients had a right-sided and/or a *RAS* or *BRAF* mutated tumour, while only a minority (16%) of them had a left-sided and *RAS* and *BRAF* wild-type tumour. This might be explained by the increased use of chemotherapy plus an anti-Epidermal Growth Factor Receptor (EGFR) monoclonal antibody as first-line treatment of patients with *RAS* and *BRAF* wild-type tumours during the accrual of the TRIBE2 study. As a consequence, the optimal candidates to first-line doublets plus anti-EGFR are under-represented in the present study and the combination of an anti-EGFR with chemotherapy remains a preferred option for these patients. On the other side, a relevant magnitude of benefit was reported among patients with a right-sided and/or a *RAS* or *BRAF* mutated tumour, thus making upfront FOLFOXIRI plus bevacizumab the best first-line option for patients in this subgroup.

Contributors

CCr, CA, DR, SL, FL, FP, RB, TPL, ET, DS, AP, FM, RG,GA, AZ, SMu, CG, AB, RM, SC, SCo, LA, GT, GM, MR, SDD, CCa, MC, GR, AM, MR, SCu, SMa, EF, EC, VZ and AF collected data and recruited patients. CCr, CA, DR, LB, and AF analysed and interpreted the data. CCr, CA, DR, FM, RM, GM, LB and AF wrote the manuscript. CCr, LB, FL and AF designed the study. CU and GF performed molecular analyses. All authors revised and approved the manuscript.

Declarations of interest

CCr received personal fees from Roche, Amgen, Bayer, Servier, research funding from Merck Serono, and has a consulting or advisory role with Roche, Bayer, Amgen. DR received personal fees from Takeda.

SL received personal fees from Roche, Lilly, Bristol-Myers Squibb, Servier, Merck Serono, research funding from Amgen, Merck Serono, and has a consulting or advisory role with Amgen, Merck Serono, Lilly, Servier. FL received personal fees from Roche, Sanofi, Bayer, Amgen, research funding from Roche, Merck Serono, Amgen, Bayer, and has a consulting or advisory role with Amgen, Sanofi, Bayer, Amal. FP received personal fees from Amgen, Merck Serono, Roche, Sanofi, Bayer, Servier, Lilly, research funding from Bristol-Myers Squibb, and has a consulting or advisory role with Amgen, Merck Serono, Bayer, Lilly, Sanofi, Roche, Servier. RB received personal fees from and has a consulting or advisory role with Bayer, AstraZeneca, Sanofi, Novartis, Amgen, Roche, Pfizer, Jansen, Bristol-Myers Squibb. GA received personal fees from and has consulting or advisory role with Merck Serono, Amgen, Roche, Servier. AZ received personal fees from and has consulting or advisory role with Amgen, Bayer, Lilly, Merck Serono, Servier, Sanofi. SCo received personal fees from Roche, Sanofi, Servier, Merck Serono, Lilly, research funding from Roche, Merck Serono, Amgen, Servier, Lilly, has a consulting or advisory role with Amgen, Sanofi, Bayer, Servier, Merck Serono, Ipsen. VZ received personal fees from Bayer, Roche, Bristol-Myers Squibb, Astellas Pharma, Servier, AstraZeneca, Lilly,

research funding from Bayer (Inst), Roche (Inst), Lilly (Inst), Bristol-Myers Squibb (Inst), Ipsen (inst), Astellas Pharma (Inst) has a consulting or advisory role with Bristol-Myers Squibb, Merck Serono. AF received personal fees from Roche, Amgen, Merck Serono, Celgene, Bayer, Sanofi, research funding from Roche (Inst), Amgen (Inst), Merck Serono (inst). CA, TPL, ET, DS, AP, FM, RG, SMu, CG, AB, RM, SC, LA, GT, GM, MR, SDD, CCa, MC, GR, AM, MR, SCu, SMa, EF, EC, GF, CU, LB report no competing interests.

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Data sharing

Qualified researchers may request to GONO Foundation access to individual patient-level data. Data will be provided with an accompanying dictionary defining each field in the set and clinical study documentation. The study protocol is already available online at http://fondazionearco.org/studio-tribe-2/. Further details on GONO Foundation global policy on sharing of clinical information will be available by emailing to info@gonogroup.org or to the corresponding author.

Panel: Research in context

Evidence before study

A previous phase 3 trial (TRIBE study) by the Italian GONO Foundation proved the superiority of the first-line triplet regimen FOLFOXIRI (fluorouracil, L-leucovorin, oxaliplatin, and irinotecan) over the doublet FOLFIRI (fluorouracil, L-leucovorin, and irinotecan) when bevacizumab was added to both regimens in patients with unresectable metastatic colorectal cancer. Based on these results, FOLFOXIRI plus bevacizumab is supported by all major clinical guidelines as a valuable first-line option for metastatic colorectal cancer patients, selected according to the pivotal TRIBE study criteria. However, some concerns raised about the use of FOLFOXIRI in the daily practice, including the actual benefit of the exposure to all the three cytotoxics as compared with the pre-planned sequential administration of the same drugs in oxaliplatin- and irinotecan-based doublets, and the feasibility and the efficacy of treatments after progression.

We searched Pubmed on July 30th, 2019, for the terms “FOLFOXIRI”, “triplet”, “doublets”, “FOLFOX”, “XELOX”, “FOLFIRI”, “XELIRI”, “bevacizumab”, “reintroduction”, “second-line”, “strategy trial”. We found only a few reports that retrospectively described a favourable outcome of second-line therapies, including the reintroduction of the triplet, given after failure of first-line FOLFOXIRI in non-randomly assigned subgroups, and no trials that prospectively compared the efficacy of the upfront use of FOLFOXIRI versus a standard sequential strategy of oxaliplatin- and irinotecan-based doublets.

Herein, we report results of the phase III TRIBE2 study, designed with the purpose to investigate whether the upfront use of FOLFOXIRI improves the clinical outcome of unresectable metastatic colorectal cancer patients, when compared with the pre-planned, sequential use of mFOLFOX6 and FOLFIRI. In both strategies bevacizumab is added upfront

and after progression, to exploit the effectiveness of a prolonged inhibition of angiogenesis, alternating short (up to 4 months) induction periods and less intensive maintenance phases.

Added value of this study

Current data provide additional evidence of the impact of the upfront use of FOLFOXIRI plus bevacizumab on the survival of unresectable metastatic colorectal cancer patients, demonstrating its superiority when compared with a sequential strategy of doublets plus bevacizumab. The efficacy of treatments after progression to FOLFOXIRI plus bevacizumab is clearly shown, and the beneficial effect of the reintroduction of the triplet in selected patients is suggested for the first time.

Implications of all the available evidence

Based on these results upfront FOLFOXIRI plus bevacizumab followed by the reintroduction of the same regimen in case of disease progression is the best therapeutic option for metastatic colorectal cancer patients who meet the study inclusion criteria and, in particular, for those with a right-sided and/or a *RAS* or *BRAF* mutated tumour.

Tables and Figures

Table 1. Baseline characteristics of patients in the intention to treat population.

Characteristic	Control group (n= 340)	Experimental group (n= 339)
Age (years)	61 (52–67)	60 (53–67)
Sex		
Male	206 (61%)	181 (53%)
Female	134 (39%)	158 (47%)
ECOG Performance Status		
0	289 (85%)	293 (86%)
1-2	51 (15%)	46 (14%)
Time to Metastases		
Synchronous	302 (89%)	302 (89%)
Metachronous	38 (11%)	37 (11%)
Prior Adjuvant chemotherapy		
No	332 (98%)	332 (98%)
Yes	8 (2%)	7 (2%)
Primary Tumour Site		
Right	129 (38%)	130 (38%)
Left or rectum	211 (62%)	209 (62%)
Number of Metastatic Sites		
1	127 (37%)	151 (45%)
>1	213 (63%)	186 (55%)
Missing data	-	2 (<1%)
Liver-Only Disease		
Yes	95 (28%)	106 (31%)
No	245 (72%)	231 (68%)
Missing data	-	2 (<1%)
Surgery on Primary Tumour		
Yes	179 (53%)	167 (49%)
No	161 (47%)	172 (51%)
RAS and *BRAF* status		
RAS and *BRAF* wild-type	70 (20%)	74 (22%)
RAS mutated	221 (65%)	215 (63%)
BRAF mutated	33 (10%)	33 (10%)
Missing data	16 (5%)	17 (5%)
Microsatellite status		
MSS/MSI-low/proficient MMR	262 (77%)	266 (79%)
MSI-high/deficient MMR	12 (4%)	14 (4%)
Missing data	66 (19%)	59 (17%)
Primary tumour site and *RAS* and *BRAF* status		
Right and/or *RAS* or *BRAF* mutated	273 (80%)	275 (81%)
Left and *RAS* and *BRAF* wild-type	53 (16%)	56 (17%)
Missing data	14 (4%)	8 (2%)

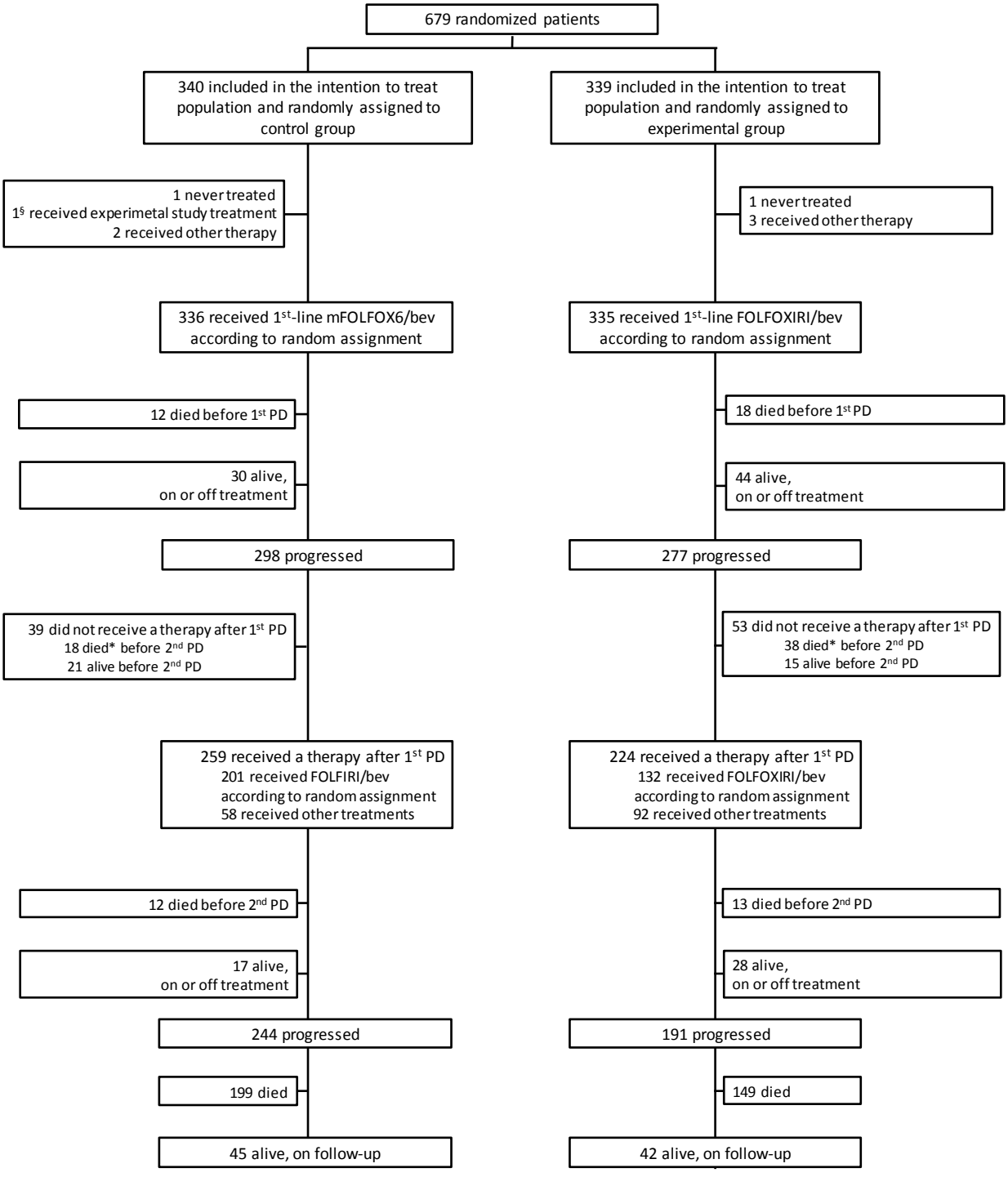
Data are median (IQR) or number (%). ECOG, Eastern Cooperative Oncology Group; MMR, mismatch repair; MSS: microsatellite stable; MSI-low, low microsatellite instability; MSI-high, high microsatellite instability. Control group indicates mFOLFOX6 plus bevacizumab, followed after disease progression by FOLFIRI plus bevacizumab. Experimental group indicates FOLFOXIRI plus bevacizumab, followed after disease progression by FOLFOXIRI plus bevacizumab.

Table 2. Most common grade ≥3 adverse events occurring during first-line in the safety population and during therapy administered after disease progression in the per protocol population, according to treatment group.

AEs, No (%)	First-line therapy			Treatment after disease progression as per protocol		
	Control group (n= 336) No (%)	Experimental group (n= 336) No (%)	p value	Control group (n= 201) No (%)	Experimental group (n= 132) No (%)	p value
Nausea	11 (3%)	20 (6%)	0·140	6 (3%)	8 (6%)	0·263
Vomiting	5 (2%)	9 (3%)	0·419	4 (2%)	4 (3%)	0·717
Diarrhoea	18 (5%)	57 (17%)	<0·001	12 (6%)	13 (10%)	0·207
Stomatitis	9 (3%)	15 (5%)	0·299	7 (3%)	6 (5%)	0·774
Neutropenia	71 (21%)	167 (50%)	<0·001	49 (24%)	34 (26%)	0·800
Febrile neutropenia	10 (3%)	22 (7%)	0·045	3 (1%)	4 (3%)	0·442
Neurotoxicity	3 (1%)	6 (2%)	0·505	0	6 (5%)	0·004
Asthenia	19 (6%)	23 (7%)	0·633	12 (6%)	10 (8%)	0·653
Anorexia	6 (2%)	4 (1%)	0·545	2 (1%)	2 (2%)	0·650
Arterial hypertension	34 (10%)	25 (7%)	0·223	4 (2%)	4 (3%)	0·717
Venous thromboembolism	19 (6%)	12 (4%)	0·204	2 (1%)	1 (1%)	1·000

Data are number (%). AEs: adverse events. First-line therapy: control group indicates induction with mFOLFOX6 plus bevacizumab, followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab; experimental group indicates induction with FOLFOXIRI plus bevacizumab, followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab. Therapy administered per protocol after disease progression: control group indicates induction with FOLFIRI plus bevacizumab, followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab; experimental group indicates induction with FOLFOXIRI plus bevacizumab, followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab.
The incidence of adverse events in the two treatment groups was compared with two-sided Fisher's exact test.

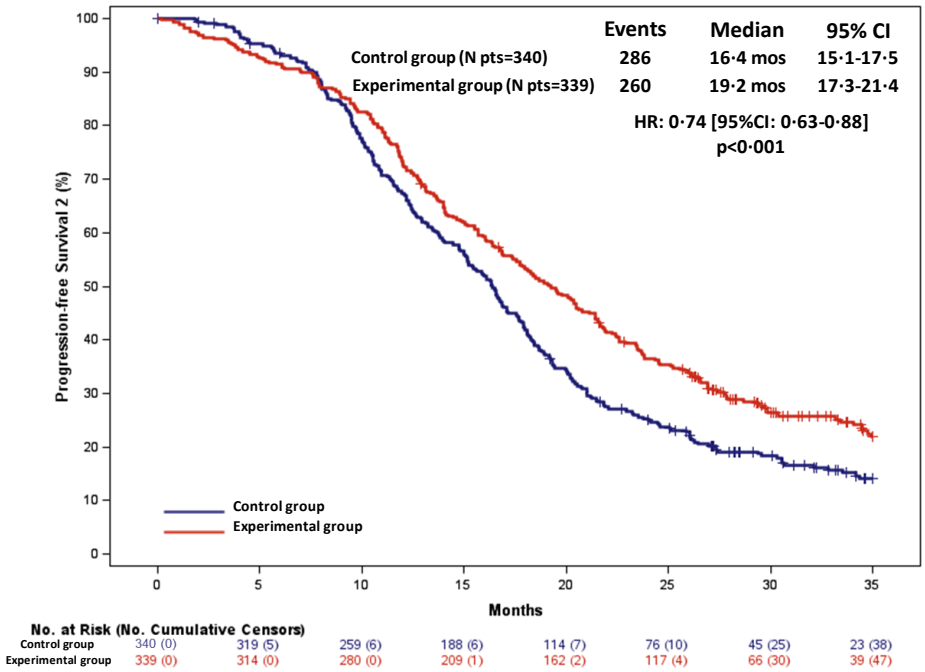
Figure 1. TRIBE2 study consort diagram.



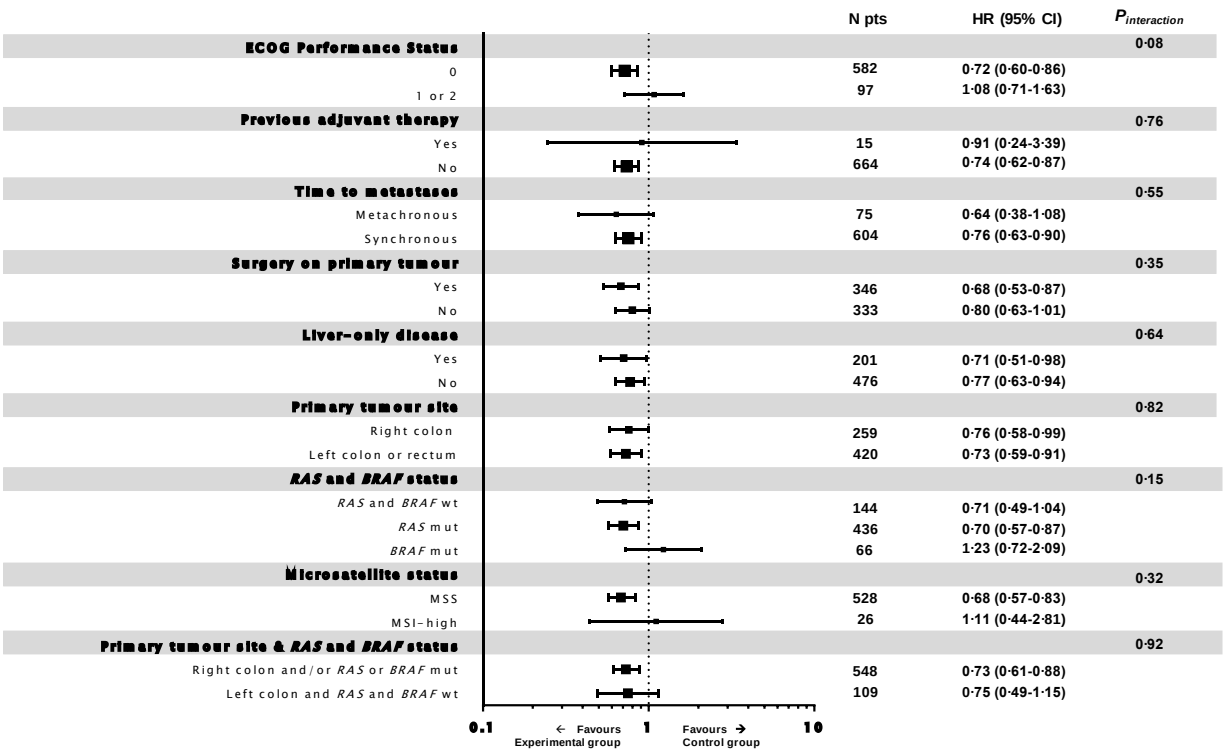
§ One patient allocated to control group received the experimental study treatment and was included in the experimental group in the safety population; *two patients in the control group and three patients in the experimental group died the same day of disease progression and were not included in the population for the analysis of 2nd progression-free survival. Control group indicates mFOLFOX6 plus bevacizumab, followed after disease progression by FOLFIRI plus bevacizumab. Experimental group indicates FOLFOXIRI plus bevacizumab, followed after disease progression by FOLFOXIRI plus bevacizumab. FOLFIRI: fluorouracil, L-leucovorin, and irinotecan; FOLFOXIRI: fluorouracil, L-leucovorin, oxaliplatin and irinotecan; FOLFOX: fluorouracil, L-leucovorin, and oxaliplatin; bev: bevacizumab; PD, progressive disease.

Figure 2. Progression-free survival 2.

A.



B.

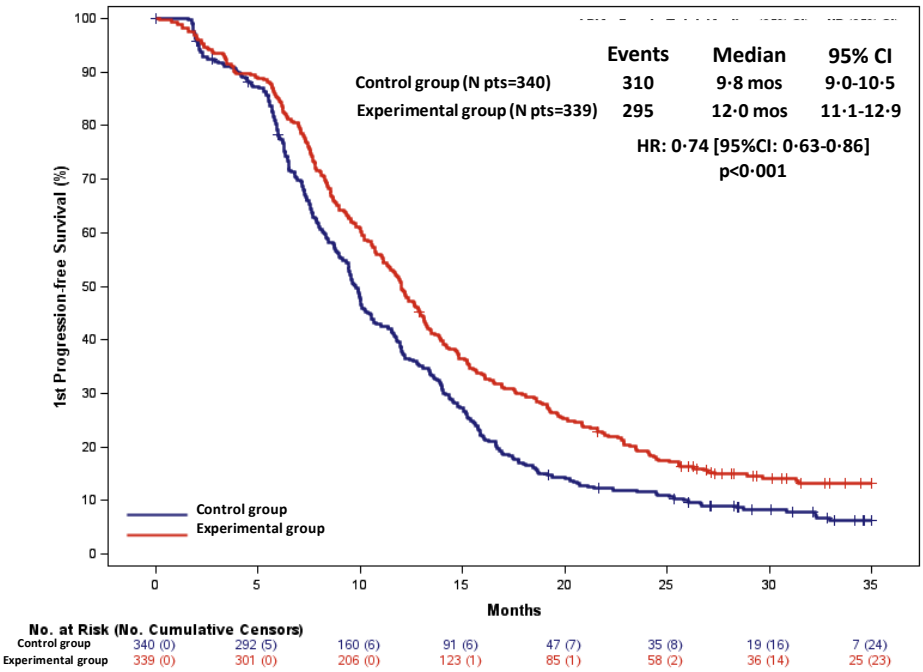


Kaplan Meier estimates of progression-free survival 2 in the intention to treat population, according to treatment group (A). Subgroup analyses of progression-free survival 2 according to clinical and molecular characteristics (B).

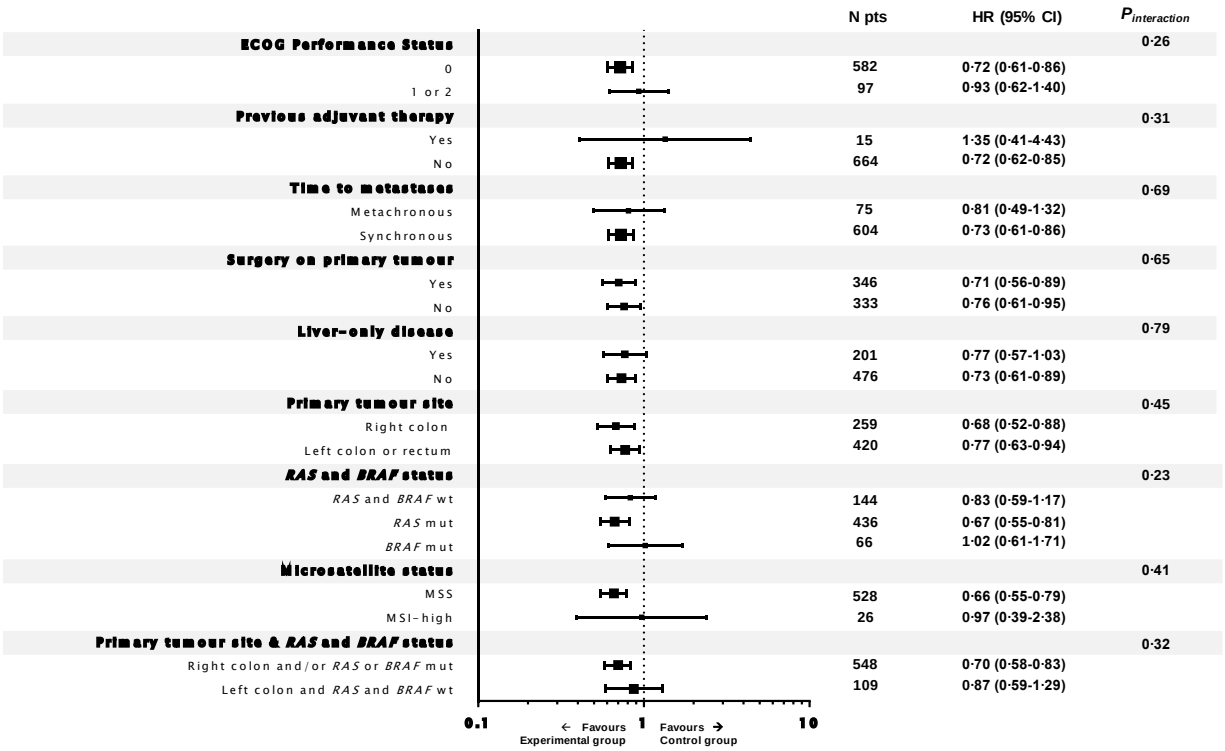
CI, confidence interval; HR, hazard ratio; mos, months; ECOG PS, Eastern Cooperative Oncology Group Performance Status; mut, mutated; wt, wild-type; MSS, microsatellite stable; MSI-high, high microsatellite instability. Control group indicates mFOLFOX6 plus bevacizumab, followed after disease progression by FOLFIRI plus bevacizumab. Experimental group indicates FOLFOXIRI plus bevacizumab, followed after disease progression by FOLFOXIRI plus bevacizumab.

Figure 3. First progression-free survival.

A.



B.



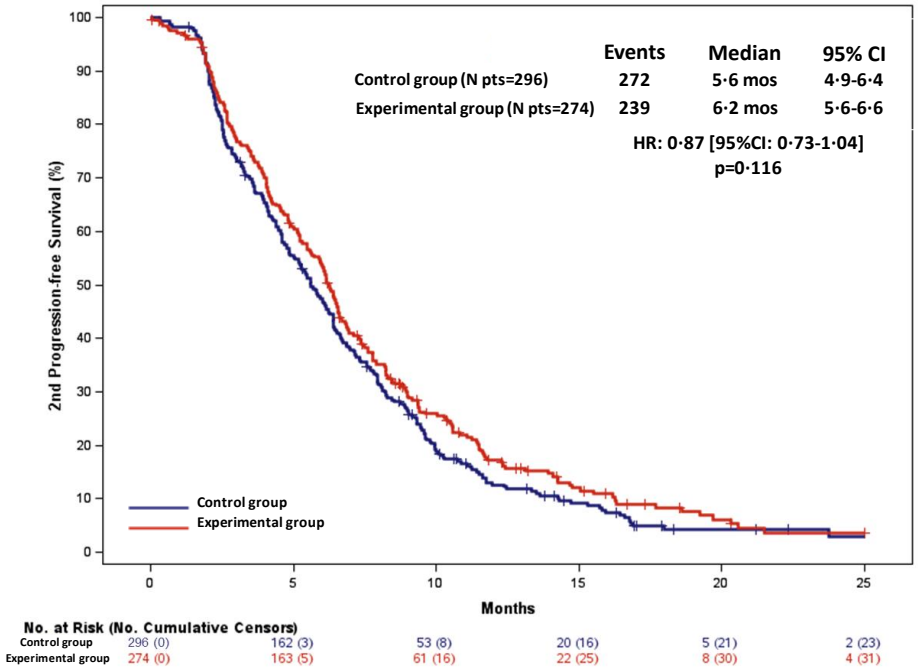
Kaplan Meier estimates of 1st progression-free survival in the in the intention to treat population, according to treatment group (A). Subgroup analyses of 1st progression-free survival according to clinical and molecular characteristics (B).

CI, confidence interval; HR, hazard ratio; mos, months; ECOG PS, Eastern Cooperative Oncology Group Performance Status; mut, mutated; wt, wild-type; MSS, microsatellite stable; MSI-high, high microsatellite instability. Control group indicates first-line induction with mFOLFOX6 plus bevacizumab, followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab.

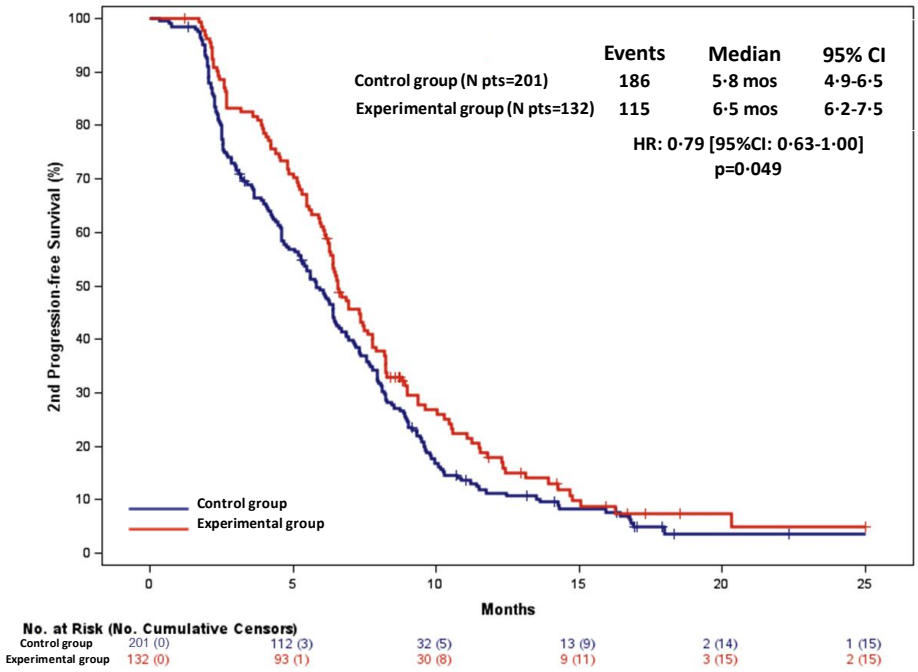
Experimental group indicates first-line induction with FOLFOXIRI plus bevacizumab, followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab.

Figure 4. Second progression-free survival.

A.



B.

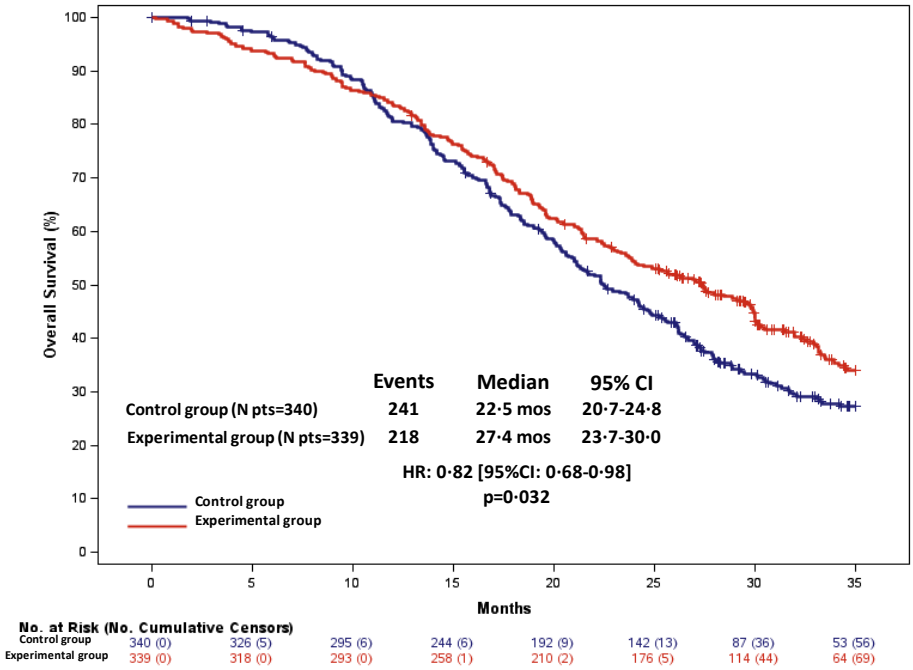


Kaplan Meier estimates of 2nd progression-free survival in the intention to treat population (*i.e.*, patients alive at the time of first-line disease progression), according to treatment group (A). Kaplan Meier estimates of 2nd progression-free survival in the per protocol population, according to treatment group (B).

CI, confidence interval; HR, hazard ratio; mos, months. Panel A. Control group indicates patients candidate to receive after disease progression FOLFIRI plus bevacizumab, according to random assignment. Experimental group indicates patients candidate to receive after disease progression FOLFOXIRI plus bevacizumab, according to random assignment. Panel B. Control group indicates patients who actually received after disease progression induction with FOLFIRI plus bevacizumab,

followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab, as per random assignment. Experimental group indicates patients who actually received after disease progression induction with FOLFOXIRI plus bevacizumab, followed by maintenance with fluorouracil/L-leucovorin plus bevacizumab, as per random assignment.

Figure 5. Overall survival.



Kaplan Meier estimates of overall survival in the intention to treat population, according to treatment group.

CI, confidence interval; HR, hazard ratio; mos, months. Control group indicates mFOLFOX6 plus bevacizumab, followed after disease progression by FOLFIRI plus bevacizumab. Experimental group indicates FOLFOXIRI plus bevacizumab, followed after disease progression by FOLFOXIRI plus bevacizumab.

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Supplementary Material

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G.O.N.O.

GRUPPO ONCOLOGICO NORD-OVEST

FIRST-LINE FOLFOXIRI PLUS BEVACIZUMAB FOLLOWED BY REINTRODUCTION OF FOLFOXIRI PLUS BEVACIZUMAB AT PROGRESSION *versus* FOLFOX PLUS BEVACIZUMAB FOLLOWED BY FOLFIRI PLUS BEVACIZUMAB AT PROGRESSION IN FIRST- AND SECOND-LINE TREATMENT OF UNRESECTABLE METASTATIC COLORECTAL CANCER

THE TRIBE-2 PHASE III STUDY

EUDRACT 2014-004436-19

Protocol Version 2.1 – September 2nd, 2014

STUDY STAFF

Principal Investigator

Prof. Alfredo Falcone	U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana Dipartimento di Ricerca Traslazionale e Nuove Tecnologie – Università di Pisa Istituto Toscano Tumori	Tel: +39.050.992466 Fax: +39.050.992069 alfredo.falcone@med.unipi.it

Principal Co-investigators and Protocol Writers

Dr. Fotios Loupakis	U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana Dipartimento di Ricerca Traslazionale e Nuove Tecnologie – Università di Pisa Istituto Toscano Tumori	Tel: +39.050.992466 Fax: +39.050.992069 fotiosloupakis@gmail.com
Dr.ssa Chiara Cremolini	U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana Dipartimento di Ricerca Traslazionale e Nuove Tecnologie – Università di Pisa Istituto Toscano Tumori	Tel: +39.050.992466 Fax: +39.050.992069 chiaracremolini@gmail.com

Clinical Trial Administration and Data Management

Dr. Luca Boni	Centro Coordinamento Sperimentazioni Cliniche – Istituto Toscano Tumori	Tel: +39.055.7947553 Fax: +39.055.7947553 luca.boni@ittumori.it
Dr.ssa Laura Delliponti	U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana Dipartimento di Ricerca Traslazionale e Nuove Tecnologie – Università di Pisa Istituto Toscano Tumori	Tel: +39.050.992192 Fax: +39.050.992069 laura.delliponti@gmail.com

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LIST OF ABBREVIATIONS AND ACRONYMS

5-FU: 5-fluorouracil
ACE: Angiotensin-Converting-Enzyme
ADR: Adverse Drug Reaction
AE: Adverse Event
AESI: Adverse Events of Special Interest
ALAT (SGPT): Alanine-Aminotransferase (Sèrum Glutamic Pyruvic Transaminase)
APTT: Activated Partial Thromboplastin Time
ASAT (SGOT): Aspartate-Aminotransferase (Sèrum Glutamic Oxaloacetic Transaminase)
Bas: At baseline
Bev: bevacizumab
CA19.9: Carbohydrate Antigen 19.9
CAPOX: Capecitabine, Oxaliplatin
CEA: CarcinoEmbryonic Antigen
CHF: Congestive Heart Failure
CNS: Central Nervous System
CR: Complete Response
CT: Computed Tomography
CTCAE:Common Terminology Criteria for Adverse Events
CVAD: Central Venous Access Device
DPYD: Dihydropyrimidine dehydrogenase
e.g.: Example given
EC: Ethics Committee
ECG: Electrocardiography
ECOG PS: Eastern Cooperative Oncology Group – Performance Status
e-CRF: electronic Case Report Form
EDTA: Ethylenediaminetetraacetic acid
EGFR: Epidermial Growth Factor Receptor
ELISA: Enzyme Linked Immunosorbent Assay
EOR: Early Objective Response
ERCC1: Excision Repair Cross-Complementation group 1
FOLFIRI: folinic-acid, 5-Fluorouracil, irinotecan
FOLFOX: folinic-acid, 5-Fluorouracil, oxaliplatin
FOLFOXIRI: folinic-acid, 5-Fluorouracil, oxaliplatin, irinotecan
G-CSF: Granulocyte – Colony Stimulating Factor
GI: Gastrointestinal
GONO: Gruppo Oncologico Nord-Ovest
GISCAD: Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente
HR: Hazard Ratio
ICH: International Conference on Harmonisation
INR: International Normalized Ratio
ISBN: International Standard Book Number
ITT: Intension To Threat

LDH: Lactate Dehydrogenase
LOHP: oxaliplatin
LV: leucovorin
mCRC: metastatic colorectal cancer
MRI: Magnetic Resonance Imaging
NA: Not Available
NCI CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events
NCIC-CTG: National Cancer Institute of Canada – Clinical Trials Group
NYHA: New York Heart Association
ORR: Overall Response Rate
OS: overall survival
pCR: pathologic Complete Response
PD: Progression Disease
PDGF: Platelet-derived Growth Factor
PFS: Progression Free Survival
PlGF: Placental Growth Factor
PR: Partial Response
PRES/RPLS: Posterior Reversible Encephalopaty Syndrome/ Reversible Posterior Leukoencephalopathy Syndrome
RECIST: Response Evaluation Criteria In Solid Tumors
RR: response rate
SADR: Serious Adverse Drug Reaction
SAE: Serious Adverse Event
SBP: Survival Beyond Progression
SC: Subcutaneously
SNP: Single Nucleotide Polymorphism
SP: Safety Population
sVEGFRs: soluble Vascular Endothelial Growth Factor Receptor 2
TE: Tracheoesophageal
TFS: Time to Failure of Strategy
TP: Thymidylate Phosphorylase
TS: Thymidylate Synthase
TTP: Time To Progression
UGT1A: UDP glucuronosyltransferase 1 A
ULN: Upper Limit of Normal
UNL: Upper-Normal Limits
VEGF: Vascular Endothelial Growth Factor
VEGFRs: VEGF receptors
WBC: White Blood Cell
Wks: weeks
XPD: Xeroderma Pigmentosum D

1. INTRODUCTION

1.1. FOLFOXIRI plus bevacizumab as first-line treatment of unresectable mCRC

A growing amount of drugs is indicated for the first-line treatment of mCRC and, in the absence of contraindications, the association of a biologic agent to a chemotherapy backbone is a standard choice as a first-line regimen. The intensity of the upfront chemotherapy is a highly debated issue and international guidelines [1,2] include one- to three-drugs regimens as possible options according to the treatment's objective (conversion vs palliative intent), disease's characteristics (indolent vs aggressive behaviour, tumor load) and patient's general conditions and comorbidities. Not only the three conventional cytotoxics (fluoropyrimidines, oxaliplatin, irinotecan), but also three targeted agents (the anti- Vascular Endothelial Growth Factor (VEGF), bevacizumab (bev) and the anti-Epidermal Growth Factor (EGFR) monoclonal antibodies, cetuximab and panitumumab) can be used in the first-line setting. Phase III randomized trials demonstrate that the addition of the antiangiogenic bev to first-line fluoropyrimidine-based monochemotherapy [3-5] as well as to oxaliplatin- [6] or irinotecan-based doublets [7] provided a significant benefit in terms of survival. Metanalyses estimating the magnitude of this benefit consistently show a reduction of the risk of death around 20% [8-10].

Bev safety profile is now well-known and easily manageable. Phase IV BEAT, BRiTE and ARIES trials included more than 5000 patients treated in the daily practice with chemotherapy plus bev and indicate that the incidence of bev-related adverse events is quite low and includes bleeding (3%), gastrointestinal perforation (1-2%), arterial thromboembolism (1-2%), hypertension (5-8%), proteinuria (1%) and wound-healing complications (1-2%) [11-13].

More recently, a phase II trial by the G.O.N.O. group evaluated the combination of bev with the three-drugs regimen FOLFOXIRI (CPT-11 165 mg/sqm d1, LOHP 85 mg/sqm d1, LV 200 mg/sqm d1 and 5-FU 3200 mg/sqm infusion over 48h). Cycles were repeated every 2 weeks, for a total of 12 cycles, followed by a maintenance treatment with 5-FU/LV and bev. According to a Phase II single-stage Fleming design, assuming

a null hypothesis of 10 months-progression free rate (10m-PFR) of 50% and an alternative hypothesis of 10m-PFR of 70%, with alpha and beta-errors of 0.05 and 0.10, the experimental treatment would have been judged to be promising if at least 33 patients, out of 53 evaluable, had been free of progression at 10 months.

At a median follow-up of 28.8 months, 42 (74%) out of 57 treated patients were actually free of progression at 10 months, with a median PFS of 13.1 months and a median OS of 30.9 months. In terms of activity, promising results were reported, with a RR of 77% and a disease control rate of 100%. Such a considerable activity translated into a radical resection rate of 26%, rising to 40% among patients with liver-only metastases. A pCR was observed in 20% of patients who underwent radical resection. The safety profile was absolutely consistent with expected toxicities and no unforeseen adverse events were reported [14].

Based on these promising findings the phase III TRIBE trial was designed. Five-hundred-eight unresectable mCRC patients were randomly assigned to receive up to 12 cycles of FOLFOXIRI plus bev or FOLFIRI plus bev, both followed by 5FU/LV plus bev until disease progression. Primary endpoint was PFS. Patients treated with FOLFOXIRI plus bev achieved a significantly longer PFS (12.1 vs 9.7 months, stratified HR: 0.75 [0.62-0.90], p=0.003) and a higher response rate (65% vs 53%, p=0.006). No significant differences in terms of secondary resection rate with radical intent were observed (26% vs 21%, p=0.327). A preliminary analysis, at a median follow up of 32.2 months, evidenced a trend toward longer OS in the experimental arm (31.0 vs 25.8 months, stratified HR: 0.79 [0.63-1.00], p=0.054).

The safety profile was consistent with results from the previous phase III trial by the G.O.N.O. group of FOLFOXIRI vs FOLFIRI. The triplet was associated with increased grade 3/4 neutropenia (50% vs 20%), diarrhea (19% vs 11%) and stomatitis (9% vs 4%) but not with higher incidence of febrile neutropenia (9% vs 6%). Bev-related adverse events were in the expected range. The incidence of serious adverse events (20.4% vs 19.7%) and treatment-related deaths (2.4% vs 1.6%) was not significantly different between treatment arms [15].

Previous impressive results achieved by the triplet FOLFOXIRI in terms of activity and secondary resections led to consider such an intensive upfront regimen as a preferable choice also when a remarkable tumor shrinkage is needed. Indeed, this suggestion has been recently confirmed by phase II OLIVIA trial [16], that randomized 80 mCRC patients with liver-only metastases, defined as initially unresectable by a multidisciplinary team, to receive FOLFOX plus Bev or FOLFOXIRI plus Bev. Overall (R0/R1/R2) resection rate, the primary endpoint, was numerically higher in the FOLFOXIRI plus bev arm (61.0% vs 48.7%, p=0.271). The triplet plus bev allowed to achieve an higher R0 resection rate (48.8% vs 23.1%, p=0.017) and an impressively higher ORR (80.5% vs 61.5%, p=0.061), with a substantial benefit also in terms of PFS (18.8 vs 12.0 months, p=0.0002).

1.2. Continuation of bevacizumab beyond progression in mCRC

More than ten years ago, preclinical experiences suggested the potential efficacy of a sustained antiangiogenic strategy beyond the first occurrence of resistance. Results from the observational studies BRiTE and ARIES provided initial clinical data in support of this hypothesis. In particular, in the large US prospective observational cohort study BRiTE 642 (44.4%) out of 1445 patients who had experienced progressive disease, received bev beyond progression, while 531 (36.7%) received no bev beyond progression [12]. A significant advantage in terms of survival beyond first progression (SBP) was noted with this strategy, that was still significant after adjusting for other prognostic factors (HR:0.49 [0.41-0.58], p<0.001). Similar results were provided by the ARIES observational study. Among 539 out of 1097 patients who received bev beyond progression significantly longer SBP was observed, compared to 417 patients who did not. Results provided by the multivariate model were consistent with those from BRiTE trial (HR: 0.41 [0.34-0.49], p<0.001) [17].

More recently, a phase III trial, named TML (Treatment across Multiple Lines - ML18147) was conducted in Europe and Saudi Arabia, randomizing mCRC patients previously treated with bev plus standard first-line chemotherapy to cross-over

chemotherapy with or without bev. Enrolled patients had experienced progressive disease less than 4 weeks prior to start of study treatment. Primary endpoint was OS. The use of bev beyond progression provided a significant advantage in terms of OS (11.2 vs 9.8 months, HR: 0.81 [0.69-0.94], p=0.0062) and PFS (5.7 vs 4.1 months, HR: 0.68 [0.59-0.78], p<0.0001), while no differences in response rate were reported (5.4% vs 3.9%, p=0.311). Adverse events were consistent with the expected toxicity profile of bev. As expected, the advantage provided by the addition of bev was independent of the *KRAS* mutational status [18-19].

Another phase III study with a similar design, the BEBYP (Bevacizumab BeYond Progression) trial, was contemporaneously conducted in Italy and prematurely stopped when results from TML were released. Primary endpoint was PFS. The continuation of bev beyond progression provided a significant advantage in terms of PFS (6.8 vs 5.0 months, HR: 0.72 [0.54-0.97], p=0.0029), while no differences in response rate (21% vs 18%, p=0.71) or OS (14.1 vs 15.5 months, HR: 0.77 [0.56-1.07], p=0.12) were reported. Nevertheless, the trial was clearly underpowered to detect an advantage in terms of survival [20].

Consistent results from both trials demonstrated the efficacy of a prolonged antiangiogenic strategy and identified the prosecution of bev in combination with a switched chemotherapy as a reasonable option for the second-line treatment of mCRC patients who have already received a bev-containing first-line regimen.

1.3. Induction and maintenance phases in the era of targeted agents

Recent evidences point out the correlation of the early objective response with survival in mCRC, thus highlighting the potential influence of the early tumor shrinkage on the subsequent steps of disease history. These findings also underscore the importance of achieving a relevant tumor shrinkage early after an intensive upfront treatment.

At the same time, the optimal duration of chemotherapy and Bev is still a matter of debate and some trials indicate that the possibility to alternate on-chemo and chemo-

free intervals is a reasonable option. Phase III randomized OPTIMOX1 (21), 2 (22), COIN (23) and GISCAD (24) trials addressed this issue, substantially evidencing that the choice not to continuously administer the treatment until the evidence of disease progression, but to alternate periods of less intensive chemotherapy or chemo-holidays can be pursued without compromising patients' prognosis.

Nowadays, in the targeted agents' era, a heated issue concerns the importance of the so called "maintenance" treatment, that is the choice to pursue the antiangiogenic until disease progression, also in the case of a partial or total interruption of the associated chemotherapy. SAKK 41/06 study is a non-inferiority trial that randomized 262 patients that did not progress after 4-6 months of chemotherapy plus Bev, to continue or not Bev alone until disease progression. The non-inferiority of the observation strategy was not demonstrated in terms of time to progression (TTP) or OS. Patients treated with Bev reported a 1.2 months absolute advantage in TTP (4.1 vs 2.9 months from randomization, HR: 0.74 [0.57-0.95], p for non-inferiority=0.470) and a 3.3 months advantage in OS (26.1 vs 22.8 months, HR: 0.83 [0.61-1.12], p for difference=0.218) (25).

In CAIRO-3 trial, patients achieving a disease stabilization or response after six cycles of CAPOX plus Bev were randomized between observation or maintenance treatment with capecitabine plus Bev. Upon the first disease progression, CAPOX plus bev had to be reintroduced and continued until the second evidence of disease progression. The primary endpoint was the PFS2, defined as the time from randomization to progression upon re-introduction of CAPOX plus bev. Patients in the maintenance arm achieved a significant benefit in terms of PFS2 (11.8 vs 10.5 months, HR: 0.81 [0.67-0.98], p=0.028), PFS (8.5 vs 4.1 months, HR: 0.44 [0.36-0.53], p<0.00001) and a non-significant advantage in OS (21.7 vs 18.2 months, HR: 0.87 [0.71-1.06], p=0.156) that becomes significant in the adjusted analysis (HR: 0.80, p=0.035) (26).

On the basis of these evidences, the opportunity to alternate induction and maintenance phases in the disease history of mCRC patients is considered a valuable option.

2. STUDY RATIONALE

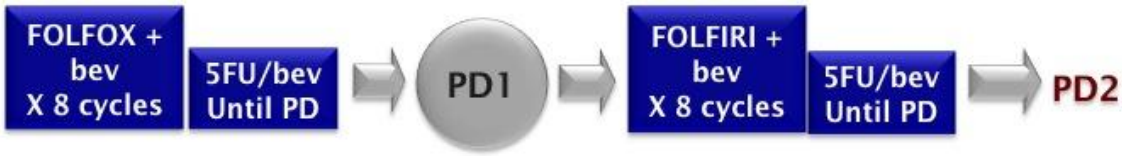
- Bev improves the efficacy of first-line chemotherapy in unresectable mCRC.
- In the phase III TRIBE trial upfront FOLFOXIRI plus bev provided a significant advantage in terms of PFS and RR compared to FOLFIRI plus bev. A trend toward better OS was also evidenced. The second-line treatment was at investigator's choice. A manageable increase in diarrhea, mucositis and neutropenia was reported, while no differences in febrile neutropenia, serious adverse events and toxic deaths were evidenced.
- A growing amount of data support the clinical relevance of achieving an early and deep tumor shrinkage.
- Phase III TML and BEBYP trials demonstrated that the continuation of bev beyond disease progression combined with a switched chemotherapy regimen provided a significant advantage in terms of OS and PFS.
- Based on recent evidences, the partial interruption of the upfront "induction" chemotherapy before disease progression and the prosecution of bev until disease progression as maintenance treatment is a valid strategy in the treatment of mCRC.

On the basis of these considerations, a first-line doublet plus bev followed by a second-line switched doublet (from oxaliplatin to irinotecan and viceversa) plus bev should be considered a standard option for mCRC patients. Only retrospectively collected data are currently available about the efficacy of first-line FOLFOXIRI plus bev followed by second-line rechallenge with FOLFOXIRI plus bev. We therefore designed the present phase III randomized trial of first-line FOLFOXIRI plus bev followed by reintroduction of FOLFOXIRI plus bev at progression versus FOLFOX plus bev followed by FOLFIRI plus bev at progression in first- and second-line treatment of unresectable mCRC patients.

3. STUDY DESIGN

This is a prospective, open-label, multicentric phase III randomized trial in which initially unresectable and previously untreated mCRC patients will be randomized to receive:

Arm A:



OR

Arm B:

The third- and subsequent lines of treatment will be at investigators' choice.

4. STUDY OBJECTIVES

4.1. Primary objective

The main objective of this trial is to compare the efficacy of the two proposed treatment strategies in terms of duration of Progression Free Survival 2 (PFS2).

4.2. Secondary objectives

Secondary objectives of this study are to compare the two proposed treatment strategies in terms of:

- Duration of Progression Free Survival (PFS);
- Duration of 2nd-Progression Free Survival (2nd-PFS);
- Duration of Time to Failure of Strategy (TFS);
- Duration of Overall Survival (OS);
- Distribution of Objective Response Rate (ORR) during first- and second-line treatment;
- Distribution of Early Objective Response (EOR) during first-line treatment;
- Distribution of the rate of secondary R0 resection of metastases;
- Safety profile;
- Translational analyses.

5. PATIENTS' SELECTION

5.1. Inclusion criteria

- Histologically proven diagnosis of colorectal cancer
- Initially unresectable metastatic colorectal cancer not previously treated with chemotherapy for metastatic disease
- At least one measurable lesion according to RECIST1.1 criteria
- Availability of a tumoral sample
- Male or female of 18-75 years of age
- ECOG PS < or = 2 if aged < 71 years, ECOG PS = 0 if aged 71-75 years
- Life expectancy of at least 12 weeks
- Previous adjuvant chemotherapy allowed only if with fluoropyrimidine monotherapy and more than 6 months elapsed between the end of adjuvant and first relapse
- Neutrophils >1.5 x 109/L, Platelets >100 x 109/L, Hgb >9 g/dl
- Total bilirubin 1.5 time the upper-normal limits (UNL) of the normal values and ASAT (SGOT) and/or ALAT (SGPT) <2.5 x UNL (or <5 x UNL in case of liver metastases) alkaline phosphatase <2.5 x UNL (or <5 x UNL in case of liver metastases)
- Creatinine clearance >50 mL/min or serum creatinine 1.5 x UNL
- Urine dipstick of proteinuria <2+. Patients discovered to have 2+ proteinuria on dipstick urinalysis at baseline, should undergo a 24-hour urine collection and must demonstrate <1 g of protein/24 hr
- Women of childbearing potential must have a negative blood pregnancy test at the baseline visit. For this trial, women of childbearing potential are defined as all women after puberty, unless they are postmenopausal for at least 12 months, are surgically sterile, or are sexually inactive.
- Subjects and their partners must be willing to avoid pregnancy during the trial and until 6 months after the last trial treatment. Male subjects with female partners of childbearing potential and female subjects of childbearing potential must, therefore,

be willing to use adequate contraception as approved by the investigator (barriere contraceptive measure or oral contraception)

- Will and ability to comply with the protocol
- Written informed consent to study procedures and to molecular analyses.

5.2. Exclusion criteria

- Radiotherapy to any site within 4 weeks before the study
- Previous adjuvant oxaliplatin-containing chemotherapy
- Previous treatment with bevacizumab
- Untreated brain metastases or spinal cord compression or primary brain tumours
- History or evidence upon physical examination of CNS disease unless adequately treated
- Symptomatic peripheral neuropathy > 2 grade NCIC-CTG criteria
- Serious, non-healing wound, ulcer, or bone fracture
- Evidence of bleeding diathesis or coagulopathy
- Uncontrolled hypertension and prior histor of hypertensive crisis or hypertensive encephalopathy
- Clinically significant (i.e. active) cardiovascular disease for example cerebrovascular accidents (≤6 months), myocardial infarction (≤6 months), unstable angina, New York Heart Association (NYHA) grade II or greater congestive heart failure, serious cardiac arrhythmia requiring medication
- Significant vascular disease (e.g. aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months of study enrolment.
- Any previous venous thromboembolism > NCI CTCAE Grade 3.
- History of abdominal fistula, GI perforation, intra-abdominal abscess or active GI bleeding within 6 months prior to the first study treatment.
- Current or recent (within 10 days prior to study treatment start) ongoing treatment with anticoagulants for therapeutic purposes

- Chronic, daily treatment with high-dose aspirin (>325 mg/day)
- Treatment with any investigational drug within 30 days prior to enrollment or 2 investigational agent half-lives (whichever is longer)
- Other co-existing malignancies or malignancies diagnosed within the last 5 years with the exception of localized basal and squamous cell carcinoma or cervical cancer in situ
- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to study treatment start, or anticipation of the need for major surgical procedure during the course of the study
- Lack of physical integrity of the upper gastrointestinal tract, malabsorption syndrome, or inability to take oral medication
- Pregnant or lactating women. Women of childbearing potential with either a positive or no pregnancy test at baseline. Postmenopausal women must have been amenorrheic for at least 12 months to be considered of non-childbearing potential. Sexually active males and females (of childbearing potential) unwilling to practice contraception (barriere contraceptive measure or oral contraception) during the study and until 6 months after the last trial treatment.

5.3 Discontinuation Criteria

A patient may be discontinued from the clinical trial at any time for any reason.
It is the right and the duty of the investigator to stop treatment in any case in which emerging effects are of unacceptable risk to the individual subject. In addition, patients have the right to voluntarily discontinue study treatment or withdraw from the study at any time for any reason. In instances where consent is withdrawn, the Investigator must clarify whether the patient is willing to continue to be followed (i.e. for survival).

Reasons for discontinuation of study treatment may include, but are not limited to, the following:

- Any medical condition that at the judgement of the Investigator or of the Sponsor may jeopardise patient‘s safety if he or she continues on study treatment;
- Major protocol violation (i.e. affecting the patients’ safety);
- Investigator or Sponsor determines it is in the best interest of the patient;
- Patient’s non-compliance to the protocol;
- Patient withdrawal of consent.

Reasons for withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent;
- Patient lost to follow-up;
- Death.

5.4 Replacement of Subjects

A subject who discontinues from the trial will not be replaced.

6. PARTICIPATING CENTERS, ENROLLMENT AND STUDY TIMELINE

About 60 Italian Oncology Units will participate to the trial. 654 patients will be randomized.

The registration and randomization procedures will be centralized at Clinical Trials Coordinating Center - Istituto Tosca++no Tumori.

Patients considered eligible and who have signed a written informed consent will be randomly assigned to one of the two treatment arms in a 1:1 ratio. Eligible patients will be stratified according to center, ECOG PS (0 *vs* 1, 2), primary tumor location (right *vs* left or rectum) and previous adjuvant chemotherapy (yes *vs* no).

The randomization will be performed by using an electronic WEB-based system.

The randomization code will consist of a unique identification code. This code must be used on all further documentation and correspondence, including electronic case record forms (e-CRFs). e-CRFs fac-simile are provided as a separate addendum to this study protocol.

It is responsibility of the principal investigator to ensure that each patient is eligible for the study before requesting randomization.

Study length is planned to be about 4.5 years since the enrollment is expected to be about 3 years, with a minimum period of follow-up of 18 months.

The end of study is defined as the time when all randomized patients will have experienced the second evidence of disease progression or will be out of treatment as per protocol, toxicity or medical decision.

The planned study timeline is as follows:

1. Submission date to health authority / ethics: November 2014
2. First Patient In: December 2014
3. Enrollment rate: 200 pts/year
4. Last Patient In: December 2017
5. Last Patient Last Visit: May 2019

6. Preliminary data on ORR and safety will be reported with no comparative intent when 150, 300 and 450 patients will have been randomized. This will happen approximately after 18, 27 and 36 months from the first patient in, respectively.
7. Efficacy interim analysis on primary endpoint: after 303 events (early 2018)
8. First data release on PFS2: Early 2020
9. Manuscript submission: Late 2020

7. STUDY TREATMENT AND PROCEDURES

7.1. Study treatment

Eligible patients will be randomized to receive:

Arm A

mFOLFOX-6 plus bev

- Bevacizumab 5 mg/kg iv over 30 minutes, day 1
- Oxaliplatin 85 mg/sqm iv over 2 hours, day 1
- L-Leucovorin 200 mg/sqm iv over 2 hours, day 1
- 5-fluoruracil 400 mg/sqm iv bolus, day 1
- 5-fluoruracil 2400 mg/sqm 48 h-continuous infusion, starting on day 1

to be repeated every 2 weeks for a maximum of 8 cycles.

If no progression occurs, patients will receive maintenance **5-FU/LV plus bev** at the same dose used at the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal.

The prosecution of bev until disease progression is recommended also in the case of interruption of 5-fluoruracil because of adverse events, patient's refusal or investigator's choice.

At the time of disease progression patients will receive **FOLFIRI plus bev***

- Bevacizumab 5 mg/kg iv over 30 minutes, day 1
- Irinotecan 180 mg/sqm iv over 2 hours, day 1
- L-Leucovorin 200 mg/sqm iv over 2 hours, day 1
- 5-fluoruracil 400 mg/sqm iv bolus, day 1
- 5-fluoruracil 2400 mg/sqm 48 h-continuous infusion, starting on day 1

to be repeated every 2 weeks for a maximum of 8 cycles.

*Doses may be modified according to patient's tolerance to 1st-line regimen.

If no progression occurs, patients will receive maintenance **5-FU/LV plus bev** at the same dose used at the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal.

The prosecution of bev until disease progression is recommended also in the case of interruption of 5-fluoruracil because of adverse events, patient's refusal or investigator's choice.

Arm B:

FOLFOXIRI plus bev

- Bevacizumab 5 mg/kg iv over 30 minutes, day 1
- Irinotecan 165 mg/sqm iv over 60 minutes, day 1
- Oxaliplatin 85 mg/sqm iv over 2 hours, day 1
- L-Leucovorin 200 mg/sqm iv over 2 hours, day 1
- 5-fluorouracil 3200 mg/sqm 48 h-continuous infusion, starting on day 1

to be repeated every 2 weeks for a maximum of 8 cycles.

If no progression occurs during FOLFOXIRI plus bev, patients will receive maintenance 5-FU/LV plus bev at the same dose used at the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal.

The prosecution of bev until disease progression is recommended also if 5-fluorouracil is interrupted because of adverse events, patient's refusal or investigator's choice.

At the time of disease progression, patients will re-introduce FOLFOXIRI plus bev at the same doses and schedule previously tolerated, for a maximum of 8 cycles. In the case of persistent neurotoxicity ≥ G2, FOLFIRI plus bev will administered for a maximum of 8 cycles.

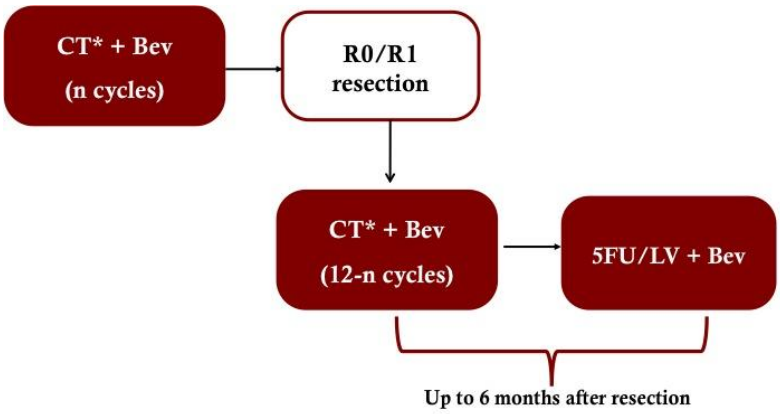
If no progression occurs during FOLFOXIRI plus bev, patients will receive maintenance 5-FU/LV plus bev at the same dose used in the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal.

The prosecution of bev until disease progression is recommended also in the case of interruption of 5-fluorouracil because of adverse events, patient's refusal or investigator's choice.

7.2. Secondary resection of metastases

Surgical radical resection of residual metastases in responsive patients is highly recommended and its feasibility should be evaluated every 2 months. It is strongly recommended to assess patients' resectability in the frame of a multidisciplinary group with a good expertise in the management of mCRC.

At least 5 weeks should elapse between the last administration of bev and the day of surgery. After resection, patients will receive post-operative therapy for 6 months (12 cycles) possibly up to 12 cycles of the same chemotherapy plus Bev regimen received before resection followed by 5FU/LV plus bev up to a total of 12 post-operative cycles (including chemotherapy plus Bev and 5FU/LV plus Bev). Post-operative treatment should start not earlier than 4 weeks after surgery. In the case of repeated procedures, post-operative treatment should start not earlier than 4 weeks after the last procedure. The choice to administered additional cycles of systemic treatment between two procedures of a pre-planned 2 stage-surgery is at investigator's choice.



* According to treatment arm

If disease progression occurs more than 1 year after the completion of the post-operative therapy, the second-line treatment according to the randomization arm is still recommended but it will be considered out of study .

7.3. Baseline and on treatment clinical evaluations

At baseline:

- Medical history, ECOG PS, physical examination (including height and weight, blood pressure and heart rate);
- ECG;
- Complete blood examination: blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT), CEA, CA19.9; pregnancy test (if clinically indicate);
- Urinanalysis;
- Contrast-Enhanced chest and abdominal CT scan, or Abdomen MRI and Chest CT if contrast-enhanced CT scan is contraindicated. To be performed no more than 28 days before randomization;

- Collection of a copy of baseline CT scan (and/or abdomen MRI), digitally stored on CD-ROM;
- Obtained written informed consent;
- Collection of a paraffin-embedded block of the primary tumor and/or metastases, or 10 slides 5 μm-thick for immunohystochemistry and 10 slides 8 μm-thick;
- Collection of blood and plasma samples.

Before every cycle of treatment (induction or maintenance), until the 2nd evidence of PD:

- Partial blood examination: Blood count and differential, bilirubin (total and direct), serum creatinine, INR/APTT (only for patients on anticoagulation therapy);
- Dipstick proteinuria;
- Collection of reported adverse events;
- ECOG PS, physical examination (including height, weight, blood pressure and heart rate).

Every 8 weeks until the 2nd evidence of PD:

- Complete blood examination: Blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium), INR/APTT, CEA, CA19.9;
- Contrast-Enhanced chest and abdominal CT scan, or Abdomen MRI and Chest CT if contrast-enhanced CT scan is contraindicated (the same technique used in the baseline assessment);
- Collection of blood and plasma samples.

At the end of the treatment and after the 2nd evidence of PD (visits scheduled according to investigator's practice):

- ECOG PS, physical examination (including height, weight, blood pressure and heart rate);

- Follow up on adverse events still ongoing at the time of 2nd PD;
- Survival follow up.

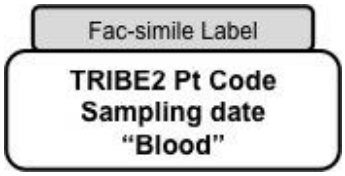
7.4. Tissue specimens collection

The collection of tissue specimens is mandatory for study entry. A paraffin-embedded block of the primary tumor and/or metastases if available, or 10 slides 5 μm-thick for immunohystochemistry and 10 slides 8 μm-thick for molecular biology analyses, are required.

Tissue specimens will be sent, together with the accompanying histological report, to the Coordinating Center (U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana), where they will be collected and adequately stored under the responsability of Dr. Loupakis.

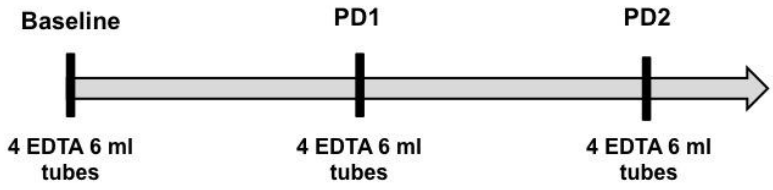
7.5. Blood sampling collection

Three 6ml EDTA tubes will be collected once at anytime before or during the treatment. They will be labelled as "TRIBE2 - Patient Code/ SNP Blood" (see fac-simile) and will be stored at -20°C until shipment to the Coordinating Center (U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana).



In addition, four 6 ml EDTA tubes will be collected at the following time-points:

- at baseline (Bas)
- at the first evidence of PD (PD1)
- at the second evidence of PD (PD2)

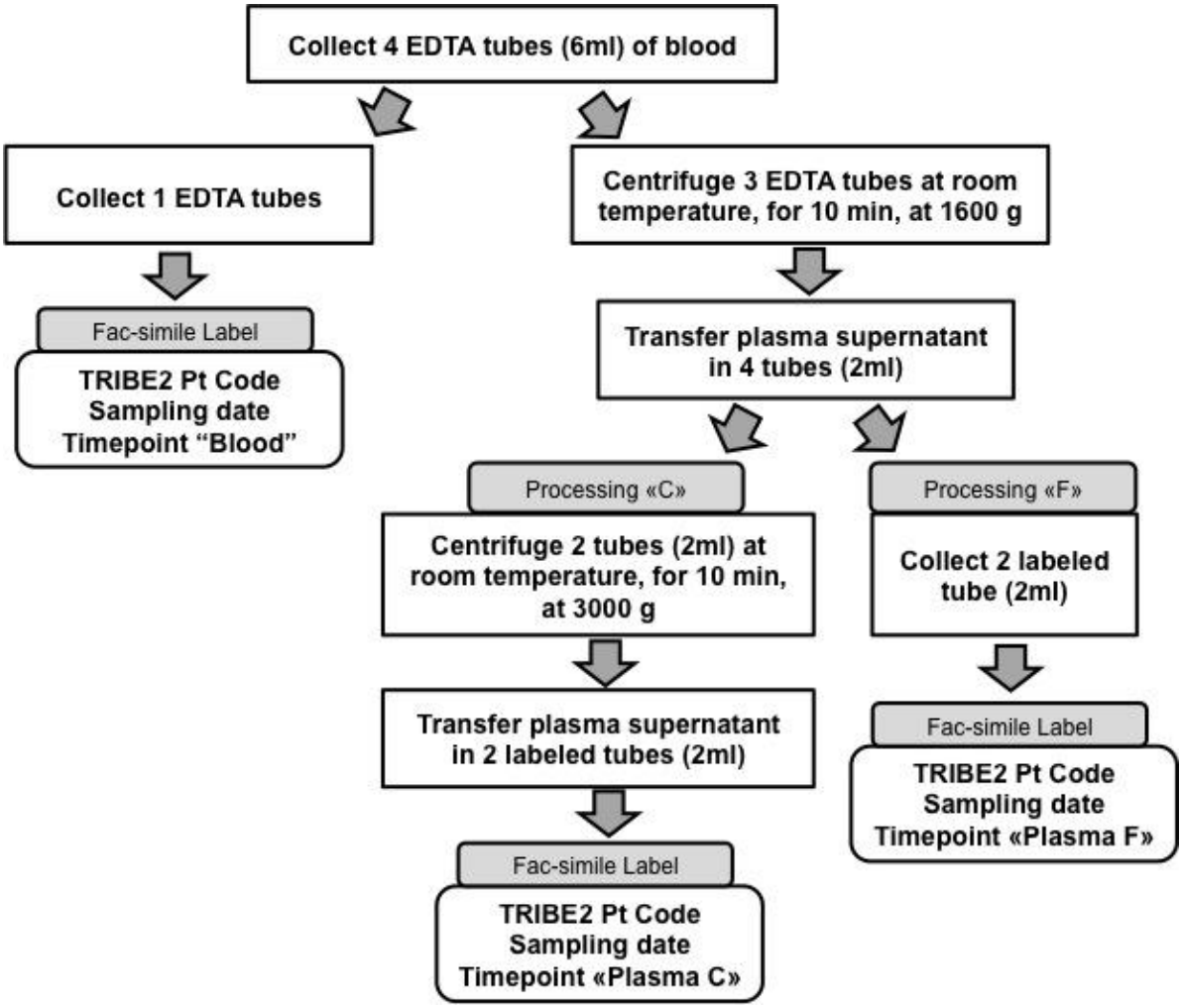


One tube will be labelled as "TRIBE2 - Patient Code/ Date/ Bas or PD1 or PD2/Blood" (see fac-simile) and directly stored at -80°C until shipment to the Coordinating Center (U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana).

Three tubes will be centrifuged as soon as possible at room temperature at 1600 g x 10 minutes and plasma supernatant will be collected and divided into four aliquots. Two aliquots will be stored at -80°C until shipment to the Coordinating Center in

tubes labelled as "TRIBE2 – Patient Code/ Date/ Bas or PD1 or PD2/ Plasma F" (see fac-simile).

The other 2 aliquots will be centrifuged at room temperature at 3000 g x 10 minutes and plasma supernatant will be collected in tubes labelled as "TRIBE2 – Patient Code/ Date/ Bas or PD1 or PD2/ Plasma C" (see fac-simile).



The shipment of blood samples will be arranged by the G.O.N.O. that will provide dry ice for the shipment.

7.6. Collection of CT scan images

Tumor response will be assessed throught contrast-enhanced chest and abdomen CT scans with a contiguous slice thickness of ≤ 7mm, that will be performed in the radiology department of the study site. Abdomen MRI and chest CT scan are allowed in the case of contraindications to the use of iodine contrast agents.
In the case of clinical suspicion of disease progression, the radiographic evaluation should be performed within a maximum of 7 days to confirm objective disease progression.

CD-ROM copies of the CT scans at baseline, at the time of the best response during the first treatment, at the time of the first and second evidence of PD will be collected at the Coordinating Center (U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana) for central review.

Site should follow their local privacy practices to de-identify all sybject identifying information (name, medical record number, ect.) prior to submitting images to Coordinating Center.
Upon receipt, the Coordinating Center will verify that this information has been completely redacted, and, if necessary, will redact any remaining identifying information.

7.7. Tabulated overview

Procedure	Screening (within 28 days before random)	Baseline	Before every cycle[1]	Every 8 wks [1]	After the 2nd evidence of PD
Informed Consent	X				
Complete medical history	X				
Inclusion/Exclusion Criteria Checked	X				
Tumor assessment (total-body CT or abdomen MRI + chest CT)	X			X	
Collection of a CD-ROM copy of CT scan	X			X	
12-lead ECG	X				
ECOG PS	X	X	X	X	
Physical examination	X		X	X	
Complete blood examination[2]	X			X	
Partial blood examination[3]		X	X		
Dipstick proteinuria	X	X	X		
Collection of a paraffin-embedded tissue sample	X				
Collection of blood samples		X		X[4]	
Adverse events and toxicity			X[5]		X[6]
Survival follow up					X

1. Until the 2nd evidence of disease progression
2. Blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT), CEA, CA19.9; pregnancy test (if clinically indicate);
3. Blood count and differential, bilirubin (total and direct), serum creatinine, INR/APTT (only for patients on anticoagulation therapy);
4. Only a the first and second evidence of PD
5. AE assessment to be started after signing of IC until 30 days after last study treatment
6. Follow up on adverse events still ongoing at the time of 2nd PD

8. SAFETY ISSUES

8.1. Dose reductions and delays

Toxicities should be evaluated according to CTCAEv4.0. Once a dose has been reduced it should not be increased at a later time.

Dose modifications for toxicities attributable to chemotherapy

TOXICITY AT THE START OF SUBSEQUENT CYCLES OF THERAPY	GRADE/ Values	Irinotecan	Oxaliplatin	5FU
WBC	< 3.000/mm^3	**Hold until resolution**		
Neutrophils	< 1.000/mm^3			
Platelets	< 100.000/mm^3			
Diarrhea	≥ 1			
Mucositis	≥ 1			
Any other non-hematological toxicity	≥ 2			
Hand/foot syndrome	3-4	100%	100%	**STOP**
Neurotoxicity	≥ 3	100%	**STOP**	100%

PREVIOUS TOXICITY	GRADE	Irinotecan	Oxaliplatin	5FU
Neutropenia >5 days	4	**75%**	**75%**	100%
Febrile Neutropenia	4			
Thrombocytopenia	3-4			
Diarrhea	3	**75%**	100%	**75%**
Diarrhea	4	**50%**	100%	**50%**
Stomatitis	3	100%	100%	**75%**
Stomatitis	4	100%	100%	**50%**
Myocardial Ischemia	NA	100%	100%	**STOP**

Dose modifications for toxicities attributable to bevacizumab

Event	Grade	Adjustment to bev
Hypertension	3	If not controlled by 3-drug medication, permanently discontinue
	4 (Hypertensive crisis and encepalopathy)	Permanently discontinue
Hemorrhage	Any grade CNS	Permanently discontinue
	≥2 (pulmonary)	Permanently discontinue
	≥3 (non-pulmonary/non-CNS)	Permanently discontinue
Venous thrombosis	3	Hold temporarily
	4	Permanently discontinue
Arterial thrombosis	Any Grade	Permanently discontinue
Congestive Heart Failure	≥ 3	Permanently discontinue
Proteinuria	2-3	- *For 2+ dipstick*: may administer bev, obtain 24-hour urine sample prior to next bev dose Suspend bev for ≥2 g /24 hours and resume when proteinuria is <2 g /24 hours and protein creatinine ratio <2.0 - *For 3+ dipstick*: obtain 24 hour urine sample prior to bev administration Suspend bev for ≥2 g /24 hours and resume when proteinuria is <2 g /24 hours and protein creatinine ratio <2.0
	4	Permanently discontinue
GI perforation	Any grade	Permanently discontinue
PRES/RPLS	Any grade	Permanently discontinue
Fistula	Any grade TE fistula	Permanently discontinue
	≥3 (other than TE)	Permanently discontinue
Febrile neutropenia/ thrombocytopenia	4	Hold temporarily
Other unspecified bev-related AE	3	Hold until recovery to ≤ Grade 1
	4	Permanently discontinue

Gastrointestinal Perforation

Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation.

Fistula

Bevacizumab should be permanently discontinued in patients who develop any grade tracheoesophageal and temporarily hold or permanently discontinued in the case of grade ≥ 2 fistula in any other site.

Surgical Procedures/Wound Healing Complications

Bevacizumab therapy should not be initiated for at least 28 days following major surgery or until the surgical wound is fully healed. In patients who experience wound healing complications during bevacizumab treatment, bevacizumab should be withheld until the wound is fully healed.

Bevacizumab therapy should be withheld 60 days before elective surgery. CVAD placement and complications will be monitored as an assessment of treatment-related complications. Date of placement of CVAD will be noted in the medical record and recorded in the eCRF. Episodes of CVAD removal or replacement will be recorded. Episodes of CVAD-related thrombosis, infection, or dysfunction will be recorded.

Necrotising fasciitis including fatal cases, has rarely been reported in patients treated with bevacizumab; usually secondary to wound healing complications, gastrointestinal perforation or fistula formation. Bevacizumab should be discontinued in patients who develop necrotising fasciitis, and appropriate treatment should be promptly initiated.

Hypertension

Patients should be monitored for the development or worsening of hypertension via frequent blood pressure measurement. Blood pressure measurements should be taken after the patient has been in a resting position for ≥ 5 minutes. Repeat

measurements of blood pressure for verification should be undertaken if the initial reading is ≥ 140 mmHg systolic and/or ≥ 90 mmHg diastolic blood pressure.

• Grade 1 hypertension: Asymptomatic, transient (< 24 hrs) increase by > 20 mmHg (diastolic) or to > 150/100 mmHg if previously within normal limits. Intervention not indicated.

• Grade 2 hypertension: Recurrent or persistent (> 24 hr) or symptomatic increase by > 20 mmHg (diastolic) or to > 150/100 mmHg if previously within normal limits. Monotherapy with ACE-inhibitor may be indicated. Once controlled to < 150/100 mmHg, patients may continue bevacizumab therapy.

• Grade 3 hypertension: Requiring more than one anti-hypertensive or more intensive therapy than previously. Addiction of diuretic to ACE-inhibitor may be indicated; if hypertension is not controlled a third anti-hypertensive drug (calcium channel blocker) should be added.

Bevacizumab should be withheld for persistent or symptomatic hypertension and should be permanently discontinued if hypertension is not controlled with triple-drug medication.

Proteinuria

All patients will have a dipstick urinalysis or 24 hour protein determination performed within 48 hours prior to the first bevacizumab dose and thereafter every 8 weeks. Adjustment of bevacizumab administration for proteinuria of ≥ 2 g/24h will occur according to the following guidelines, listed below:

- < 2+ (dipstick): no additional evaluation is required.
- ≥ 2+ (dipstick): Collect 24-hour urine to determine the total protein within 3 days prior to the next scheduled dose:

- 24-hour proteinuria ≤ 2 g: Administer bevacizumab as scheduled.
- 24-hour proteinuria > 2 g: Bevacizumab treatment should be withheld pending next 24 hour total protein.

Repeat 24-hour urine protein ≤ 2 g: Administer bevacizumab as schedule. 24-hour protein should be further monitored prior to each administration of bevacizumab until it has decreased to ≤ 1 g/24h.

Repeat 24-hour urine protein > 2 g: Bevacizumab dose should be withheld until 24-hour protein has decreased to ≤ 2 g. 24-hour protein should be further monitored prior to each administration of bevacizumab until it has decreased to ≤ 1 g/24 h.

Nephrotic syndrome (Grade 4, CTCAEv4.0): Discontinue bevacizumab treatment.

Thrombosis/Embolism

All toxicity will be graded according to CTCAEv4.0 guidelines. For patients who develop thrombosis/embolism the following action is recommended:

Bevacizumab should be permanently discontinued in patients who develop arterial thromboembolic events of any grade and in patients to develop grade 3 venous thrombosis

Congestive heart failure

Caution should be exercised when treating patients with clinically significant cardiovascular disease or pre-existing congestive heart failure with bevacizumab, such as pre-existing coronary heart disease or concomitant cardiotoxic therapy.

Events consistent with congestive heart failure were reported in clinical trials with symptoms ranging from asymptomatic declines in left ventricular ejection fraction to symptomatic congestive heart failure, requiring treatment or hospitalisation. Patients developing ≥ G3 congestive heart failure should permanently discontinue bevacizumab treatment.

Haemorrhage

Patients who develop grade ≥2 pulmonary or CNS (any grade) or grade ≥3 hemorrhage should discontinue bevacizumab treatment.

Patients who develop grade 3 non-pulmonary and non – CNS hemorrhage should hold bevacizumab until all of the following criteria are met:

- The bleeding has resolved and haemoglobin is stable.

- There is no bleeding diathesis that would increase the risk of therapy.
- There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.

Posterior Reversible Encephalopathy Syndrome (PRES/RPLS)

Bevacizumab should be permanently discontinued in patients who develop any grade PRES/RPLS

8.2. Concomitant medications and management of specific adverse events

Acute colinergic syndrome

Atropine sulfate can be used, at the discretion of the investigator, as secondary prophylaxis or therapy of early onset cholinergic syndrome induced by irinotecan. Secondary prophylactic or therapeutic administration of 0.25-1 mg of subcutaneous atropine can be considered (unless clinically contraindicated) in patients experiencing rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping, or diarrhea (occurring during or shortly after infusion of irinotecan).

Antiemetic prophylaxis

To be chosen on the basis of the chemotherapy regimen according to the center's guidelines.

Diarrhea

Irinotecan can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Early diarrhea (occurring during or shortly after infusion of irinotecan) is cholinergic in nature. It is usually transient and only infrequently is severe. It may be accompanied by symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be ameliorated by administration of atropine (0.25 mg SC). Atropine should not be given prophylactically during cycle 1.

Late diarrhea (generally occurring more than 24 hours after administration of irinotecan) can be prolonged, may lead to dehydration and electrolyte imbalance, and can be lifethreatening. Patients and patients' caregivers should be carefully informed of possible severe toxic effects such as diarrhea and abdominal cramps. Each patient should be instructed to have loperamide readily available and to begin treatment for late diarrhea (generally occurring more than 24 hours after administration of irinotecan) at the first episode of poorly formed or loose stools or the earliest onset of

bowel movements more frequent than normally expected for the patient. The patient should also be instructed to notify the Investigator if diarrhea or abdominal cramps occur. If diarrhea persists for more than 24 hours despite loperamide, the patient should be instructed to take a fluoroquinolone antibiotic and to re- contact the treating Investigator. The patient should be hospitalised for parenteral support and loperamide should be replaced by another anti-diarrheal treatment (e.g. octreotide). Patients should have a supply of fluoroquinolone antibiotic available at home. The recommended dosage regimen for loperarnide previously used in irinotecan clinical trials consists of the following: 4 mg at the first onset of late diarrhea and then 2 mg every 2 hours until the patient is diarrhea-free for at least 12 hours. Note: This dosage regimen exceeds the usual dosage recommendations for loperamide. Premedication with loperamide is not recommended. If diarrhea occurs it is of vital importance that measures are taken to avoid dehydration and electrolyte imbalance. Patients should be supported as clinically indicated. The use of drugs with laxative properties should be avoided because of the potential for exacerbation of diarrhea. Patients should be advised to contact their Investigator to discuss any laxative use. Abdominal cramps should be treated the same as for diarrhea.

Extravasation

No severe extravasation reactions have been observed so far with CPT-11 and Oxaliplatin. As a general recommendation, in the event of extravasation, the following advice should be observed (like for any drug):

1. stop the infusion immediately,
2. do not remove the needle or cannula,
3. aspirate as much infiltrated drug as possible from the subcutaneous site with the same needle,
4. apply ice to the area for 15 to 20 minutes every 4 to 6 hours for the first 72 hours,
5. watch the area closely during the following days in order to determine whether any further treatment is necessary.

Hematopoietic growth factors

G-CSF is not recommended as primary prophylaxis, but it can be used in secondary prophylaxis in case of:

- Precedent febrile neutropenia;
- Precedent grade 4 neutropenia lasting 5 days or more;
- More than 2 delays of the planned therapy due to neutropenia.

Prohibited treatment

High dose aspirin (>325 mg/day) and anticoagulants for therapeutic purpose are not allowed in combination with Bevacizumab.

9. STATISTICAL METHODS

This is a prospective, open-label, multicentric, randomized phase III study in which patients, stratified according to center, ECOG PS (0 vs 1, 2), primary tumor location (right vs left or rectum) and previous adjuvant chemotherapy, will be randomized to receive one of two treatment strategies, as specified in the Paragraph "Study design".

9.1. Primary endpoint

The primary endpoint is Progression Free Survival 2 (PFS2).

PFS2 will be defined as beginning with randomization and ending with the first of the following events: a) death; b) disease progression on any treatment given after 1st progression. For patients that will not receive any treatment within 3 months after 1st progression, PFS2 will be equal to PFS. The determination of disease progression will be based on investigator-reported measurements. Disease status will be evaluated according to RECIST 1.1 criteria.

Censoring rules for PFS2 will be: end of study without PD, loss at follow-up. Curative surgery for metastasis will not result in censoring for PFS2.

PFS2 will be analyzed both in the intention-to-treat population (primary analysis) and in the per-protocol population.

9.2. Secondary endpoints

Secondary endpoints of this study are the following:

Progression free survival (PFS) is defined as the time from randomization to the first documentation of objective disease progression or death due to any cause, whichever occurs first. PFS will be censored on the date of the last evaluable on study tumor assessment documenting absence of progressive disease for patients who are alive, on study and progression free at the time of the analysis. Alive patients having no tumor assessments after baseline will have time to event censored on the date of randomization.

2nd-Progression free survival (2nd-PFS) is defined as the time from the beginning of the second-line treatment to the documentation of objective disease progression or death due to any cause, whichever occurs first. 2nd-PFS will be censored on the date of the last evaluable on study tumor assessment documenting absence of progressive disease for patients who are alive, on study and 2nd-progression free at the time of the analysis. 2nd-PFS will be analyzed both in the intention-to-treat population (whichever 2nd-line treatment will be adopted) and in the per-protocol population.

Time to failure of strategy (TFS) is defined as the time time from randomization to the first of the following events: death; patient requires the addition of a new therapeutic agent (i.e. an agent not included in the original strategy); patient experiences disease progression while being treated with all agents that are components of the initial treatment strategy (except for agents which cannot be used because of persistent toxicity or contraindications); or patient experiences disease progression during a partial or complete treatment holiday from initial treatment strategy and receives no further therapy within 3 months. Subjects who did not have an event as stated above while on study will be censored at the last evaluable radiographic assessment date. TFS will be analyzed both in the intention-to-treat population (primary analysis) and in the per-protocol population.

Overall survival (OS) is defined as the time from randomization to the date of death due to any cause. For patients still alive at the time of analysis, the OS time will be censored on the last date the patients were known to be alive.

Objective Response Rate is defined as the percentage of patients, relative to the total of enrolled subjects, achieving a complete (CR) or partial (PR) response, according to RECIST 1.1 criteria, during the induction and the maintenance phases of treatment. The determination of clinical response will be based on investigator reported measurements. Responses will be evaluated every 8 weeks.

Early Objective Response Rate is defined as the percentage of patients, relative to the total of the enrolled subjects, achieving a ≥ 20% decrease in the sum of diameters of RECIST target lesions at week 8 compared to baseline.

R0 Resection Rate is defined as the percentage of patients, relative to the total of enrolled subjects, undergoing secondary R0 resection of metastases. Secondary R0 surgery is defined as microscopically margin free complete surgical removal of all residual disease, performed during treatment or after its completion, allowed by tumoral shrinkage and/or disappearance of one or more lesions.

Overall Toxicity Rate is defined as the percentage of patients, relative to the total of enrolled subjects, experiencing any adverse event, according to National Cancer Institute Common Toxicity Criteria (version 4.0), during the induction and the maintenance phases of treatment.

Toxicity Rate is defined as the percentage of patients, relative to the total of enrolled subjects, experiencing a specific adverse event of grade 3/4, according to National Cancer Institute Common Toxicity Criteria (version 4.0), during the induction and the maintenance phases of treatment.

9.3. Study populations for primary and secondary analyses

Intention to treat population (ITT)

The ITT population will include all randomized patients. The ITT population will be the population for evaluating all primary and secondary endpoints, with the exception of toxicity rate and overall toxicity rate.

Safety population (SP)

The SP will include all patients who receive at least one dose of the study medication designated according to the randomization arm. The SP will be the population for evaluating treatment administration/compliance and safety.

Per-protocol population

The per-protocol population will include patients that proceeded according to the protocol, receiving at least one cycle of FOLFOX plus bev as first-line treatment and at least one cycle of FOLFIRI plus bev as second-line treatment (arm A) and at least one cycle of FOLFOXIRI plus bev as first-line treatment and at least one cycle of 5-FU +/- oxaliplatin +/- irinotecan plus bev as second-line treatment (arm B).

9.4. Analysis of endpoints

Analysis of primary endpoint

The primary analysis of PFS2 will be performed in the ITT population. An unstratified log rank test will be used to compare PFS2 time between the two treatment arms with a two-sided alpha level equal to 0.0131 and 0.0455 at the interim and/or final analyses, respectively. Hazard ratios and 95 percent confidence intervals will be calculated with the use of the Cox proportional-hazards model. Survival curves will be calculated according to Kaplan–Meier method. A log-rank test stratified by means of the same factors as used for randomization will also be performed, as well as a multivariable model including all the baseline variables that will result significantly ($p<0.05$) related to PFS2 at the univariate analyses.

Analysis of secondary endpoints

A two-sided log-rank test will be used to compare study arms in terms of PFS, TFS and OS. Hazard ratios and 95 percent confidence intervals will be calculated with the use of the Cox proportional-hazards model. Survival curves will be calculated according to Kaplan– Meier methods. Log-rank tests stratified by the same factors as

used for randomization will also be performed, as well as multivariable models including all the significant baseline variables.

Best overall response rate will be calculated as the number of patients with a CR or PR as best response divided by the total number of enrolled patients. The corresponding exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

R0 resection rate will be calculated as the number of patients undergoing secondary R0 resection of metastases divided by the total number of randomized patients in each arm. The corresponding exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

Toxicity rates and overall toxicity rate will be calculated as the number of patients experiencing a specific adverse event of grade 3/4 or any adverse event of grade 3/4 divided by the total number of randomized patients and it will be summarized by the two arms of treatment and also by each study medication/type of treatment and by periods (before first PD and after first PD). Also a separate summary of AE grade ¾ will be provided for patients undergoing secondary R0 resection of metastases. The corresponding exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

9.5. Sample size

Based on the assumption that PFS2 of each arm follows an exponential distribution and the true hazard ratio (HR) for PFS2 is 0.77 between experimental group (arm B) vs. control group (arm A), 466 events are required for a two-sided unstratified log-rank test with $\alpha = 0.05$ to have 80% power. Assuming a proportion of PFS2 equal to 50% at 15 months in arm A, this treatment effect can be translated to a 9% absolute improvement in PFS2 at 15 months in arm B. Assuming an accrual rate of 200 subjects/year, a minimum follow up period equal to 1.5 years and an overall dropout

rate equal to 5%, it is estimated that the enrollment of 654 subjects, randomized in a 1:1 ratio, is required.

We plan a group sequential design with 1 interim analysis to assess the primary efficacy endpoint. The analyses will take place at 2/3 (303 events) of the primary events using an O'Brien Fleming alpha-spending rule. The interim analysis will assess superiority of experimental arm to control group for the primary endpoint because the study will only be considered for early termination if superiority is met. The first interim analysis will have a two sided alpha level of 0.0131. According to the O'Brien Fleming spending rule this will leave a two sided alpha level of 0.0455 for the final analysis. The total type I error rate will be only slightly increased.

10. ETHICAL ISSUES

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Sponsor and Investigator abide by GCP guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRB(s) will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the IEC/IRB approval must be obtained and also forwarded to the Sponsor. The responsible unit (eg, IEC/IRB, head of the study center/medical institution) must supply to the Sponsor, upon request, a list of the IEC/IRB members involved in the vote and a statement to confirm that the IEC/IRB is organized and operates according to GCP and applicable laws and regulations.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by either the Sponsor or the investigator without agreement by both parties. However, the investigator or the Sponsor may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IEC/IRB/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and, if appropriate, the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution/Sponsor. Any deviations from the protocol must be explained and documented by the investigator.

10.1. Informed Consent

The investigator must explain to each patient (or legally authorised representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each patient must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect her subsequent medical treatment or relationship with physician. The informed consent will be given by means of standard written statement, written in non-technical language. The patient should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the document, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign documents. No patient can enter the study before her informed consent has been obtained. The informed consent is part of the protocol and must be submitted by the investigator with to the local ethical committee.

A copy of the patient's signed written consent will be kept by the center in the proper section of the Investigator Site File.

10.2. Patient protection

The names of patients will not be recorded; a sequential identification number will be attributed to each patient registered in the trial. This number will identify the patient and must be included on all electronic Case Report Forms.

In order to avoid identification errors, patients initials (maximum of 2 letters) and date of birth will also be reported on the Case Report Forms.

Investigators will guarantee that all persons involved in this study will respect the confidentiality of any information concerning the trial subject.

All parties involved in this clinical trial will maintain the strict confidentiality to assure that neither the person nor the family privacy of the patient participating in

the trial is violated; appropriate measures shall be taken to avoid the access of non authorized persons to the trial data. The processing of the personal data of patients taking part in the trial, and in particular regarding data concerning consent, shall comply with local law on the privacy (Legge delega 127/2001) and with the European Directive on the Privacy of data (95/46/EC).
The patient can withdraw consent whenever he wants and further data will not be collected, even if the already collected data will be used for the study's analyses.

10.3. Confidential subject information for samples storage

For the storage of biological samples, specific means will be taken to ensure the subject's right to privacy and the pertinent guidance documents and regulations will be considered.

Subjects may withdraw their consent to store the biological samples. If the patient withdraws his consent from the study within 5 years, the biological samples will be destroyed. After 5 years, biological samples will be anonymized completely. At that time the samples cannot be identified in any way. The samples will be maintained for potential analysis for 15 years from the acquisition. Samples will be destroyed according to GONO policies and procedures.

Samples will be collected and sent to the laboratory designated for the trial where they will be processed.
Tumor tissue samples, blood and plasma samples will be stored at Oncologia Medica 2 Universitaria of Azienda Ospedaliero-Universitaria Pisana – Translational Research and New Technologies Department– University of Pisa, under the responsibility of Dr. Loupakis.

To maintain privacy of information collected from samples obtained for storage and future analysis, GONO has developed secure policies and procedures to maintain subject privacy. At the clinical site, a unique Code will be placed on the blood sample

for transfer to the storage facility. The Code is a random number used only to identify the biosample of each subject. No other personal identifiers will appear on the sample tube. The first Code will be replaced with a Sample Code at the Central Laboratory or at the GONO designated facility. This sample is now a single coded sample. The Sample Code is stored separately from all previous sample identifiers. A secure code, hereinafter referred to as a "first coding key", will be utilized to match the Sample Code to the original blood code and subject number to allow clinical information collected during the course of the trial to be associated with the biosample. This "first coding key" will be transferred by the central laboratory or GONO designated facility under secure procedures to the GONO designated as the entrusted keyholder to maintain confidentiality of the biosamples. The Sample Code will be logged into the primary biorepository database, and in this database this identifier will not have identifying demographic data or identifying clinical information (i.e., race, sex, age, diagnosis, lab values) associated with it. The sample will be stored in a designated repository site with secure policies and procedures for sample storage and usage.

10.4. Ethics Committe (EC)

The Investigator must submit this protocol to the local Ethics Committee and is required to forward a copy of the written approval to the CRP.
The EC approval must report, the identification of the trial (title, protocol number and version), the documents evaluated (protocol, informed consent material, advertisement when applicable) and the date of their version.

10.5. Administrative responsabilities

The Coordinating Center (U.O. Oncologia 2 Universitaria – Polo Oncologico Azienda Ospedaliero-Universitaria Pisana, AOUP) and the Data Center (Centro Coordinamento Sperimentazioni Cliniche of Istituto Toscano Tumori – Azienda Ospedaliero-Universitaria Careggi) will be responsible for:

- reviewing the protocol
- centralizing databases
- centralizing data validation according to Data Validation Plan
- controlling the quality of the reported data
- emitting Data Query Forms
- generating study program reports
- generating the Statistical Analysis Plan
- perform statistical analysis

10.6. Trial sponsorship and financing

- The present study is an investigator-initiated trial, carried out by participating clinicians, who have the intellectual ownership of the results.
- The study is sponsored by Gruppo Oncologico Nord-Ovest (G.O.N.O.) Cooperative Group Via G. Mameli, 3 – Genoa (ITALY), who will provide the economical support for costs related to data management, statistical analysis and the other activities of central and group coordinating centers.
- Roche SpA will provide vials of Bevacizumab beyond progression in both arms and partial financial support for study costs.
- No funds can be provided to ethical committees and single participating centers.
- The study will be conducted according to the current regulations.

11. STUDY MONITORING

11.1. Quality assurance

Each participating Investigator will be responsible for ensuring data quality as planned in the Data Validation Plan document. Each reported information will be systematically checked for consistency, completeness and accuracy by the Coordinating Data Center that will issue Data Query Forms in case of inconsistent data. Local quality control will be provided by coordinating centers of each participating group, which will be responsible of monitoring the centers belonging to their group.

11.2. Responsabilities of the investigators

The Investigators undertake to perform the study in accordance with ICH Good Clinical Practice and Good Clinical Practice for Trials on Medicinal Products in the European Community (ISBN 92 - 825-9563-3).

The Investigator is required to ensure his compliance to the procedures required by the protocol with respect to the investigational drug schedule and visit schedule. The Investigator agrees to provide all information requested in the Case Report Form in an accurate and legible manner according to the instructions provided.

The Investigator has responsibilities to the Health Authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity and validity of the data recorded on the case report forms. The main duty of the Trial Monitor is to help the Investigator and the Coordinators to maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

At regular intervals during the study, the center will be contacted, through site visits, letters or telephone calls, to review the study progress, the investigators and subjects adherence to protocol requirements.

During each monitoring visits, the following points will be scrutinized:

- subject informed consent
- subject recruitment and follow-up

- study drug allocation
- subject compliance to the study treatment
- study treatment accountability
- Adverse Event documentation and reporting

11.3. Source documents requirements

According to the guidelines on ICH Good Clinical Practice, the monitor of the study will check the case report form entries against the source documents. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

Considering the primary end point of the study, independent review of objective response will be performed by an external panel. For this reason, a copy (either on CD or radiological film) of each CT or RMN scan performed during the study will be required.

11.4. Use and completion of electronic case report forms (e-CRFs)

It is the responsibility of the Investigator to prepare and maintain adequate and accurate e-CRFs for each patient enrolled in the study. All e-CRFs should be completed to ensure accurate interpretation of data.

12. ADVERSE EVENTS

12.1. Definition of adverse event

An adverse event is defined in the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment." (ICH E6:1.2). See below (specific table), for guidelines to drug-event relationship assessment.

The investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study subject represents a change from values before the study. In general, abnormal laboratory findings without clinical significance (based on the investigator's judgment) should not be recorded as adverse events; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered adverse events.

Patients will be instructed by the Investigator to report the occurrence of any adverse event.

Assessment of drug-event relationship

Relationship	Description
unrelated	There is no evidence of any causal relationship
unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event

	(e.g. the patient's clinical condition, other concomitant treatments).
probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

12.2. Definition of Adverse Drug Reactions (ADR)

All untoward and unintended responses to a medicinal product related to any dose administered.

The phrase "responses to a medicinal product" means that a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

A serious ADR (SADR) is an ADR that meets the definition of serious (provided below).

12.3. Definition of Serious Adverse Event

A serious adverse event (SAE) is defined as an adverse event that:

- is fatal
- is life threatening (places the subject at immediate risk of death):
- requires in-patient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- other significant medical hazard

A hospitalization meeting the regulatory definition for "serious" is any inpatient hospital admission that includes a minimum of an overnight stay in a health care

facility. Any adverse event that does not meet one of the definitions of serious (i.e., emergency room visit, outpatient surgery, or requires urgent investigation) may be considered by the investigator to meet the "other significant medical hazard" criterion for classification as a serious adverse event. Examples include allergic bronchospasm, convulsions, and blood dyscrasias.

Hospitalization for the performing of protocol-required procedures or administration of study treatment is not classified as an SAE.

All adverse events which do not meet any of the criteria for serious should be regarded as non-serious adverse events.

All serious adverse events occurring during the study treatment period must be reported within 24 hours according to the procedure described below. Any late SAE (occurring after this 30 days period) possibly or probably related to the study treatment should follow the same reporting procedure.

Progression of colorectal cancer leading to one of the above should not be reported as a serious adverse event.

12.4 ADVERSE EVENTS OF SPECIAL INTEREST (AESI) to bevacizumab

Non-serious and serious adverse events of special interest are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event) Adverse events of special interest for this study include the following:

- Hypertension ≥ grade 3
- Proteinuria ≥ grade 3
- GI perforation, abscesses and fistulae (any grade)
- Wound healing complications ≥ grade 3
- Haemorrhage ≥ grade 3 (any grade CNS bleeding; ≥ grade 2 haemoptysis)
- Arterial thromboembolic events (any grade)
- Venous thromboembolic events ≥ grade 3

- PRES (or RPLS; any grade)
- CHF ≥ grade 3
- Non-GI fistula or abscess ≥ grade 2

Other Non-Serious or Serious AESIs for this study include the following:

a. *Cases of potential drug-induced liver injury* that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law. The finding of an elevated ALT or AST (> 3 × baseline value) in combination with either an elevated total bilirubin (> 2 × ULN) or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia is considered to be an indicator of severe liver injury. Therefore, investigators must report as an adverse event the occurrence of either of the following:

- treatment-emergent ALT or AST > 3 × baseline value in combination with total bilirubin > 2 × ULN (of which ≥ 35% is direct bilirubin);
- treatment-emergent ALT or AST > 3 × baseline value in combination with clinical jaundice.

The most appropriate diagnosis or, (if a diagnosis cannot be established) the abnormal laboratory values, should be recorded on the Adverse Event eCRF page and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event), either as a serious adverse event or a non-serious adverse event of special interest.

b. *Suspected transmission of an infectious agent by the study drug*, as defined below:

Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

Regardless of relationship or severity, these events will be recorded if they start from the time of the first dose (including partial dose) of study treatment until 6 months after the last study treatment. AESIs will be followed until resolution. All these AESIs must be reported to the Sponsor immediately (i.e. no more than 24 hours after learning of the event).

12.5. Deaths reporting procedure

Any death occurring between the *randomization* and 30 days following the *treatment* must be reported to the Sponsor within 24 hours, regardless of the relation to study drug(s). Deaths occurring later than 30 days after the treatment should be reported on the death report form section of the e-CRF regardless of cause.

12.6. Pregnancies reporting procedure

The investigator must report to the sponsor any pregnancy occurring in a study subject, or in his partner, during the subject's participation in this study. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE.

For a study subject, the outcome of the pregnancy should be followed up carefully, and any abnormal outcome of the mother or the child should be reported.

For the pregnancy of a study subject's partner, all efforts should be made to obtain similar information on course and outcome, subject to the partner's consent.

12.7. Reporting procedure

a. *Reporting Procedures for All Adverse Events*

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by subjects are properly captured in the subjects' medical records.

The following adverse event attributes must be assigned by the investigator: adverse event diagnosis or syndrome(s) (if known, signs or symptoms if not known); event description (with detail appropriate to the event); dates of onset and resolution; severity; outcome, assessment of relatedness to study treatment; and action taken.
Medically significant adverse events considered related to the treatment by the investigator or the sponsor will be followed until resolved or considered stable.
It will be left to the investigator's clinical judgment to determine whether an adverse event is related and of sufficient severity to require the subject's removal from treatment or from the study. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable adverse event. If either of these situations arises, the subject should be strongly encouraged to undergo an end-of-study assessment and be under medical supervision until symptoms cease or the condition becomes stable.

b. *Serious Adverse Events Reporting Procedures*
Serious adverse events will be collected and recorded throughout the study period, defined as through to 6 months after the last dose of the treatment or the end of the study (including the follow-up period), whichever is longer.
The investigator should notify the Sponsor of all serious adverse events occurring at the site(s) in accordance with local procedures, statutes and the European Clinical Trial Directive (where applicable). The Sponsor will medically review all SAEs.
The Sponsor will ensure the notification of the appropriate Ethics Committees, Competent Authorities and participating Investigators of all serious adverse events occurring at the site(s) in accordance with local legal requirements, statutes and the European Clinical Trial Directive.

12.8. Follow-up

Patients withdrawn from the study treatment due to any adverse event will be followed at least until the outcome is determined, even if it implies that the follow-up

continues after the patients has left the trial, and where appropriate until the end of the planned period of follow-up.

In case of serious adverse event, the patient must be followed until clinical recovery is complete and laboratory results have returned to normal, or until symptoms have stabilized. This may imply that the follow-up will continue after the patient has left the trial.

Further information will be noted on the SAE form, by ticking the box marked "follow-up" and will be sent to the Coordinating Center as information becomes available.

12.9 Post-study follow up

After study drug treatment ends, anti-cancer medications taken by the patient should be documented in the eCRF.

Patients will be evaluated approximately every month to determine their survival status. Telephone follow-up is acceptable. Site staff must use caution when contacting the patient's family for this information, especially if they are no longer under the care of the investigator, so as to not inadvertently cause any distress to the family of a patient who is no longer alive.

During this period, If the investigator becomes aware of a serious adverse event with a suspected causal relationship to the investigational medicinal product that occurs after the end of the clinical trial, the investigator shall, without undue delay, report the serious adverse event to the Sponsor.

The investigator should report these events directly to the Sponsor, by completing the Serious Adverse Event / Adverse Event of Special Interest Reporting Form that will be sent to the Coordinating Center.

Subjects who withdraw consent from study drug treatment should enter the post-study follow-up period (unless consent to follow-up is specifically withdrawn).

Details should be documented on the specified Serious Adverse Event Form.

Please fax the report to 050.992069
and mail a .pdf scan version to:
tribe2study@gmail.com

The Sponsor will also send the report to national authorities, Ethic Committees (EC) and investigators as appropriate, according to local regulations.

In addition, the Sponsor shall supply Roche with a copy of all above mentioned safety report regardless of the causality assessment concerning the Pharmaceutical Product administration.

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14. APPENDICES

14.1. Study Synopsis (English Version)

Title

First-line FOLFOXIRI plus bevacizumab followed by reintroduction of FOLFOXIRI plus bevacizumab at progression versus FOLFOX plus bevacizumab followed by FOLFIRI plus bevacizumab at progression in first- and second line treatment of unresectable metastatic colorectal cancer patients

Version

2.1 – 2nd September 2014

Sponsor

Gruppo Oncologico Nord-Ovest G.O.N.O.

Coordinating Investigator

Prof. Alfredo Falcone

U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana
Dipartimento di Ricerca Traslazionale e Nuove Tecnologie – Università di Pisa
Istituto Toscano Tumori

Principal Co-Investigators and Protocol Writers

Dr. Fotios Loupakis

Dr. Chiara Cremolini

U.O. Oncologia Medica 2 Universitaria – Azienda Ospedaliero-Universitaria Pisana
Dipartimento di Ricerca Traslazionale e Nuove Tecnologie – Università di Pisa
Istituto Toscano Tumori

Phase of study

Phase III

Indication

Metastatic colorectal cancer (mCRC)

Study Rationale

- The association of chemotherapy and bevacizumab (bev) is a standard option for the first-line treatment of unresectable metastatic colorectal cancer patients. In particular, an oxaliplatin-based doublet plus Bev is a widely used regimen in this setting.

- Recent results from phase III TRIBE trial demonstrated that the triplet FOLFOXIRI plus bev, compared to first-line FOLFIRI plus bev, provides a significant advantage in terms of PFS and RR and a benefit in terms of OS with a trend towards significance. At the same time, phase II OLIVIA trial showed that FOLFOXIRI plus bev allows to achieve an higher R0 resection rate, with encouraging results in terms of PFS, in the setting of unresectable mCRC patients with liver-limited disease.

- A sustained inhibition of angiogenesis across different lines of treatment is an efficacious strategy in the management of mCRC. As demonstrated by both ML18147 and BEBYP trials, the prosecution of bev beyond the clinical evidence of disease progression provides a survival benefit.

- The optimal duration of chemotherapy plus bev is highly debated. Phase III OPTIMOX1, 2, COIN, CONcePT and GISCAD trials address this issue, substantially evidencing that the choice not to continuously administer the treatment until the evidence of disease progression, but to alternate periods of less intensive chemotherapy or chemo-holidays can be pursued without compromising patients' prognosis.

- Both phase III randomized SAKK 41/06 and CAIRO-3 trials addressed the issue of maintenance with bev. SAKK 41/06 did not demonstrate the non-inferiority of interrupting instead of prosecuting bev until the time of progression. CAIRO-3 trial met its primary endpoint PFS2 evidencing an advantage by the prosecution of Capecitabine plus Bev until disease progression, compared to observation.

- The opportunity to alternate induction periods, able to rapidly induce a relevant tumor shrinkage, and maintenance phases in the disease history of mCRC patients could be considered a valuable strategy.

  On the basis of these considerations, a first-line doublet plus bev followed by a second-line switched doublet (from oxaliplatin to irinotecan and viceversa) plus bev is a standard option for mCRC patients. Only retrospectively collected data are currently available about the efficacy of first-line FOLFOXIRI plus bev followed by second-line rechallenge with FOLFOXIRI plus bev. We therefore designed the present phase III randomized trial of first-line FOLFOXIRI plus bev followed by reintroduction of FOLFOXIRI plus bev at progression versus FOLFOX plus bev followed by FOLFIRI plus bev at progression in first- and second-line treatment of unresectable mCRC patients.

Primary objective

To compare the two proposed strategies in terms of Progression Free Survival 2 (PFS2)

Secondary objectives

To compare the two proposed strategies in terms of :

- 1st-line Progression-free survival (PFS)
- 2nd-line Progression-free survival (2nd-PFS)
- Time to failure of strategy (TFS)
- Overall Survival (OS)
- Response rate in 1st and 2nd-line
- Early Objective Response
- R0 Resection Rate
- Safety profile
- Translational analyses.

Definition of primary endpoint

PFS2 will be defined as beginning with randomization and ending with the first of the following events: a) death; b) disease progression on any treatment given after 1st progression. For patients that will not receive any treatment within 3 months after 1st progression, PFS2 will be equal to PFS. Censoring rules for PFS2 will be: end of study without PD, loss at follow-up. Curative surgery for metastases will not result in censoring for PFS2

Definition of secondary endpoints

PFS is defined as the time from randomization to the first documentation of objective disease progression or death due to any cause, whichever occurs first. The determination of disease progression will be based on investigator-reported measurements. Disease status will be evaluated according to RECIST 1.1 criteria.

2nd PFS is defined as the time from the beginning of the second-line treatment to the documentation of objective disease progression or death due to any cause, whichever occurs first. The determination of disease progression will be based on investigator-reported measurements. Disease status will be evaluated according to RECIST 1.1 criteria. 2nd-PFS will be analyzed both in the intention-to-treat population (whichever 2nd-line treatment will be adopted) and in the per-protocol population.
TFS is defined as the time from randomization to the first of the following events: death; patient requires the addition of a new therapeutic agent (i.e. an agent not included in the original strategy); patient experiences disease progression while being treated with all agents that are components of the initial treatment strategy (except for agents which cannot be used because of persistent toxicity or contraindications); or patient experiences disease progression during a partial or complete treatment holiday from initial treatment strategy and receives no further therapy within 3 months. Subjects who did not have an event as stated above while on study will be censored at the last evaluable radiographic assessment date.
OS is defined as the time from randomization to the date of death due to any cause.

Objective response rate is defined as the percentage of patients, relative to the total of enrolled subjects, achieving a complete (CR) or partial (PR) response, according to RECIST 1.1 criteria.
R0 resection rate is defined as the percentage of patients, relative to the total of enrolled subjects, undergoing secondary R0 resection of metastases.
Overall toxicity rate is defined as the percentage of patients, relative to the total of enrolled subjects, experiencing any adverse event, according to National Cancer Institute Common Toxicity Criteria (version 4.0), during the induction and the maintenance phases of treatment.

Statistical considerations

Based on the assumption that PFS2 of each arm follows an exponential distribution and the true hazard ratio (HR) for PFS2 is 0.77 between experimental group (arm B) vs. control group (arm A), 466 events are required for a two-sided unstratified log-rank test with $\alpha = 0.05$ to have 80% power.
Assuming a proportion of PFS2 equal to 50% at 15 months in arm A, this treatment effect can be translated to a 9% absolute improvement in PFS2 at 15 months in arm B.
Assuming an accrual rate of 200 subjects/year, a minimum follow up period equal to 1.5 years and an overall dropout rate equal to 5%, it is estimated that the enrollment of 654 subjects, randomized in a 1:1 ratio, is required.
The primary analyses of PFS2 will be performed in the ITT population. An unstratified log rank test will be used to compare PFS2 time between the two treatment arms with a two alpha level equal to 0.0131 and 0.0455 at the interim and/or final analyses, respectively.
We plan a group sequential design with 1 interim analysis to assess the primary efficacy endpoint. The analyses will take place at 2/3 (303 events) of the primary events using an O'Brien Fleming alpha-spending rule. The interim analysis will assess superiority of experimental arm to control group for the primary endpoint because the study will only be considered for early termination if superiority is met. The first interim analysis will have a two sided alpha level of 0.0131. According to the O'Brien Fleming spending rule this will leave a two sided alpha level of 0.0455 for the final analysis. The total type I error rate will be only slightly increased.

Inclusion Criteria

- Histologically proven diagnosis of colorectal cancer
- Initially unresectable metastatic colorectal cancer not previously treated with chemotherapy for metastatic disease
- At least one measurable lesion according to RECIST1.1 criteria
- Availability of a tumoral sample
- Male or female of 18-75 years of age
- ECOG PS < or = 2 if aged < 71 years, ECOG PS = 0 if aged 71-75 years
- Life expectancy of at least 12 weeks
- Previous adjuvant chemotherapy allowed only if with fluoropyrimidine monotherapy and more than 6 months elapsed between the end of adjuvant and first relapse
- Neutrophils >1.5 x 109/L, Platelets >100 x 109/L, Hgb >9 g/dl
- Total bilirubin 1.5 time the upper-normal limits (UNL) of the normal values and ASAT (SGOT) and/or ALAT (SGPT) <2.5 x UNL (or <5 x UNL in case of liver metastases) alkaline phosphatase <2.5 x UNL (or <5 x UNL in case of liver metastases)
- Creatinine clearance >50 mL/min or serum creatinine 1.5 x UNL
- Urine dipstick of proteinuria <2+. Patients discovered to have 2+ proteinuria on dipstick urinalysis at baseline, should undergo a 24-hour urine collection and must demonstrate <1 g of protein/24 hr
- Women of childbearing potential must have a negative blood pregnancy test at the baseline visit. For this trial, women of childbearing potential are defined as all women after puberty, unless they are postmenopausal for at least 12 months, are surgically sterile, or are sexually inactive.
- Subjects and their partners must be willing to avoid pregnancy during the trial and until 6 months after the last trial treatment. Male subjects with female partners of childbearing potential and female subjects of childbearing potential must, therefore, be willing to use adequate contraception as approved by the investigator (barriere contraceptive measure or oral contraception)
- Will and ability to comply with the protocol
- Written informed consent to study procedures and to molecular analyses

Exclusion criteria

- Radiotherapy to any site within 4 weeks before the study
- Previous adjuvant oxaliplatin-containing chemotherapy
- Previous treatment with bevacizumab
- Untreated brain metastases or spinal cord compression or primary brain tumours
- History or evidence upon physical examination of CNS disease unless adequately treated
- Symptomatic peripheral neuropathy > 2 grade NCIC-CTG criteria
- Serious, non-healing wound, ulcer, or bone fracture
- Evidence of bleeding diathesis or coagulopathy
- Uncontrolled hypertension and prior histor of hypertensive crisis or hypertensive encephalopathy
- Clinically significant (i.e. active) cardiovascular disease for example cerebrovascular accidents (≤6 months), myocardial infarction (≤6 months), unstable angina, New York Heart Association (NYHA) grade II or greater congestive heart failure, serious cardiac arrhythmia requiring medication
- Significant vascular disease (e.g. aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months of study enrolment
- Any previous venous thromboembolism > NCI CTCAE Grade 3
- History of abdominal fistula, GI perforation, intra-abdominal abscess or active GI bleeding within 6 months prior to the first study treatment.
- Current or recent (within 10 days prior to study treatment start) ongoing treatment with anticoagulants for therapeutic purposes
- Chronic, daily treatment with high-dose aspirin (>325 mg/day)
- Treatment with any investigational drug within 30 days prior to enrollment or 2 investigational agent half-lives (whichever is longer)
- Other co-existing malignancies or malignancies diagnosed within the last 5 years with the exception of localized basal and squamous cell carcinoma or cervical cancer in situ
- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to study treatment start, or anticipation of the need for major surgical procedure during the course of the study
- Lack of physical integrity of the upper gastrointestinal tract, malabsorption syndrome, or inability to take oral medication
- Pregnant or lactating women. Women of childbearing potential with either a positive or no pregnancy test at baseline. Sexually active males and females (of childbearing potential) unwilling to practice contraception during the study (barriere contraceptive measure or oral contraception).

Study treatment

Arm A - mFOLFOX-6 plus bev (to be repeated every 2 weeks for a maximum of 8 cycles)

- Bevacizumab 5 mg/kg iv over 30 minutes, day 1
- Oxaliplatin 85 mg/sqm iv over 2 hours, day 1
- L-Leucovorin 200 mg/sqm iv over 2 hours, day 1
- 5-fluoruracil 400 mg/sqm iv bolus, day 1
- 5-fluoruracil 2400 mg/sqm 48 h-continuous infusion, starting on day 1

If no progression occurs, patients will receive maintenance **5-FU/LV plus bev** at the same dose used at the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal. The prosecution of bev until disease progression is recommended also in the case of interruption of 5-fluoruracil because of adverse events, patient's refusal or investigator's choice. At the time of disease progression patients will receive **FOLFIRI plus bev* (**to be repeated every 2 weeks for a maximum of 8 cycles):

- Bevacizumab 5 mg/kg iv over 30 minutes, day 1
- Irinotecan 180 mg/sqm iv over 2 hours, day 1
- L-Leucovorin 200 mg/sqm iv over 2 hours, day 1
- 5-fluoruracil 400 mg/sqm iv bolus, day 1
- 5-fluoruracil 2400 mg/sqm 48 h-continuous infusion, starting on day 1

*Doses may be modified according to patient's tolerance to 1st-line regimen.
If no progression occurs, patients will receive maintenance **5-FU/LV plus bev** at the same dose used at the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal. The prosecution of bev until disease progression is recommended also in the case of interruption of 5-fluoruracil because of adverse events, patient's refusal or investigator's choice.

Arm B - FOLFOXIRI plus bev (to be repeated every 2 weeks for a maximum of 8 cycles):

- Bevacizumab 5 mg/kg iv over 30 minutes, day 1
- Irinotecan 165 mg/sqm iv over 60 minutes, day 1
- Oxaliplatin 85 mg/sqm iv over 2 hours, day 1
- L-Leucovorin 200 mg/sqm iv over 2 hours, day 1
- 5-fluorouracil 3200 mg/sqm 48 h-continuous infusion, starting on day 1

If no progression occurs during FOLFOXIRI plus bev, patients will receive maintenance 5-FU/LV plus bev at the same dose used at the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal. The prosecution of bev until disease progression is recommended also if 5-fluorouracil is interrupted because of adverse events, patient's refusal or investigator's choice. At the time of disease progression, patients will re-introduce FOLFOXIRI plus bev at the same doses and schedule previously tolerated, for a maximum of 8 cycles. If persistent neurotoxicity ≥G2, FOLFIRI plus bev will administered for a maximum of 8 cycles. If no progression occurs during FOLFOXIRI plus bev, patients will receive maintenance 5-FU/LV plus bev at the same dose used in the last cycle of the induction treatment. 5-FU/LV plus bev will be repeated biweekly until disease progression, unacceptable toxicity or patient's refusal. The prosecution of bev until disease progression is recommended also in the case of interruption of 5-fluorouracil because of adverse events, patient's refusal or investigator's choice.

Study Design Diagram

Arm A:

OR

Arm B:

Study procedures overview

Procedure	Screening (within 28 days before random)	Baseline	Before every cycle[1]	Every 8 wks [1]	After the 2nd evidence of PD
Informed Consent	X				
Complete medical history	X				
Inclusion/Exclusion Criteria Checked	X				
Tumor assessment (total-body CT or abdomen MRI + chest CT)	X			X	
Collection of a CD-ROM copy of CT scan	X			X	
12-lead ECG	X				
ECOG PS	X	X	X	X	
Physical examination	X		X	X	
Complete blood examination[2]	X			X	
Partial blood examination[3]		X	X		
Dipstick proteinuria	X	X	X		
Collection of a paraffin-embedded tissue sample	X				
Collection of blood samples		X		X[4]	
Adverse events and toxicity			X[5]		X[6]
Survival follow up					X

1. Until the 2nd evidence of disease progression
2. Blood count and differential, bilirubin (total and direct), AST, ALT, alkaline phosphatase, albumin, LDH, serum creatinine, glucose, electrolytes (sodium, potassium, calcium), International normalized ratio (INR)/Activated partial Thromboplastin Time (APTT), CEA, CA19.9; pregnancy test (if clinically indicate);
3. Blood count and differential, bilirubin (total and direct), serum creatinine, INR/APTT (only for patients on anticoagulation therapy);
4. Only a the first and second evidence of PD
5. AE assessment to be started after signing of IC until 30 days after last study treatment
6. Follow up on adverse events still ongoing at the time of 2nd PD

Dose reductions and delays for chemotherapeutic agents

TOXICITY AT THE START OF SUBSEQUENT CYCLES OF THERAPY	GRADE/ Values	Irinotecan	Oxaliplatin	5FU
WBC	< 3.000/mm^3	**Hold until resolution**		
Neutrophils	< 1.000/mm^3			
Platelets	< 100.000/mm^3			
Diarrhea	≥ 1			
Mucositis	≥ 1			
Any other non-hematological toxicity	≥ 2			
Hand/foot syndrome	3-4	100%	100%	**STOP**
Neurotoxicity	≥ 3	100%	**STOP**	100%

PREVIOUS TOXICITY	GRADE	Irinotecan	Oxaliplatin	5FU
Neutropenia >5 days	4	**75%**	**75%**	100%
Febrile Neutropenia	4			
Thrombocytopenia	3-4			
Diarrhea	3	**75%**	100%	**75%**
Diarrhea	4	**50%**	100%	**50%**
Stomatitis	3	100%	100%	**75%**
Stomatitis	4	100%	100%	**50%**
Myocardial Ischemia	NA	100%	100%	**STOP**

Dose reductions and delays for bevacizumab

Event	Grade	Adjustment to bev
Hypertension	3	If not controlled by 3-drug medication, permanently discontinue
	4 (Hypertensive crisis and encepalopathy)	Permanently discontinue
Hemorrhage	Any grade CNS	Permanently discontinue
	≥2 (pulmonary)	Permanently discontinue
	≥3 (non-pulmonary/non-CNS)	Permanently discontinue
Venous thrombosis	3	Hold temporarily
	4	Permanently discontinue
Arterial thrombosis	Any Grade	Permanently discontinue
Congestive Heart Failure	≥ 3	Permanently discontinue
Proteinuria	2-3	- *For 2+ dipstick*: may administer bev, obtain 24-hour urine sample prior to next bev dose Suspend bev for ≥2 g /24 hours and resume when proteinuria is <2 g /24 hours and protein creatinine ratio <2.0 - *For 3+ dipstick*: obtain 24 hour urine sample prior to bev administration Suspend bev for ≥2 g /24 hours and resume when proteinuria is <2 g /24 hours and protein creatinine ratio <2.0
	4	Permanently discontinue
GI perforation	Any grade	Permanently discontinue
PRES/RPLS	Any grade	Permanently discontinue
Fistula	Any grade TE fistula	Permanently discontinue
	≥3 (other than TE)	Permanently discontinue
Febrile neutropenia/ thrombocytopenia	4	Hold temporarily
Other unspecified bev-related AE	3	Hold until recovery to ≤ Grade 1
	4	Permanently discontinue

Total number of centers

About 60 Italian Oncology Units

Study length

Study length is planned to be about 4.5 years since the enrollment is expected to be about 3 years, with a minimum period of follow-up of 18 months

Enrollment and data management

Registration, randomization and data collection are centralized at Ufficio Sperimentazioni Cliniche - Unit of Oncology, Azienda Ospedaliero-Universitaria Pisana and Clinical Trials Coordinating Center Istituto Toscano Tumori

14.2. RECIST 1.1

Response and progression will be evaluated in this study using the RECIST criteria version 1.1. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used**.**

Measurable Disease

Tumor lesions: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) with a minimum size of

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm) or MRI. If scans with slice thicknesses greater than 5mm are used, the minimum size should be twice the slice thickness.
- 20 mm by chest x-ray
- 10 mm caliper measurement by clinical examination (lesions which cannot be accurately measured with calipers should be recorded as non-measurable

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components that can be evaluated by CT or MRI, can be considered as measurable lesions if the soft tissue component meets the definition of measurability. All tumor measurements must be recorded in millimetres (or decimal fractions of centimetres). Tumor lesions situated in a previously irradiated area are not considered measurable unless there has been demonstrated progression in the lesion.

Non-Measurable Disease: All other lesions (or sites of disease), including small lesions

(longest diameter <10 mm or pathological lymph nodes with ≥10 to < 15mm short axis) are considered non-measurable disease. Leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitic involvement of skin or lung, inflammatory breast disease, abdominal masses/ abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques and blastic bone lesions are all non-measurable.

Target Lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs should be identified as *target lesions* and be recorded and measured at baseline. These 5 lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs and should be suitable for reproducible repeated measurements. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for *all target lesions* will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize any objective tumor regression of the measurable dimension of the disease. If there are >5 measurable lesions, those not selected as *target lesions* will be considered together with non-measurable disease as *non-target lesions*.

Non-target Lesions: All non-measurable lesions (or sites of disease) plus any measurable lesions over and above the 5 listed as *target lesions*. Measurements are not required but these lesions should be noted at baseline and should be followed as "present", "absent" or in rare cases "unequivocal progression".

Best Response: All subjects will have their BEST RESPONSE on study classified as outlined below:

Complete Response (CR): Disappearance of all clinical and radiological evidence of tumor (both *target* and *non-target*). Any pathological lymph nodes (whether target or non target) must have a reduction in short axis to < 10mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target

lesions taking as reference the baseline sum, no unequivocal progression of existing non target lesions and no appearance of new lesions.

Stable Disease (SD): Steady state of disease. Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, no unequivocal progression of existing non target lesions and no appearance of new lesions.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm. Unequivocal progression of existing non target lesions or the appearance of one or more new lesions will also constitute progressive disease.

Table 1: Response for patients with Target and Non-Target Lesions

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
PD	Any	Yes or No	PD
Any	Any	Yes	PD
Any	PD	Yes or No	PD

Table 2: Response for patients with Non-Target Lesions only

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/Non-PD	No	Non-CR/non- PD*
Not evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* Non-CR/non-PD is preferred over "stable disease" for non-target disease.

Methods of Measurement - The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Clinical Lesions - Clinical lesions will only be considered measurable when they are superficial (e.g. skin nodules, palpable lymph nodes) and ≥ 10mm diameter as assessed using calipers. For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is recommended.

Chest X-ray - Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, chest CT is preferable.

CT / MRI - CT is the best currently available and reproducible methods to measure target lesions selected for response assessment. CT scans should be performed with cuts of 5 mm or less in slice thickness. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable. This applies to the chest, abdomen and pelvis. Head & neck and extremities usually require specific protocols.

Ultrasound - Ultrasound is not useful in assessment of lesion size and should not be used as method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised.

Endoscopy / Laparoscopy - The utilization of these techniques for objective tumor evaluation is not advised.

Cytology / Histology - These techniques can be used to differentiate between PR and CR in rare cases (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

14.3. NCI Common Terminology Criteria for Adverse Events

This study will utilize the NCI Common Terminology Criteria for Adverse Events Version 4.0 for toxicity and serious adverse event reporting. A copy of the CTC Version 4.0 can be downloaded from the CTEP home page:

http/ctep.cancer.gov:protocolDevelopment:electronic_applications:ctc.htm - ctc_40)

All appropriate treatment areas should have access to a copy of the CTC Version 4.0.

14.4 SAE report form

Please send completed form to tribe2study@gmail.com

SPONSOR USE ONLY
Receipt date of this report (stamp or date)

Serious Adverse Event Report Form

TRIBE2 study

EUDRACT 2014-004436-19

TYPE OF REPORT	
☐ Initial	☐ Follow-up

A. REPORTER INFORMATION

Reporter's First Name	Reporter's Last Name
Investigator's First Name (if different from Reporter)	Investigator's Last Name (if different from Reporter)
Address	City
Country	Phone Number
E-Mail:	Fax Number

B. SUBJECT INFORMATION

Subject ID

_____ / _____ / _____

Study name / Center name. / Patient code

Subject Initials	Sex	Height	Weight
	☐ Female ☐ Male	cm	kg

Date of Birth (dd/mmm/yyyy) OR Age at Time of Adverse Event (Specify unit, e.g. years months, etc.)

/ /

Ethnicity/Race ☐ American Indian/Alaska native ☐ Asian ☐ Black or African American ☐ Caucasian/White ☐ Hispanic or Latino

☐ Native Hawaiian or other Pacific islander ☐ other _____

C. RELEVANT MEDICAL HISTORY

Condition/Disorder	Start Date (dd/mmm/yyyy)	End Date (dd/mmm/yyyy)	Ongoing
	/ /	/ /	☐
	/ /	/ /	☐
	/ /	/ /	☐
	/ /	/ /	☐
	/ /	/ /	☐

D. CONCOMITANT MEDICATIONS

Drug Trade Name	Single Dose	Frequency of Administration	Route	Start Date (dd/mmm/yyyy)	Stop Date (dd/mmm/yyyy)	Indication
				/ /	/ /	
				/ /	/ /	
				/ /	/ /	
				/ /	/ /	
				/ /	/ /	

E. INVESTIGATIONAL MEDICINAL PRODUCT(S)

Indication of Investigational Medicinal Product(s)

1 Investigational Medicinal Product Name / Route of Administration:

Not yet administered:	
Date of first administration: (dd/mmm/yyyy) / /	Dose/Unit:
Date of most recent administration prior to SAE: (dd/mmm/yyyy) / /	Dose/Unit:
Number of cycles prior to SAE:	

ACTIONS TAKEN REGARDING THIS INVESTIGATIONAL MEDICINAL PRODUCT

☐ Temporary discontinued on: ____/____/____	Event subsided?	☐ Yes	☐ No	☐ Unknown
. . . . .	If "yes", how long after cessation of treatment? _____			
☐ If temporary discontinued, restarted on: ____/____/____ .	At previous dose?	☐ Yes	☐ No	☐ Unknown
. . . . .	Event subsequently reappeared?	☐ Yes	☐ No	☐ Unknown
☐Permanently discontinued on: ____/____/____.				
☐ Dose Reduced on: ____/____/____	Event subsided?	☐ Yes	☐ No	☐ Unknown
☐ Treatment Continued without Change				
☐ Not Applicable				
☐ Unknown				

2	Investigational Medicinal Product Name / Route of Administration	
Not yet administered:		
Date of first administration: (dd/mmm/yyyy) / / (hh:mm) /		Dose/Unit:
Date of most recent administration prior to SAE: (dd/mmm/yyyy) / / (hh:mm) /		Dose/Unit:
Number of cycles prior to SAE:		

ACTIONS TAKEN REGARDING THIS INVESTIGATIONAL MEDICINAL PRODUCT

☐ Temporary discontinued on: ____/____/____	Event subsided?	☐ Yes	☐ No	☐ Unknown
. . . . .	If "yes", how long after cessation of treatment? _____			
☐ If temporary discontinued, restarted on: ____/____/____	At previous dose?	☐ Yes	☐ No	☐ Unknown
. . . . .	Event subsequently reappeared?	☐ Yes	☐ No	☐ Unknown
☐Permanently discontinued on: ____/____/____.				
☐ Dose Reduced on: ____/____/____	Event subsided?	☐ Yes	☐ No	☐ Unknown
☐ Treatment Continued without Change				
☐ Not Applicable				
☐ Unknown				

F. ADVERSE EVENT(S) *(If there are more than three adverse events, reprint this page as many times as is necessary.)*						
*Report adverse event **diagnosis (ses)**, if not available provide sign(s) and symptom(s)*	AE _____:		AE _____:		AE _____:	
Onset Date and Time (dd/mmm/yyyy hh:mm)	____/____/____ __:__		____/____/____ __:__		____/____/____ __:__	
Resolution Date (dd/mmm/yyyy)	____/____/____		____/____/____		____/____/____	
Duration, if less than 24h	_____ ☐ hr ☐ min		_____ ☐ hr ☐ min		_____ ☐ hr ☐ min	
SEVERITY						
Severity Grade *Use either NCI-CTC grading **OR** Qualitative Scale*	☐ 1	☐ Mild	☐ 1	☐ Mild	☐ 1	☐ Mild
	☐ 2	☐ Moderate	☐ 2	☐ Moderate	☐ 2	☐ Moderate
	☐ 3	☐ Severe	☐ 3	☐ Severe	☐ 3	☐ Severe
	☐ 4	☐ Life-threatening	☐ 4	☐ Life-threatening	☐ 4	☐ Life-threatening
	☐ 5	☐ Death	☐ 5	☐ Death	☐ 5	☐ Death
SERIOUSNESS						
Resulted in Death	☐		☐		☐	
Is Life-Threatening	☐		☐		☐	
Requires/Prolongs Hospitalization	☐		☐		☐	
Persistent/Significant Disability/Incapacity	☐		☐		☐	
Medically Significant	☐		☐		☐	
Is Congenital Anomaly/Birth Defect	*Parent-Child/Foetus Report Form must be completed*		*Parent-Child/Foetus Report Form must be completed*		*Parent-Child/Foetus Report Form must be completed*	
OUTCOME						
Unknown (only applicable if subject is lost to follow-up)	☐		☐		☐	
Fatal (AE resulted in death)	☐		☐		☐	
Ongoing	☐		☐		☐	
Resolved without Sequelae	☐		☐		☐	
Resolved with Sequelae	☐ Specify:		☐ Specify:		☐ Specify:	

RELATION TO THE INVESTIGATIONAL MEDICINAL PRODUCT(S) / STUDY TREATMENT *If an event is unrelated, please indicate any other causality factors in the appropriate section and/or provide further details in the narrative (Description of Adverse Event(s)).*						
	Related	Unrelated	Related	Unrelated	Related	Unrelated
Investigational Medicinal Product 1	☐	☐	☐	☐	☐	☐
Investigational Medicinal Product 2	☐	☐	☐	☐	☐	☐

G. DESCRIPTION OF ADVERSE EVENT(S)

Provide a detailed description of AE, i.e. clinical course of event(s), signs, symptoms, laboratory results, treatment of AE, etc.

1) In Case of Death

Cause of Death: ☐ AE ☐ Other If "other", specify:

Date of Death: ____/____/____ Autopsy performed? ☐ Yes ☐ No If "yes", please attach autopsy report if available.

2) In Case of Hospitalization or Prolonged Hospitalization

Admission Date: ____/____/____ Discharge Date: ____/____/____ ☐ Not Discharged

H. RELEVANT TESTS/PROCEDURES/LABORATORY TESTS TO CONFIRM ADVERSE EVENT

I. OTHER RELEVANT RISK FACTORS

☐ Alcohol Use	☐ Physical Therapy	☐ Contraceptive	☐ Smoking
☐ Pace Maker	☐ Drug Dependence	☐ Radiation Therapy	☐ Diet
☐ Metabolic Disorders	☐ Drug Abuse	☐ Obesity	☐ Allergy
☐ Implants	☐ Other, specify: _____		

J. CAUSALITY FACTORS OTHER THAN TRIAL TREATMENT

☐ Concomitant Medication, please specify suspected drug:_____(record details in section D)

☐ Medical History, please specify disease:_____(record details in section C)

☐ Disease Under Study ☐ Disease Progression; specify:_____

☐ Trial Procedure ☐ Other; specify:_____

K. INVESTIGATOR SIGNATURE

Investigator's Signature

Date of Report: