Regorafenib Assessment in Refractory advanced Colorectal Cancer: RegARd-C study protocol.

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Regorafenib Assessment in Refractory advanced Colorectal Cancer: RegARd-C study protocol.

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Abstract

Introduction

Regorafenib was recently approved for patients with pretreated advanced colorectal cancer (aCRC), despite a moderate improvement of the patients’ outcome, and significant toxicities. Based on previous studies showing that early FDG-PET-based metabolic response assessment (MRA) might adequately select patients unlikely to benefit from treatment, the RegARd-C trial uses early MRA to identify likely non-responders to regorafenib in a population of aCRC patients and guide a comprehensive evaluation of genomic and epigenetic determinants of resistance to treatment.

Methods and analysis

RegARd-C is a multicentric prospective study. Its primary objective is to identify non-beneficiers from regorafenib given at 160 mg/day, 3 weeks/4 in a population of patients with pretreated aCRC. Baseline PET is repeated at D14 of the first treatment course. MRA is blinded for the investigators. Overall Survival (OS) is the primary endpoint and will be correlated with metabolic parameters and (epi)genetic alterations assessed from tumor and serial blood samples. A target sample size of 105 evaluable patients (70 as derivation set and 35 as validation set), is considered as sufficient to validate an expected hazard ratio (HR) for OS of responders compared to non-responders significantly < 1 (with 80% power and 1-sided 5% alpha in case of a true HR ≤ 0.59 and a responders rate of 47%).

Ethics and dissemination

The study was approved by the Institut Jules Bordet’s competent ethics committee and comply with the Helsinki’s declaration or the belgian laws and regulations, whichever provides the greatest protection for the patient, and follows the International Conference on Harmonization E 6 (R1) Guideline for Good Clinical Practice, reference number CPMP/ICH/135/95.

The protocol and the trials results, even inconclusive, will be presented at international oncology congresses, and published in peer-reviewed journals. Genomic and epigenetic data will be made available in public open datasets.
Registration

EudraCT number: 2012-005655-16

ClinicalTrials.gov number: NCT01929616

Funding

This study is supported by an unrestricted grant from Bayer Healthcare Pharmaceutical.

Key Words:

Colorectal Cancer, FDG PET, PET-CT, Next-generation sequencing

Strengths and limitations of this study:

1. Strengths

• Prospective multicentric academic trial
• Inclusion/exclusion criteria compatible with the study objectives (to determine biomarkers predictive of the patient’s outcome under treatment by regorafenib monotherapy in metastatic colorectal cancer refractory to all known medications with FDG-PET-assessable diseases)
• All PET-CT centers accredited according to EARL (European Association for Nuclear Medicine Research Limited)
• Central review and quality-control by an Imaging Core laboratory of the FDG-PET/CT data
• Prospective collection of biological specimen (frozen and paraffin-embedded tissue blocks, sequential frozen plasmatic samples for ctDNA research, whole blood) for comprehensive genomic and epigenetic analysis guided by metabolic imaging definition of responding/non-responding disease
• Statistical hypothesis foreseeing a exploration set and a validation set and allowing the validation of a metabolic response hypothesis based on previous work taking in account the tumoral heterogeneity in response.
2. Weaknesses
   • Non-randomized design
A. Introduction

Colorectal Cancer

With a 35/100.000/year incidence rate in the developed world, colorectal cancer affects about 150.000 people per year in Western Europe.[1] About half of the patients will develop a metastatic disease, carrying a grim prognosis if unresectable with curative intent. Progress in chemotherapy has been substantial during the last decade, allowing rare, but well-advertised secondary resections of primarily unresectable metastatic disease. In the palliative setting, chemotherapy aims essentially at extending survival and the use of all available drugs (fluoropyrimidines, oxaliplatin, irinotecan, bevacizumab, anti-EGFR antibodies) either successively or concomitantly has increased the median OS of patients to more than 25 months.[2-6] However, no single drug or any combination is able to cure metastatic disease, and the tumor will eventually become resistant to all known medications, leading to the patient’s death.

Regorafenib

Regorafenib (BAY 73-4506) is a novel oral diphenylurea-based multikinase inhibitor, shown in preclinical studies as a potent inhibitor of several angiogenic and stromal receptor tyrosine kinases (RTKs), including vascular endothelial growth factor receptor (VEGFR)-1, -2, -3, platelet-derived growth factor receptor (PDGFR)-β, fibroblast growth factor receptor (FGFR)-1, and TIE2. In addition, regorafenib inhibits various oncogenic RTKs (c-KIT and RET) and intracellular signaling kinases (cRAF/RAF-1, B-RAF, and B-RAF V600E mutant). The exact mode of action of regorafenib remains however unknown, even if it is probably associated with antiangiogenic and antiproliferative effects, mostly through RAF inhibition. Data from a phase I trial [7 8] has established the recommended dose at 160mg/day 3 weeks out of 4. A recent phase III trial in aCRC refractory to all known medications (CORRECT) [9], has randomized 760 patients between regorafenib (n=505) and placebo (n= 255), showing a small but statistically significant advantage for OS (median 6.4 months versus 5 months, one-sided p-value 0.005) and progression-free survival (PFS) (median 1.9 months versus 1.7 months, one-sided p-value <0.000001) for regorafenib. This drug has the potential to become a standard
therapy for the treatment of mCRC patients who have been previously treated with all approved therapies. Nevertheless, the toxicity of this medication is important next to the palliative situation in which it was developed. Both the limited survival benefit and the clinically meaningful toxicity highlight the need to develop tools able to quickly identify the patients unlikely to benefit from the treatment in order to spare them from unnecessary side effects.

**Early FDG PET-CT**

Standard radiological response measurements (RECIST criteria, modified RECIST, WHO) rely entirely upon measuring the size of the tumor with CT, ultrasound, or MRI, and are only applicable under restrictive conditions (well defined lesions, adequate minimum size, at least six weeks of chemotherapy). Response rates in advanced solid tumors correlate poorly with other patient outcomes, such as PFS and OS.[10 11] Several techniques with the potential to detect early response are emerging: serial FDG PET-CT, dynamic MRI (DCE-MRI) and diffusion MR techniques, and circulating tumor cells (CTCs). Among these, FDG PET-CT is the most studied and promising. It is widely available in Belgium, and its value in detecting early metabolic changes predictive of later outcome is currently being assessed.[12 13] Recent data suggest that use of serial FDG PET-CT imaging to assess tumor metabolism can reliably detect refractory disease. Our research group prospectively studied 41 patients with mCRC undergoing first- or second-line chemotherapy.[13] Serial FDG PET-CT was performed at baseline and 14 days after the first cycle of chemotherapy. The metabolic changes were compared to the morphologic response evaluated by CT according to RECIST criteria. A RECIST response was observed in 10/23 (43%) PET responding patients and in 0/17 (0%) PET non-responding patients (p=0.002). The metabolic assessment’s predictive performance for RECIST response was 100% for sensitivity (95% confidence interval [CI] 69% to 100%), 57% for specificity (95% CI 37% to 75%), 43% for positive predictive value (95% CI 23% to 66%), and 100% for negative predictive value (95% CI 80% to 100%). Comparing patients who had metabolic responses to those who did not, the hazard ratio (HR) was 0.28 (95% CI 0.10–0.76) for OS and 0.57 (95% CI 0.27–1.21) for PFS. This suggests that FDG PET-CT may be used for the early detection of non-responding patients. Additionally, we conducted another FDG PET-CT driven metabolic study with a similar design in
92 patients with advanced refractory CRC treated with a combination of sorafenib and capecitabine (SoMore study).[14] The aim of this metabolic study was to identify patients unlikely to benefit from the therapy. The metabolic analysis of this population will be used as a model for regorafenib. The most important finding related to this metabolic analysis was the identification of a prognostic value for early metabolic homogeneous response.[15] Indeed, among 79 patients who underwent baseline PET examination as well as day 14 examination, 37 patients (47%) were found responding homogeneously in all their lesions to treatment with combined sorafenib-capecitabine. These patients had prolonged survival after day 14 compared to all the other patients (HR=0.59, 95% CI: 0.37-0.96, p=0.03).

**Genomic and Epigenomic assessments**

Recent advances in Next Generation Sequencing (NGS) technologies and data analysis have enabled to explore patients’ specific genomic/ somatic alterations in their cancer genome in a relatively timely, costly and clinically efficient manner. The design of the RegARd-C study provides a unique opportunity to identify and characterize molecular factors that could predict PFS and OS for patients treated with regorafenib. Metabolic imaging could enable us to define the subpopulations of patients unlikely to benefit from this medication, thereby increasing the likelihood of finding determinants of resistance to therapy. Molecular translational research could also provide tools to distinguish patients who will or will not benefit from therapy, for instance by analyzing the genetic and epigenetic differences between the metabolically non-responding patients and the remaining subpopulation. Moreover, as tumor cells liberate naked DNA (circulating tumor DNA, ctDNA) into the blood stream after necrosis or apoptosis, we will investigate whether tumor-specific rearrangements can be detected in plasma, which will be used as “liquid biopsy”. Although “liquid biopsies” are minimally invasive and may represent a molecular assessment of the overall cancer, the detection of ctDNA still has some difficulties that we will take into account: the distinction between ctDNA and cell-free DNA and the very low proportion of ctDNA which may hinder its exact quantification.

**Aim of the study**
RegARd-R aims at identifying in a population of patients bearing advanced, refractory CRC, those who draw no benefit from treatment with regorafenib. OS will be used as primary endpoint. Covariates that will be analyzed in relationship with OS will be metabolic parameters obtained at baseline and very early following treatment initiation as well as genetic, epigenetic and molecular aberrations (before and after treatment) assessed from tumor biopsies and serial blood samples, in addition to known clinical and pathological factors. The molecular aberrations will be investigated using gene expression profiling, RNA and exome sequencing, and methylation profiling of the tumor biopsies and repeated blood samples taken during therapy.

B. Methods and analysis

Study Design

The study was designed as a single-arm, prospective, non-randomized, non-comparative, open label phase II trial, with all patients being accrued in one stage. No early stopping rules are used. A FDG PET-CT will be performed at baseline (D-7-D0) and repeated after 14 to 17 days from start of the first cycle. The clinicians will remain blinded to the assessment of FDG PET-CT response. Clinical and biological evaluation will be made every cycle, starting at day 28 of the second cycle. RECIST 1.1-based radiological assessment (CT or MRI) will be made every 2 cycles, starting at day 28 of the second cycle. Treatment will be continued until disease progression, unacceptable toxicity or any other reason (study withdrawal, lost to follow-up, death,…) (see Figure 1 for an overview of the study design).

Objectives

To identify in a population of patients bearing advanced, refractory CRC, those who draw no benefit from treatment with regorafenib. OS will be used as primary endpoint.

Secondary objectives are to 1) analyze PFS and response rate (RR) in relationship with the same covariates as for OS, 2) assess regorafenib efficacy (OS, PFS, RR) and safety profile in this study population, 3) assess the Disease control rate (DCR =
Complete response [CR] + partial response [PR] + stable disease [SD]), 4) compare the relative benefit (OS, PFS) of regorafenib according to history of treatment with bevacizumab, and 5) validate the relationship that was found, in a previous study [14] conducted in the same patient population treated with sorafenib and capecitabine, between OS and early metabolic consistent response in all the patient’s lesions.

Patient Selection Criteria

Inclusion Criteria

Participants must

- have histologically proven adenocarcinoma of the colon or the rectum that is metastatic or unresectable and for which standard treatments do not exist or are no longer effective. The tumor should be refractory to all standard chemotherapy agents (fluoropyrimidines, irinotecan, and oxaliplatin) and anti-EGFR monoclonal antibodies in case of RAS wild type (cetuximab, or panitumumab) administered before study entry. Patients treated with oxaliplatin in an adjuvant setting should have progressed during adjuvant therapy or within 6 months of completion of this treatment. Patients who do not meet strictly these criteria, but for whom further treatment with oxaliplatin would be prohibited (for instance allergic reaction, residual neurotoxicity grade ≥ 2) are allowed for inclusion. Prior treatment with bevacizumab and/or aflibercept is allowed but not mandatory;

- have signed a written informed consent (approved by an Independent Ethics Committee [IEC] and obtained prior to any study specific screening procedures);

- be aged 18 or older;

- have a life expectancy of greater than 12 weeks;

- have an ECOG performance status ≤ 1;

- have normal organ and bone marrow function as defined below: Leukocytes > 3,000/mcL, with an absolute neutrophil count > 1,500/mcL, platelets >
100,000/mcL, Hb≥9g/dl, total bilirubin ≤ 1.5 × institutional ULN, AST/ALT/P-Alk levels ≤ 2.5 × institutional ULN (≤ 5x institutional ULN in case of liver metastatic involvement), P-Alk levels ≤5 x institutional ULN, international normalized ratio of Prothrombin Time (INR) ≤ 1.5 x institutional ULN, creatinine within 1.5 × normal institutional upper limits, or creatinine clearance > 30mL/min.

- Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

- Presence of a previously collected frozen or FFPE primary or metastatic tumor. Frozen tissue from the primitive tumor is preferred. If no archived tissue is available for the patient, the tissue should be collected freshly in the primary (preferentially) or a metastatic lesion before study entry.

- Presence of at least one metabolically measurable tumoral lesion on FDG PET-CT fulfilling following criteria: Size ≥ 1.5cm and FDG uptake above the background liver uptake.

**Exclusion Criteria**

Excluded from the study are patients identified with any of the following conditions or characteristics:

- Prior treatment with sorafenib or regorafenib.

- Participants with previous cancer that are not disease-free for at least for 5 years prior to registration, EXCEPT for curatively treated cervical cancer in situ, non-melanoma skin cancer and superficial bladder tumors [Ta (Non-invasive tumor), Tis (Carcinoma in situ) and T1 (Tumor invades lamina propria)].

- Participants who have had chemotherapy or targeted therapy within 2 weeks prior to entering the study.
• Participants who have had a major surgery or radiotherapy within 4 weeks prior to entering the study.

• Unresolved toxicity higher than NCI-CTCAE (version 4.0) Grade 1 attributed to any prior therapy/procedure excluding alopecia and oxaliplatin induced neurotoxicity ≤Grade 2.

• Participants receiving any experimental agents.

• Participants with known brain metastases.

• Bleeding diathesis, history of cardiovascular ischemic disease or cerebrovascular incident within the last six months.

• Any hemorrhage or bleeding event NCI-CTCAE v.4 Grade ≥3 within 4 weeks prior to the start of study medication.

• Uncontrolled concurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure (New York Heart Association (NYHA) class ≥2), unstable angina pectoris (defined by angina symptoms at rest or new-onset angina started within the last 3 months), cardiac arrhythmia requiring antiarrhythmic therapy (beta blockers or digoxin are permitted).

• Uncontrolled hypertension (defined by systolic blood pressure >150 mmHg or diastolic blood pressure >90 mmHg despite optimal medical management).

• Patients with phaeochromocytoma.

• Patients with seizure disorder requiring medication.

• Any history of organ allograft.

• Pleural effusion or ascites affecting respiration (NCI CTCAEv.4 Grade ≥ 2 dyspnea).

• Uncontrolled diabetes.

• Non-healing wound, ulcer, or bone fracture.

• Known history of human immunodeficiency virus (HIV) infection, or active hepatitis B or C, or chronic hepatitis B or C requiring treatment with antiviral therapy.

• Interstitial lung disease with ongoing signs and symptoms.

• Renal failure requiring hemo-or peritoneal dialysis.

• Dehydration NCI-CTCAE v.4 grade> 1.
• Substance abuse, medical, psychological or social conditions that may interfere with the patient’s ability to understand informed consent and participation in the study or evaluation of the study results.

• Known hypersensitivity to the study drug or excipients in the formulation.

• Any illness or medical conditions that are unstable or could jeopardize the safety of the patient and his/her compliance in the study.

• Pregnant or lactating women.

• Subjects unable to swallow oral medications.

• Subjects with thrombotic, embolic, venous, or arterial events, such as cerebrovascular accident (including transient ischemic attacks) deep vein thrombosis or pulmonary embolism within 6 months of start of study treatment within 6 months of informed consent.

• Persistent proteinuria > Grade 3 NCI-CTCAE v4.0 (> 3.5 g/24 hours, measured by urine protein: creatinine ratio on a random urine sample).

**Intervention to be measured:**

**FDG-PET/CT Imaging**

Increased glycolysis is one of the hallmarks of cancer. FDG, an analogue of glucose labeled with a positron emitting isotope of Fluor (F\(^{18}\)), is actively taken up in cancer cells of many tumor types. The positrons emitted by the FDG are detected by a dedicated camera, enabling the visualization of cellular glycolytic activity.[16] The criteria listed below define the minimal requirements for metabolic imaging assessment of tumor response:

• Blood glucose < 150 mg/dl at the time of FDG administration. Insulin or oral antidiabetic medication is not allowed on the days of FDG-PET/CT imaging.

• Delay between the first FDG-PET/CT imaging and the start of regorafenib ≤ 7 days. The second FDG-PET/CT imaging is performed on day D14 (ideal range: D14-D17). In case of treatment interruption, the second PET scan can be delayed until maximum D21 of first cycle. It must be performed while the patient is under the study medication since at least 4 days.

All participating FDG-PET/CT centres were required to obtain the FDG-PET/CT’s EARL accreditation and keep it for the whole duration of the study.[17] All images are
centralized by an imaging core lab with central quality control by an imaging expert and central review by two independent reviewers. The results of the metabolic assessment remains blinded for the clinical investigator.

**Translational Research Genomic Analysis**

Blood samples for plasma preparation (2 x 9 mL for each time point) will be collected (and frozen) at baseline (D-7↔D0) and simultaneously with the second PET. An extra 9 mL whole blood sample will be collected at baseline in order to distinguish somatic from germline mutations. Plasma samples will also be obtained every 2 cycles, starting from the first cycle and (simultaneously with biology) up to 1 year after the start of therapy. Moreover, a final plasma sample will be obtained at disease progression (see Figure 2 for overview of sample collection). Optional fresh frozen tumor tissue from a FDG PET targetable metastatic or primary lesion will be obtained before study entry and at disease progression. Previously collected frozen or FFPE primary or metastatic tumor needs to be available at the study entry. The translational research group will study the genetic and epigenetic aberrations that are associated with the patient’s outcome (PFS, OS) and with metabolic response after treatment with regorafenib. The molecular aberrations will be investigated using gene expression profiling, RNA and exome sequencing, and methylation profiling of the tumor biopsies and repeated blood samples taken during therapy.

**Follow-up**

Follow-up procedures, performed every 2 months will include physical examination, vital signs and ECOG performance status, laboratory tests and blood samples for translational research analysis, as well as radiological assessment of the tumours.

According to the European Union (EU) Clinical Trial Directive for each participating European Union (EU) country, the end of the study will be reached when the last visit under treatment protocol of the last subject for all centers has occurred. The patients will continue to be followed afterwards every 2 months till disease progression or death or refusal, whichever occurs first. Patients may be offered any further treatment available, at the investigator’s discretion.

**Statistical Considerations**
Analyses will be carried out looking at constructing signatures that are prognostic for OS as well as PFS and RR. Baseline data will be used for some models and all eligible patients (intent to treat population) will be included in these analyses. Models integrating data in the short follow-up after treatment will also be developed. In that later situation, only patients having undergone the necessary examinations will be included in the analyses. Time zero for measuring time to event variables will be adapted and will be dependent on the timing of further examinations. Analyses will be adjusted for multiplicity. As no formal hypothesis can be done on the discriminating value of genomic biomarkers, it was decided to target a sample size of 105 evaluable patients and to use the first 70 patients as derivation set and the 35 last patients as validation set. It should be stressed however that the total sample size of 105 evaluable patients is sufficient to validate the hypothesis generated by the SoMore study.\[14\] To detect in a validation series that the HR for responders compared to non-responders is significantly < 1 (with a power of 80% in case of a true HR≤ 0.59 and a 1-sided alpha level of 5% and a rate of responders of 47%), we need to observe 89 events. We then plan to carry out a validation comparison between early homogeneous responders and the other patients once we will have reached 89 events for these 105 evaluable patients. For this validation comparison, there will be no split of the total sample. In order to reach this number of evaluable patients, the overall sample size of registered patients will be adapted during the study accrual. Taking into account an expected 20-25% drop-out rate between registration and the time of further examinations, between 124 and 140 patients will be accrued. The analyses for derivation will be carried out when at least 80% of events (deaths) will have been observed. The analyses for validation will occur also when 80% of events will be reached. The study is designed as a single-arm study, with all patients being accrued in one stage. No early stopping rules will be used. The clinicians will remain blinded to the assessment of FDG PET-CT response and of the whole molecular analyses.

C. ETHICS AND DISSEMINATION

Ethical Considerations

Patient Protection
The principal investigator ensures that this study conforms to the Declaration of Helsinki (available at [http://www.wma.net/en/30publications/10policies/b3/](http://www.wma.net/en/30publications/10policies/b3/)) or the

The competent ethics committee of the Institut Jules Bordet approved the protocol, as required by applicable national legislation.

**Dissemination**

The protocol and the trials results, even inconclusive, will be presented at international oncology congresses, and published in peer-reviewed journals. Genomic and epigenetic data will be made available in public open datasets.

**Trial Sponsorship, Financing and Insurance**

This study is supported by an unrestricted grant from Bayer Healthcare Pharmaceutical.

**Discussion and Conclusions**

Regorafenib, an oral multi kinase inhibitor that shares with sorafenib several targets involved in tumour angiogenesis, oncogenesis, and tumour microenvironment, improves the patients’ outcome, but at the cost of significant toxicities, underscoring the need to identify those who will benefit from therapy. A previous study (SoMore trial) showed that early FDG PET-based metabolic response assessment may adequately discriminate patients with chemorefractory aCRC unlikely to benefit from a sorafenib-capecitabine combination. RegARd-C aims to explore early FDG PET-based metabolic response assessment in patients treated with regorafenib 1) as a clinical tool to spare them from needless toxicity from a drug that gives them little or no benefit and 2) as a translational tool able to guide comprehensive genomic and epigenetic research on the determinants of drug resistance. This translational...
research will be conducted on fresh or archived tumoral tissues and on serial blood samples looking for free ctDNA. For a subset of patients, fresh frozen tumoral tissues taken by PET-guided biopsy procedures on metabolically resistant lesions will be available.

**List of abbreviations**

aCRC, advanced colorectal cancer; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; CR, Complete Response; CRC, Colorectal Cancer; CT, Computed Tomography; DCE-MRI, Dynamic Contrast-Enhanced Magnetic Resonance Imaging; DCR, Disease Control Rate; FDG PET-CT, FluoroDeoxyGlucose-Positron Emission Tomography-Computed Tomography; FFPE, Formalin Fixed Paraffin Embedded; FGFR, Fibroblast Growth Factor Receptor; HIV, Human Immunodeficiency Virus; HR, Hazard Ratio; IC, Informed Consent; IEC, Independent Ethic Committee; INR, International normalized ratio; mCRC, metastatic ColoRectal Cancer; MRI, Magnetic Resonance Imaging; NCI-CTCAE, National Cancer Institute – Common Terminology Criteria for Adverse Event; NGS, Next generation sequencing; NYHA, New York Heart Association; OS, Overall Survival; PDGFR, Platelet-Derived Growth Factor Receptor; PFS, Progression Free Survival; PIC, Patient Information and Consent forms; PR, Partial Response; PFS, Progression-free Survival; PT, Prothrombin time; RECIST, Response Evaluation Criteria in Solid Tumors; Response Rate; RTKs, Receptor Tyrosine Kinases; SD, Stable Disease; TIE, Tyrosine kinase with Immunoglobulin and EGF homology domains; ULN, Upper Limit of Normal; US, UltraSonography; VEGFR, Vascular Endothelial Growth Factor Receptor.

**Competing interests**

The authors report no conflicts of interest.

**Authors’ contributions**

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A) Contribution to protocol writing
B) Manuscript design
C) Trial set-up
D) Manuscript writing
E) Coordination of PET imaging network
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Figure legends

**Figure 1. Study design.** FDG PET = FluoroDeoxyGlucose-Positron Emission Tomography, FFPE = Formalin Fixed Paraffin Embedded, OS = Overall survival, PD = Disease progression.

**Figure 2. Schedule of assessments.** FDG PET = FluoroDeoxyGlucose-Positron Emission Tomography, FFPE = Formalin Fixed Paraffin Embedded.
*In case of treatment interruption, the second PET scan can be delayed until maximum D21 of first cycle. It must be performed while the patient is under the study medication since at least 4 days. Plasma sample will be taken simultaneously with the second PET scan.
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Regorafenib Assessment in Refractory advanced Colorectal Cancer: RegARd-C study protocol.

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Abstract

Introduction

Regorafenib was recently approved for patients with pretreated advanced colorectal cancer (aCRC), despite a moderate improvement of the patients’ outcome, and significant toxicities. Based on previous studies showing that early FDG-PET-based metabolic response assessment (MRA) might adequately select patients unlikely to benefit from treatment, the RegARd-C trial uses early MRA to identify likely non-responders to regorafenib in a population of aCRC patients and guide a comprehensive evaluation of genomic and epigenetic determinants of resistance to treatment.

Methods and analysis

RegARd-C is a multicentric prospective study. Its primary objective is to identify non-benefitters from regorafenib given at 160 mg/day, 3 weeks/4 in a population of patients with pretreated aCRC. Baseline PET is repeated at D14 of the first treatment course. MRA is blinded for the investigators. Overall Survival (OS) is the primary endpoint and will be correlated with metabolic parameters and (epi)genetic alterations assessed from tumor and serial blood samples. A target sample size of 105 evaluable patients (70 as derivation set and 35 as validation set), is considered as sufficient to validate an expected hazard ratio (HR) for OS of metabolic responders compared to metabolic non-responders significantly < 1 (with 80% power and 1-sided 5% alpha in case of a true HR≤ 0.59 and a responders rate of 47%).

Ethics and dissemination

The study was approved by the Institut Jules Bordet’s competent ethics committee and comply with the Helsinki’s declaration or the belgian laws and regulations, whichever provides the greatest protection for the patient, and follows the International Conference on Harmonization E 6 (R1) Guideline for Good Clinical Practice, reference number CPMP/ICH/135/95.

The protocol and the trials results, even inconclusive, will be presented at international oncology congresses, and published in peer-reviewed journals. Genomic and epigenetic data will be made available in public open datasets.
Registration

EudraCT number: 2012-005655-16

ClinicalTrials.gov number: NCT01929616

Funding

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Key Words:

Colorectal Cancer, FDG PET, PET-CT, Next-generation sequencing

Strengths and limitations of this study:

1. Strengths
   - Prospective multicentric academic trial
   - Inclusion/exclusion criteria compatible with the study objectives (to determine biomarkers predictive of the patient’s outcome under treatment by regorafenib monotherapy in metastatic colorectal cancer refractory to all known medications with FDG-PET-assessable diseases)
   - All PET-CT centers accredited according to EARL (European Association for Nuclear Medicine Research Limited)
   - Central review and quality-control by an Imaging Core laboratory of the FDG-PET/CT data
   - Prospective collection of biological specimen (frozen and paraffin-embedded tissue blocks, sequential frozen plasmatic samples for ctDNA research, whole blood) for comprehensive genomic and epigenetic analysis guided by metabolic imaging definition of responding/non-responding disease
• Statistical hypothesis foreseeing an exploration set and a validation set and allowing the validation of a metabolic response hypothesis based on previous work taking into account the tumoral heterogeneity in response.

2. Weaknesses
• Non-randomized design
A. Introduction

Colorectal Cancer

With a 35/100,000/year incidence rate in the developed world, colorectal cancer affects about 150,000 people per year in Western Europe.[1] About half of the patients will develop a metastatic disease, carrying a grim prognosis if unresectable with curative intent. Progress in chemotherapy has been substantial during the last decade, allowing rare, but well-advertised secondary resections of primarily unresectable metastatic disease. In the palliative setting, chemotherapy aims essentially at extending survival and the use of all available drugs (fluoropyrimidines, oxaliplatin, irinotecan, bevacizumab, anti-EGFR antibodies) either successively or concomitantly has increased the median OS of patients to more than 25 months.[2-6] However, no single drug or any combination is able to cure metastatic disease, and the tumor will eventually become resistant to all known medications, leading to the patient’s death.

Regorafenib

Regorafenib (BAY 73-4506) is a novel oral diphenylurea-based multikinase inhibitor, shown in preclinical studies as a potent inhibitor of several angiogenic and stromal receptor tyrosine kinases (RTKs), including vascular endothelial growth factor receptor (VEGFR)-1, -2, -3, platelet-derived growth factor receptor (PDGFR)-β, fibroblast growth factor receptor (FGFR)-1, and TIE2. In addition, regorafenib inhibits various oncogenic RTKs (c-KIT and RET) and intracellular signaling kinases (cRAF/RAF-1, B-RAF, and B-RAF V600E mutant). The exact mode of action of regorafenib remains however unknown, even if it is probably associated with antiangiogenic and antiproliferative effects, mostly through RAF inhibition. Data from a phase I trial [7-8] has established the recommended dose at 160mg/day 3 weeks out of 4. A recent phase III trial in aCRC refractory to all known medications (CORRECT) [9], has randomized 760 patients between regorafenib (n=505) and placebo (n= 255), showing a small but statistically significant advantage for OS (median 6.4 months versus 5 months, one-sided p-value 0.005) and progression-free survival (PFS) (median 1.9 months versus 1.7 months, one-sided p-value <0.000001) for regorafenib. This drug has the potential to become a standard
therapy for the treatment of mCRC patients who have been previously treated with all approved therapies. Nevertheless, the toxicity of this medication is important next to the palliative situation in which it was developed. Both the limited survival benefit and the clinically meaningful toxicity highlight the need to develop tools able to quickly identify the patients unlikely to benefit from the treatment in order to spare them from unnecessary side effects.

**Early FDG PET-CT**

Standard radiological response measurements (RECIST criteria, modified RECIST, WHO) rely entirely upon measuring the size of the tumor with CT, ultrasound, or MRI, and are only applicable under restrictive conditions (well defined lesions, adequate minimum size, at least six weeks of chemotherapy). Response rates in advanced solid tumors correlate poorly with other patient outcomes, such as PFS and OS.[10 11] Several techniques with the potential to detect early response are emerging: serial FDG PET-CT, dynamic MRI (DCE-MRI) and diffusion MR techniques, and circulating tumor cells (CTCs). Among these, FDG PET-CT is the most studied and promising. It is widely available in Belgium, and its value in detecting early metabolic changes predictive of later outcome is currently being assessed.[12 13] Recent data suggest that use of serial FDG PET-CT imaging to assess tumor metabolism can reliably detect refractory disease. Our research group prospectively studied 41 patients with mCRC undergoing first- or second-line chemotherapy.[13] Serial FDG PET-CT was performed at baseline and 14 days after the first cycle of chemotherapy. The metabolic changes were compared to the morphologic response evaluated by CT according to RECIST criteria. A RECIST response was observed in 10/23 (43%) PET responding patients and in 0/17 (0%) PET non-responding patients (p=0.002). The metabolic assessment’s predictive performance for RECIST response was 100% for sensitivity (95% confidence interval [CI] 69% to 100%), 57% for specificity (95% CI 37% to 75%), 43% for positive predictive value (95% CI 23% to 66%), and 100% for negative predictive value (95% CI 80% to 100%). Comparing patients who had metabolic responses to those who did not, the hazard ratio (HR) was 0.28 (95% CI 0.10–0.76) for OS and 0.57 (95% CI 0.27–1.21) for PFS. This suggests that FDG PET-CT may be used for the early detection of non-responding patients. Additionally, we conducted another FDG PET-CT driven metabolic study with a similar design in
92 patients with advanced refractory CRC treated with a combination of sorafenib and capecitabine (SoMore study). [14] The aim of this metabolic study was to identify patients unlikely to benefit from the therapy. The metabolic analysis of this population will be used as a model for regorafenib. The most important finding related to this metabolic analysis was the identification of a prognostic value for early metabolic homogeneous response. [15] Indeed, among 79 patients who underwent baseline PET examination as well as day 14 examination, 37 patients (47%) were found responding homogeneously in all their lesions to treatment with combined sorafenib-capecitabine. These patients had prolonged survival after day 14 compared to all the other patients (HR=0.59, 95% CI: 0.37-0.96, p=0.03).

Genomic and Epigenomic assessments

Recent advances in Next Generation Sequencing (NGS) technologies and data analysis have enabled to explore patients’ specific genomic/ somatic alterations in their cancer genome in a relatively timely, costly and clinically efficient manner. The design of the RegARd-C study provides a unique opportunity to identify and characterize molecular factors that could predict PFS and OS for patients treated with regorafenib. Metabolic imaging could enable us to define the subpopulations of patients unlikely to benefit from this medication, thereby increasing the likelihood of finding determinants of resistance to therapy. Molecular translational research could also provide tools to distinguish patients who will or will not benefit from therapy, for instance by analyzing the genetic and epigenetic differences between the metabolically non-responding patients and the remaining subpopulation. Moreover, as tumor cells liberate naked DNA (circulating tumor DNA, ctDNA) into the blood stream after necrosis or apoptosis, we will investigate whether tumor-specific rearrangements can be detected in plasma, which will be used as “liquid biopsy”. Although “liquid biopsies” are minimally invasive and may represent a molecular assessment of the overall cancer, the detection of ctDNA still has some difficulties that we will take into account: the distinction between ctDNA and cell-free DNA and the very low proportion of ctDNA which may hinder its exact quantification.

Aim of the study
RegARdUC aims at identifying in a population of patients bearing advanced, refractory CRC, those who draw no benefit from treatment with regorafenib. OS will be used as primary endpoint. Covariates that will be analyzed in relationship with OS will be metabolic parameters obtained at baseline and very early following treatment initiation as well as genetic, epigenetic and molecular aberrations (before and after treatment) assessed from tumor biopsies and serial blood samples, in addition to known clinical and pathological factors. The molecular aberrations will be investigated using gene expression profiling, RNA and exome sequencing, and methylation profiling of the tumor biopsies and repeated blood samples taken during therapy.

B. Methods and analysis

Study Design

The study was designed as a single-arm, prospective, non-randomized, non-comparative, open label phase II trial, with all patients being accrued in one stage. No early stopping rules are used. A written informed consent has to be obtained by an investigator from the patient, before any screening and inclusion procedure. A FDG PET-CT will be performed at baseline (D-7-D0) and repeated after 14 to 17 days from start of the first cycle. The clinicians will remain blinded to the assessment of FDG PET-CT response. Clinical and biological evaluation will be made every cycle, starting at day 28 of the second cycle. RECIST 1.1-based radiological assessment (CT or MRI) will be made every 2 cycles, starting at day 28 of the second cycle. Treatment will be continued until disease progression, unacceptable toxicity or any other reason (study withdrawal, lost to follow-up, death...) (see Figure 1 for an overview of the study design).

Objectives

To identify in a population of patients bearing advanced, refractory CRC, those unlikely to draw a substantial benefit from treatment with regorafenib. OS will be used as primary endpoint.
Secondary objectives are to 1) analyze PFS and response rate (RR) in relationship with the same covariates as for OS, 2) assess regorafenib efficacy (OS, PFS, RR) and safety profile in this study population, 3) assess the Disease control rate (DCR = Complete response [CR] + partial response [PR] + stable disease [SD]), 4) compare the relative benefit (OS, PFS) of regorafenib according to history of treatment with bevacizumab, and 5) validate the relationship that was found, in a previous study [14] conducted in the same patient population treated with sorafenib and capecitabine, between OS and early metabolic consistent response in all the patient’s lesions.

**Patient Selection Criteria**

**Inclusion Criteria**

Participants must

- have histologically proven adenocarcinoma of the colon or the rectum that is metastatic or unresectable and for which standard treatments do not exist or are no longer effective. The tumor should be refractory to all standard chemotherapy agents (fluoropyrimidines, irinotecan, and oxaliplatin) and anti-EGFR monoclonal antibodies in case of RAS wild type (cetuximab, or panitumumab) administered before study entry. Patients treated with oxaliplatin in an adjuvant setting should have progressed during adjuvant therapy or within 6 months of completion of this treatment. Patients who do not meet strictly these criteria, but for whom further treatment with oxaliplatin would be prohibited (for instance allergic reaction, residual neurotoxicity grade ≥ 2) are allowed for inclusion. Prior treatment with bevacizumab and/or aflibercept is allowed but not mandatory;

- have signed a written informed consent (approved by an Independent Ethics Committee [IEC] and obtained prior to any study specific screening procedures);

- be aged 18 or older;

- have a life expectancy of greater than 12 weeks;

- have an ECOG performance status ≤ 1;
• have normal organ and bone marrow function as defined below: Leukocytes > 3,000/mcL, with an absolute neutrophil count > 1,500/mcL, platelets > 100,000/mcL, Hb ≥ 9g/dl, total bilirubin ≤ 1.5 × institutional ULN, AST/ALT/P-Alk levels ≤ 2.5 × institutional ULN (≤ 5x institutional ULN in case of liver metastatic involvement), P-Alk levels ≤ 5 x institutional ULN, international normalized ratio of Prothrombin Time (INR) ≤ 1.5 x institutional ULN, creatinine within 1.5 × normal institutional upper limits, or creatinine clearance > 30mL/min.

• Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

• Presence of a previously collected frozen or FFPE primary or metastatic tumor. Frozen tissue from the primitive tumor is preferred. If no archived tissue is available for the patient, the tissue should be collected freshly in the primary (preferentially) or a metastatic lesion before study entry.

• Presence of at least one metabolically measurable tumoral lesion on FDG PET-CT fulfilling following criteria: Size ≥ 1.5cm and FDG uptake above the background liver uptake.

**Exclusion Criteria**

Excluded from the study are patients identified with any of the following conditions or characteristics:

• Prior treatment with sorafenib or regorafenib.

• Participants with previous cancer that are not disease-free for at least for 5 years prior to registration, EXCEPT for curatively treated cervical cancer in situ, non-melanoma skin cancer and superficial bladder tumors [Ta (Non-invasive tumor), Tis (Carcinoma in situ) and T1 (Tumor invades lamina propria)].
• Participants who have had chemotherapy or targeted therapy within 2 weeks prior to entering the study.
• Participants who have had a major surgery or radiotherapy within 4 weeks prior to entering the study.
• Unresolved toxicity higher than NCI-CTCAE (version 4.0) Grade 1 attributed to any prior therapy/procedure excluding alopecia and oxaliplatin induced neurotoxicity ≤Grade 2.
• Participants receiving any experimental agents.
• Participants with known brain metastases.
• Bleeding diathesis, history of cardiovascular ischemic disease or cerebrovascular incident within the last six months.
• Any hemorrhage or bleeding event NCI-CTCAE v.4 Grade ≥3 within 4 weeks prior to the start of study medication.
• Uncontrolled concurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure (New York Heart Association (NYHA) class ≥2), unstable angina pectoris (defined by angina symptoms at rest or new-onset angina started within the last 3 months), cardiac arrhythmia requiring antiarrhythmic therapy (beta blockers or digoxin are permitted).
• Uncontrolled hypertension (defined by systolic blood pressure >150 mmHg or diastolic blood pressure >90 mmHg despite optimal medical management).
• Patients with phaeochromocytoma.
• Patients with seizure disorder requiring medication.
• Any history of organ allograft.
• Pleural effusion or ascites affecting respiration (NCI CTCAEv.4 Grade ≥ 2 dyspnea).
• Uncontrolled diabetes.
• Non-healing wound, ulcer, or bone fracture.
• Known history of human immunodeficiency virus (HIV) infection, or active hepatitis B or C, or chronic hepatitis B or C requiring treatment with antiviral therapy.
• Interstitial lung disease with ongoing signs and symptoms.
• Renal failure requiring hemo-or peritoneal dialysis.
• Dehydration NCI-CTCAE v.4 grade > 1.
• Substance abuse, medical, psychological or social conditions that may interfere with the patient’s ability to understand informed consent and participation in the study or evaluation of the study results.
• Known hypersensitivity to the study drug or excipients in the formulation.
• Any illness or medical conditions that are unstable or could jeopardize the safety of the patient and his/her compliance in the study.
• Pregnant or lactating women.
• Subjects unable to swallow oral medications.
• Subjects with thrombotic, embolic, venous, or arterial events, such as cerebrovascular accident (including transient ischemic attacks) deep vein thrombosis or pulmonary embolism within 6 months of start of study treatment within 6 months of informed consent.
• Persistent proteinuria > Grade 3 NCI-CTCAE v4.0 (> 3.5 g/24 hours, measured by urine protein: creatinine ratio on a random urine sample).

**Intervention to be measured:**

**FDG-PET/CT Imaging**

Increased glycolysis is one of the hallmarks of cancer. FDG, an analogue of glucose labeled with a positron-emitting isotope of Fluor (F\(^{18}\)), is actively taken up in cancer cells of many tumor types. The positrons emitted by the FDG are detected by a dedicated camera, enabling the visualization of cellular glycolytic activity.\[16\] The criteria listed below define the minimal requirements for metabolic imaging assessment of tumor response:

• Blood glucose < 150 mg/dl at the time of FDG administration. Insulin or oral antidiabetic medication is not allowed on the days of FDG-PET/CT imaging.
• Delay between the first FDG-PET/CT imaging and the start of regorafenib ≤ 7 days. The second FDG-PET/CT imaging is performed on day D14 (ideal range: D14-D17). In case of treatment interruption, the second PET scan can be delayed until maximum D21 of first cycle. It must be performed while the patient is under the study medication since at least 4 days.

All participating FDG-PET/CT centres were required to obtain the FDG-PET/CT’s EARL accreditation and keep it for the whole duration of the study.\[17\] All images are
centralized by an imaging core lab with central quality control by an imaging expert and central review by two independent reviewers. The results of the metabolic assessment remains blinded for the clinical investigator, and will follow a predetermined 3-step methodology previously described.[13 15]

**Translational Research Genomic Analysis**

Blood samples for plasma preparation (2 x 9 mL for each time point) will be collected (and frozen) at baseline (D-7↔D0) and simultaneously with the second PET. An extra 9 mL whole blood sample will be collected at baseline in order to distinguish somatic from germline mutations. Plasma samples will also be obtained every 2 cycles, starting from the first cycle and (simultaneously with biology) up to 1 year after the start of therapy. Moreover, a final plasma sample will be obtained at disease progression (see Figure 2 for overview of sample collection). Optional fresh frozen tumor tissue from a FDG PET targetable metastatic or primary lesion will be obtained before study entry and at disease progression. Previously collected frozen or FFPE primary or metastatic tumor needs to be available at the study entry. The translational research group will study the genetic and epigenetic aberrations that are associated with the patient’s outcome (PFS, OS) and with metabolic response after treatment with regorafenib. The molecular aberrations will be investigated using gene expression profiling, RNA and exome sequencing, and methylation profiling of the tumor biopsies and repeated blood samples taken during therapy.

**Follow-up**

Follow-up procedures, performed every 2 months will include physical examination, vital signs and ECOG performance status, laboratory tests and blood samples for translational research analysis, as well as radiological assessment of the tumours.

According to the European Union (EU) Clinical Trial Directive for each participating European Union (EU) country, the end of the study will be reached when the last visit under treatment protocol of the last subject for all centers has occurred. The patients will continue to be followed afterwards every 2 months till disease progression or death or refusal, whichever occurs first. Patients may be offered any further treatment available, at the investigator’s discretion.
Statistical Considerations

Analyses will be carried out looking at constructing signatures that are prognostic for OS as well as PFS and RR. Baseline data will be used for some models and all eligible patients (intent to treat population) will be included in these analyses. Models integrating data in the short follow-up after treatment will also be developed. In that later situation, only patients having undergone the necessary examinations will be included in the analyses. Time zero for measuring time to event variables will be adapted and will be dependent on the timing of further examinations. Analyses will be adjusted for multiplicity. As no formal hypothesis can be done on the discriminating value of genomic biomarkers, it was decided to target a sample size of 105 evaluable patients and to use the first 70 patients as derivation set and the 35 last patients as validation set. It should be stressed however that the total sample size of 105 evaluable patients is sufficient to validate the hypothesis generated by the SoMore study.[14] To detect in a validation series that the HR for responders compared to non-responders is significantly < 1 (with a power of 80% in case of a true HR ≤ 0.59 and a 1-sided alpha level of 5% and a rate of responders of 47%), we need to observe 89 events. We then plan to carry out a validation comparison between early homogeneous responders and the other patients once we will have reached 89 events for these 105 evaluable patients. For this validation comparison, there will be no split of the total sample. In order to reach this number of evaluable patients, the overall sample size of registered patients will be adapted during the study accrual. Taking into account an expected 20-25% drop-out rate between registration and the time of further examinations, between 124 and 140 patients will be accrued. The analyses for derivation will be carried out when at least 80% of events (deaths) will have been observed. The analyses for validation will occur also when 80% of events will be reached. The study is designed as a single-arm study, with all patients being accrued in one stage. No early stopping rules will be used. The clinicians will remain blinded to the assessment of FDG PET-CT response and of the whole molecular analyses.

C. ETHICS AND DISSEMINATION

Ethical Considerations
Patient Protection


The competent ethics committee of the Institut Jules Bordet approved the protocol, as required by applicable national legislation.

Dissemination

The protocol and the trials results, even inconclusive, will be presented at international oncology congresses, and published in peer-reviewed journals. Genomic and epigenetic data will be made available in public open datasets.

Trial Sponsorship, Financing and Insurance

This study is supported by an unrestricted grant from Bayer Healthcare Pharmaceutical, which provided regorafenib but played no further role in this study design; data collection, study management, and will play no role in results analysis, and interpretation of data; nor in writing of the report; nor in the decision to submit the report for publication, and has no kind of authority over any of these activities.

Discussion and Conclusions

Regorafenib, an oral multi kinase inhibitor that shares with sorafenib several targets involved in tumour angiogenesis, oncogenesis, and tumour microenvironment, improves the patients’ outcome, but at the cost of significant toxicities, underscoring the need to identify those who will benefit from therapy. A previous study (SoMore trial) showed that early FDG PET-based metabolic response assessment may
adequately discriminate patients with chemorefractory aCRC unlikely to benefit from a sorafenib-capecitabine combination. RegARd-C aims to explore early FDG PET-based metabolic response assessment in patients treated with regorafenib 1) as a clinical tool to spare them from needless toxicity from a drug that gives them little or no benefit and 2) as a translational tool able to guide comprehensive genomic and epigenetic research on the determinants of drug resistance. This translational research will be conducted on fresh or archived tumoral tissues and on serial blood samples looking for free ctDNA. For a subset of patients, fresh frozen tumoral tissues taken by PET-guided biopsy procedures on metabolically resistant lesions will be available.

**List of abbreviations**

aCRC, advanced colorectal cancer; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; CR, Complete Response; CRC, Colorectal Cancer; CT, Computed Tomography; DCE-MRI, Dynamic Contrast-Enhanced Magnetic Resonance Imaging; DCR, Disease Control Rate; FDG PET-CT, FluoroDeoxyGlucose-Positron Emission Tomography-Computed Tomography; FFPE, Formalin Fixed Paraffin Embedded; FGFR, Fibroblast Growth Factor Receptor; HIV, Human Immunodeficiency Virus; HR, Hazard Ratio; IC, Informed Consent; IEC, Independent Ethic Committee; INR, International normalized ratio; mCRC, metastatic ColoRectal Cancer; MRI, Magnetic Resonance Imaging; NCI-CTCAE, National Cancer Institute – Common Terminology Criteria for Adverse Event; NGS, Next generation sequencing; NYHA, New York Heart Association; OS, Overall Survival; PDGFR, Platelet-Derived Growth Factor Receptor; PFS, Progression Free Survival; PIC, Patient Information and Consent forms; PR, Partial Response; PFS, Progression-free Survival; PT, Prothrombin time; RECIST, Response Evaluation Criteria in Solid Tumors; Response Rate; RTKs, Receptor Tyrosine Kinases; SD, Stable Disease; TIE, Tyrosine kinase with Immunoglobulin and EGF homology domains; ULN, Upper Limit of Normal; US, UltraSonography; VEGFR, Vascular Endothelial Growth Factor Receptor.

**Competing interests**
The authors report no conflicts of interest.

**Authors' contributions**

1. Alain Hendlisz, MD (Corresponding author) (A, B, C, D)
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6. Thomas Guiot, PhD (A, C, E)
7. Camilo Garcia, MD (A, B, C, D, E)
8. Patrick Flamen, MD, PhD (A, B, C, D, E)

A) Contribution to protocol writing
B) Manuscript design
C) Trial set-up
D) Manuscript writing
E) Coordination of PET imaging network

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Figure legends

**Figure 1. Study design.** FDG PET = FluoroDeoxyGlucose-Positron Emission Tomography, FFPE = Formalin Fixed Paraffin Embedded, OS = Overall survival, PD = Disease progression.

**Figure 2. Schedule of assessments.** FDG PET = FluoroDeoxyGlucose-Positron Emission Tomography, FFPE = Formalin Fixed Paraffin Embedded.
*In case of treatment interruption, the second PET scan can be delayed till maximum D21 of first cycle. It must be performed while the patient is under the study medication since at least 4 days. Plasma sample will be taken simultaneously with the second PET scan.

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Abstract

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Key Words:

Colorectal Cancer, FDG PET, PET-CT, Next-generation sequencing

Strengths and limitations of this study:

1. Strengths

- Prospective multicentric academic trial
- Inclusion/exclusion criteria compatible with the study objectives (to determine biomarkers predictive of the patient’s outcome under treatment by regorafenib monotherapy in metastatic colorectal cancer refractory to all known medications with FDG-PET-assessable diseases)
- All PET-CT centers accredited according to EARL (European Association for Nuclear Medicine Research Limited)
- Central review and quality-control by an Imaging Core laboratory of the FDG-PET/CT data
- Prospective collection of biological specimen (frozen and paraffin-embedded tissue blocks, sequential frozen plasmatic samples for ctDNA research, whole blood) for comprehensive genomic and epigenetic analysis guided by metabolic imaging definition of responding/non-responding disease
• Statistical hypothesis foreseeing a exploration set and a validation set and allowing the validation of a metabolic response hypothesis based on previous work taking in account the tumoral heterogeneity in response.

2. Weaknesses

• Non-randomized design
A. Introduction

Colorectal Cancer

With a 35/100,000/year incidence rate in the developed world, colorectal cancer affects about 150,000 people per year in Western Europe.[1] About half of the patients will develop a metastatic disease, carrying a grim prognosis if unresectable with curative intent. Progress in chemotherapy has been substantial during the last decade, allowing rare, but well-advertised secondary resections of primarily unresectable metastatic disease. In the palliative setting, chemotherapy aims essentially at extending survival and the use of all available drugs (fluoropyrimidines, oxaliplatin, irinotecan, bevacizumab, anti-EGFR antibodies) either successively or concomitantly has increased the median OS of patients to more than 25 months.[2-6] However, no single drug or any combination is able to cure metastatic disease, and the tumor will eventually become resistant to all known medications, leading to the patient’s death.

Regorafenib

Regorafenib (BAY 73-4506) is a novel oral diphenylurea-based multikinase inhibitor, shown in preclinical studies as a potent inhibitor of several angiogenic and stromal receptor tyrosine kinases (RTKs), including vascular endothelial growth factor receptor (VEGFR)-1, -2, -3, platelet-derived growth factor receptor (PDGFR)-β, fibroblast growth factor receptor (FGFR)-1, and TIE2. In addition, regorafenib inhibits various oncogenic RTKs (c-KIT and RET) and intracellular signaling kinases (cRAF/RAF-1, B-RAF, and B-RAF V600E mutant). The exact mode of action of regorafenib remains however unknown, even if it is probably associated with antiangiogenic and antiproliferative effects, mostly through RAF inhibition. Data from a phase I trial [7 8] has established the recommended dose at 160mg/day 3 weeks out of 4. A recent phase III trial in aCRC refractory to all known medications (CORRECT) [9], has randomized 760 patients between regorafenib (n=505) and placebo (n= 255), showing a small but statistically significant advantage for OS (median 6.4 months versus 5 months, one-sided p-value 0.005) and progression-free survival (PFS) (median 1.9 months versus 1.7 months, one-sided p-value <0.000001)) for regorafenib. This drug has the potential to become a standard
therapy for the treatment of mCRC patients who have been previously treated with all approved therapies. Nevertheless, the toxicity of this medication is important next to the palliative situation in which it was developed. Both the limited survival benefit and the clinically meaningful toxicity highlight the need to develop tools able to quickly identify the patients unlikely to benefit from the treatment in order to spare them from unnecessary side effects.

**Early FDG PET-CT**

Standard radiological response measurements (RECIST criteria, modified RECIST, WHO) rely entirely upon measuring the size of the tumor with CT, ultrasound, or MRI, and are only applicable under restrictive conditions (well defined lesions, adequate minimum size, at least six weeks of chemotherapy). Response rates in advanced solid tumors correlate poorly with other patient outcomes, such as PFS and OS.[10-11] Several techniques with the potential to detect early response are emerging: serial FDG PET-CT, dynamic MRI (DCE-MRI) and diffusion MR techniques, and circulating tumor cells (CTCs). Among these, FDG PET-CT is the most studied and promising. It is widely available in Belgium, and its value in detecting early metabolic changes predictive of later outcome is currently being assessed.[12-13] Recent data suggest that use of serial FDG PET-CT imaging to assess tumor metabolism can reliably detect refractory disease. Our research group prospectively studied 41 patients with mCRC undergoing first- or second-line chemotherapy.[13] Serial FDG PET-CT was performed at baseline and 14 days after the first cycle of chemotherapy. The metabolic changes were compared to the morphologic response evaluated by CT according to RECIST criteria. A RECIST response was observed in 10/23 (43%) PET responding patients and in 0/17 (0%) PET non-responding patients (p=0.002). The metabolic assessment’s predictive performance for RECIST response was 100% for sensitivity (95% confidence interval [CI] 69% to 100%), 57% for specificity (95% CI 37% to 75%), 43% for positive predictive value (95% CI 23% to 66%), and 100% for negative predictive value (95% CI 80% to 100%). Comparing patients who had metabolic responses to those who did not, the hazard ratio (HR) was 0.28 (95% CI 0.10–0.76) for OS and 0.57 (95% CI 0.27–1.21) for PFS. This suggests that FDG PET-CT may be used for the early detection of non-responding patients. Additionally, we conducted another FDG PET-CT driven metabolic study with a similar design in
92 patients with advanced refractory CRC treated with a combination of sorafenib and capecitabine (SoMore study).[14] The aim of this metabolic study was to identify patients unlikely to benefit from the therapy. The metabolic analysis of this population will be used as a model for regorafenib. The most important finding related to this metabolic analysis was the identification of a prognostic value for early metabolic homogeneous response.[15] Indeed, among 79 patients who underwent baseline PET examination as well as day 14 examination, 37 patients (47%) were found responding homogeneously in all their lesions to treatment with combined sorafenib-capecitabine. These patients had prolonged survival after day 14 compared to all the other patients (HR=0.59, 95% CI: 0.37-0.96, p=0.03).

**Genomic and Epigenomic assessments**

Recent advances in Next Generation Sequencing (NGS) technologies and data analysis have enabled to explore patients’ specific genomic/ somatic alterations in their cancer genome in a relatively timely, costly and clinically efficient manner. The design of the RegARd-C study provides a unique opportunity to identify and characterize molecular factors that could predict PFS and OS for patients treated with regorafenib. Metabolic imaging could enable us to define the subpopulations of patients unlikely to benefit from this medication, thereby increasing the likelihood of finding determinants of resistance to therapy. Molecular translational research could also provide tools to distinguish patients who will or will not benefit from therapy, for instance by analyzing the genetic and epigenetic differences between the metabolically non-responding patients and the remaining subpopulation. Moreover, as tumor cells liberate naked DNA (circulating tumor DNA, ctDNA) into the blood stream after necrosis or apoptosis, we will investigate whether tumor-specific rearrangements can be detected in plasma, which will be used as “liquid biopsy”. Although “liquid biopsies” are minimally invasive and may represent a molecular assessment of the overall cancer, the detection of ctDNA still has some difficulties that we will take into account: the distinction between ctDNA and cell-free DNA and the very low proportion of ctDNA which may hinder its exact quantification.

**Aim of the study**
RegARd-C aims at identifying in a population of patients bearing advanced, refractory CRC, those who draw no benefit from treatment with regorafenib. OS will be used as primary endpoint. Covariates that will be analyzed in relationship with OS will be metabolic parameters obtained at baseline and very early following treatment initiation as well as genetic, epigenetic and molecular aberrations (before and after treatment) assessed from tumor biopsies and serial blood samples, in addition to known clinical and pathological factors. The molecular aberrations will be investigated using gene expression profiling, RNA and exome sequencing, and methylation profiling of the tumor biopsies and repeated blood samples taken during therapy.

B. Methods and analysis

Study Design

The study was designed as a single-arm, prospective, non-randomized, non-comparative, open label phase II trial, with all patients being accrued in one stage. No early stopping rules are used. A written informed consent has to be obtained by an investigator from the patient, before any screening and inclusion procedure. A FDG PET-CT will be performed at baseline (D-7-D0) and repeated after 14 to 17 days from start of the first cycle. The clinicians will remain blinded to the assessment of FDG PET-CT response. Clinical and biological evaluation will be made every cycle, starting at day 28 of the second cycle. RECIST 1.1-based radiological assessment (CT or MRI) will be made every 2 cycles, starting at day 28 of the second cycle. Treatment will be continued until disease progression, unacceptable toxicity or any other reason (study withdrawal, lost to follow-up, death…) (see Figure 1 for an overview of the study design).

Study Organization-Role of the Coordinating Center

The role of the Coordinating Center (CC) is to coordinates the trial in all participating centers and to work with all study investigators and research staff:

• to collect and clean clinical trial data
• to monitor the safety and timely study progress
The CC is responsible for the clinical trial administration, trial progress, data management including database design and data cleaning, the statistical analysis, safety handling, IT support, drug supply, sample collection and manuscript preparation.

The CC includes the principal investigator (AH), the principal co-investigator (AD), the nuclear medicine principal investigator (PF), the research administration staff, the biostatistician (MPa), programmers, study leader (RH), data managers (AK), consultants, research nurses (FH), administrative support staff (JV), monitors (SJ).

For RegARd-C, there are no endpoint adjudication committee or steering committee.

Objectives

To identify in a population of patients bearing advanced, refractory CRC, those unlikely to draw a substantial benefit from treatment with regorafenib. OS will be used as primary endpoint.

Secondary objectives are to 1) analyze PFS and response rate (RR) in relationship with the same covariates as for OS, 2) assess regorafenib efficacy (OS, PFS, RR) and safety profile in this study population, 3) assess the Disease control rate (DCR = Complete response [CR] + partial response [PR] + stable disease [SD]), 4) compare the relative benefit (OS, PFS) of regorafenib according to history of treatment with bevacizumab, and 5) validate the relationship that was found, in a previous study [14] conducted in the same patient population treated with sorafenib and capecitabine, between OS and early metabolic consistent response in all the patient’s lesions.

Patient Selection Criteria

Inclusion Criteria

Participants must

- have histologically proven adenocarcinoma of the colon or the rectum that is metastatic or unresectable and for which standard treatments do not exist or are no longer effective. The tumor should be refractory to all standard chemotherapy agents (fluoropyrimidines, irinotecan, and oxaliplatin) and anti-EGFR monoclonal antibodies in case of RAS wild type (cetuximab, or panitumumab) administered before study entry. Patients treated with
oxaliplatin in an adjuvant setting should have progressed during adjuvant therapy or within 6 months of completion of this treatment. Patients who do not meet strictly these criteria, but for whom further treatment with oxaliplatin would be prohibited (for instance allergic reaction, residual neurotoxicity grade ≥ 2) are allowed for inclusion. Prior treatment with bevacizumab and/or aflibercept is allowed but not mandatory;

- have signed a written informed consent (approved by an Independent Ethics Committee [IEC] and obtained prior to any study specific screening procedures);
- be aged 18 or older;
- have a life expectancy of greater than 12 weeks;
- have an ECOG performance status ≤ 1;
- have normal organ and bone marrow function as defined below: Leukocytes > 3,000/mcL, with an absolute neutrophil count > 1,500/mcL, platelets > 100,000/mcL, Hb≥9g/dl, total bilirubin ≤ 1.5 × institutional ULN, AST/ALT/P-Alk levels ≤ 2.5 × institutional ULN (≤ 5x institutional ULN in case of liver metastatic involvement), P-Alk levels ≤5 x institutional ULN, international normalized ratio of Prothrombin Time (INR) ≤ 1.5 × institutional ULN, creatinine within 1.5 × normal institutional upper limits, or creatinine clearance > 30mL/min.

- Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

- Presence of a previously collected frozen or FFPE primary or metastatic tumor. Frozen tissue from the primitive tumor is preferred. If no archived tissue is available for the patient, the tissue should be collected freshly in the primary (preferentially) or a metastatic lesion before study entry.
• Presence of at least one metabolically measurable tumoral lesion on FDG PET-CT fulfilling following criteria: Size ≥ 1.5cm and FDG uptake above the background liver uptake.

**Exclusion Criteria**

Excluded from the study are patients identified with any of the following conditions or characteristics:

• Prior treatment with sorafenib or regorafenib.
• Participants with previous cancer that are not disease-free for at least for 5 years prior to registration, EXCEPT for curatively treated cervical cancer in situ, non-melanoma skin cancer and superficial bladder tumors [Ta (Non-invasive tumor), Tis (Carcinoma in situ) and T1 (Tumor invades lamina propria)].
• Participants who have had chemotherapy or targeted therapy within 2 weeks prior to entering the study.
• Participants who have had a major surgery or radiotherapy within 4 weeks prior to entering the study.
• Unresolved toxicity higher than NCI-CTCAE (version 4.0) Grade 1 attributed to any prior therapy/procedure excluding alopecia and oxaliplatin induced neurotoxicity ≤Grade 2.
• Participants receiving any experimental agents.
• Participants with known brain metastases.
• Bleeding diathesis, history of cardiovascular ischemic disease or cerebrovascular incident within the last six months.
• Any hemorrhage or bleeding event NCI-CTCAE v.4 Grade ≥3 within 4 weeks prior to the start of study medication.
• Uncontrolled concurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure (New York Heart Association (NYHA) class ≥2), unstable angina pectoris (defined by angina symptoms at rest or new-onset angina started within the last 3 months), cardiac arrhythmia requiring antiarrhythmic therapy (beta blockers or digoxin are permitted).
• Uncontrolled hypertension (defined by systolic blood pressure >150 mmHg or diastolic blood pressure >90 mmHg despite optimal medical management).
• Patients with phaeochromocytoma.
• Patients with seizure disorder requiring medication.
• Any history of organ allograft.
• Pleural effusion or ascites affecting respiration (NCI CTCAEv.4 Grade ≥ 2 dyspnea).
• Uncontrolled diabetes.
• Non-healing wound, ulcer, or bone fracture.
• Known history of human immunodeficiency virus (HIV) infection, or active hepatitis B or C, or chronic hepatitis B or C requiring treatment with antiviral therapy.
• Interstitial lung disease with ongoing signs and symptoms.
• Renal failure requiring hemo- or peritoneal dialysis.
• Dehydration NCI-CTCAE v.4 grade> 1.
• Substance abuse, medical, psychological or social conditions that may interfere with the patient’s ability to understand informed consent and participation in the study or evaluation of the study results.
• Known hypersensitivity to the study drug or excipients in the formulation.
• Any illness or medical conditions that are unstable or could jeopardize the safety of the patient and his/her compliance in the study.
• Pregnant or lactating women.
• Subjects unable to swallow oral medications.
• Subjects with thrombotic, embolic, venous, or arterial events, such as cerebrovascular accident (including transient ischemic attacks) deep vein thrombosis or pulmonary embolism within 6 months of start of study treatment within 6 months of informed consent.
• Persistent proteinuria > Grade 3 NCI-CTCAE v4.0 (> 3.5 g/24 hours, measured by urine protein: creatinine ratio on a random urine sample).

**Intervention to be measured:**

**FDG-PET/CT Imaging**

Increased glycolysis is one of the hallmarks of cancer. FDG, an analogue of glucose labeled with a positron-emitting isotope of Fluor (F\(^{18}\)), is actively taken up in cancer
cells of many tumor types. The positrons emitted by the FDG are detected by a
dedicated camera, enabling the visualization of cellular glycolytic activity.[16] The
criteria listed below define the minimal requirements for metabolic imaging
assessment of tumor response:

- Blood glucose < 150 mg/dl at the time of FDG administration. Insulin or oral
  antidiabetic medication is not allowed on the days of FDG-PET/CT imaging.
- Delay between the first FDG-PET/CT imaging and the start of regorafenib ≤
  7 days. The second FDG-PET/CT imaging is performed on day D14 (ideal
  range: D14-D17). In case of treatment interruption, the second PET scan can
  be delayed until maximum D21 of first cycle. It must be performed while the
  patient is under the study medication since at least 4 days.

All participating FDG-PET/CT centres were required to obtain the FDG-PET/CT’s
EARL accreditation and keep it for the whole duration of the study.[17] All images are
centralized by an imaging core lab with central quality control by an imaging expert
and central review by two independent reviewers. The results of the metabolic
assessment remains blinded for the clinical investigator, and will follow a
predetermined 3-step methodology previously described.[13 15]

**Translational Research Genomic Analysis**

Blood samples for plasma preparation (2 x 9 mL for each time point) will be collected
(and frozen) at baseline (D-7->D0) and simultaneously with the second PET. An
extra 9 mL whole blood sample will be collected at baseline in order to distinguish
somatic from germline mutations. Plasma samples will also be obtained every 2
cycles, starting from the first cycle and (simultaneously with biology) up to 1 year
after the start of therapy. Moreover, a final plasma sample will be obtained at disease
progression (see Figure 2 for overview of sample collection). Optional fresh frozen
tumor tissue from a FDG PET targetable metastatic or primary lesion will be obtained
before study entry and at disease progression. Previously collected frozen or FFPE
primary or metastatic tumor needs to be available at the study entry. The translational
research group will study the genetic and epigenetic aberrations that are associated
with the patient’s outcome (PFS, OS) and with metabolic response after treatment
with regorafenib. The molecular aberrations will be investigated using gene expression profiling, RNA and exome sequencing, and methylation profiling of the tumor biopsies and repeated blood samples taken during therapy.

**Follow-up**

Follow-up procedures, performed every 2 months will include physical examination, vital signs and ECOG performance status, laboratory tests and blood samples for translational research analysis, as well as radiological assessment of the tumours.

According to the European Union (EU) Clinical Trial Directive for each participating European Union (EU) country, the end of the study will be reached when the last visit under treatment protocol of the last subject for all centers has occurred. The patients will continue to be followed afterwards every 2 months till disease progression or death or refusal, whichever occurs first. Patients may be offered any further treatment available, at the investigator’s discretion.

**Statistical Considerations**

Analyses will be carried out looking at constructing signatures that are prognostic for OS as well as PFS and RR. Baseline data will be used for some models and all eligible patients (intent to treat population) will be included in these analyses. Models integrating data in the short follow-up after treatment will also be developed. In that later situation, only patients having undergone the necessary examinations will be included in the analyses. Time zero for measuring time to event variables will be adapted and will be dependent on the timing of further examinations. Analyses will be adjusted for multiplicity. As no formal hypothesis can be done on the discriminating value of genomic biomarkers, it was decided to target a sample size of 105 evaluable patients and to use the first 70 patients as derivation set and the 35 last patients as validation set. It should be stressed however that the total sample size of 105 evaluable patients is sufficient to validate the hypothesis generated by the SoMore study.[14] We will use a binary assessment of PET metabolic response based on previous results obtained from SoMore, sharing the same inclusion criteria. We will define OS and PFS from the time of second PET and analyze the relationship with PET metabolic response with univariate and multivariate Cox regression models. To
detect in a validation series that the HR for responders compared to non-responders is significantly < 1 (with a power of 80% in case of a true HR≤ 0.59 and a 1-sided alpha level of 5% and a rate of responders of 47%), we need to observe 89 events. We then plan to carry out a validation comparison between early homogeneous responders and the other patients once we will have reached 89 events for these 105 evaluable patients. For this validation comparison, there will be no split of the total sample. In order to reach this number of evaluable patients, the overall sample size of registered patients will be adapted during the study accrual. Taking into account an expected 20-25% drop-out rate between registration and the time of further examinations, between 124 and 140 patients will be accrued. The analyses for derivation will be carried out when at least 80% of events (deaths) will have been observed. The analyses for validation will occur also when 80% of events will be reached. The study is designed as a single-arm study, with all patients being accrued in one stage. No early stopping rules will be used. The clinicians will remain blinded to the assessment of FDG PET-CT response and of the whole molecular analyses.

C. ETHICS AND DISSEMINATION

Ethical Considerations

Patient Protection
The principal investigator ensures that this study conforms to the Declaration of Helsinki (available at http://www.wma.net/en/30publications/10policies/b3/) or the laws and regulations of the country, whichever provides the greatest protection of the patient. The study follows the International Conference on Harmonization E 6 (R1) Guideline for Good Clinical Practice, reference number CPMP/ICH/135/95 (available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf).

The competent ethics committee of the Institut Jules Bordet approved the protocol, as required by applicable national legislation.

Dissemination

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml
The protocol and the trials results, even inconclusive, will be presented at international oncology congresses, and published in peer-reviewed journals. Genomic and epigenetic data will be made available in public open datasets.

**Trial Sponsorship, Financing and Insurance**

This study is supported by an unrestricted grant from Bayer Healthcare Pharmaceutical, which provided regorafenib but played no further role in this study design; data collection, study management, and will play no role in results analysis, and interpretation of data; nor in writing of the report; nor in the decision to submit the report for publication, and has no kind of authority over any of these activities.

**Discussion and Conclusions**

Regorafenib, an oral multi kinase inhibitor that shares with sorafenib several targets involved in tumour angiogenesis, oncogenesis, and tumour microenvironment, improves the patients’ outcome, but at the cost of significant toxicities, underscoring the need to identify those who will benefit from therapy. A previous study (SoMore trial) showed that early FDG PET-based metabolic response assessment may adequately discriminate patients with chemorefractory aCRC unlikely to benefit from a sorafenib-capecitabine combination. RegARd-C aims to explore early FDG PET-based metabolic response assessment in patients treated with regorafenib 1) as a clinical tool to spare them from needless toxicity from a drug that gives them little or no benefit and 2) as a translational tool able to guide comprehensive genomic and epigenetic research on the determinants of drug resistance. This translational research will be conducted on fresh or archived tumoral tissues and on serial blood samples looking for free ctDNA. For a subset of patients, fresh frozen tumoral tissues taken by PET-guided biopsy procedures on metabolically resistant lesions will be available.

**List of abbreviations**
aCRC, advanced colorectal cancer; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; CR, Complete Response; CRC, Colorectal Cancer; CT, Computed Tomography; DCE-MRI, Dynamic Contrast-Enhanced Magnetic Resonance Imaging; DCR, Disease Control Rate; FDG PET-CT, FluoroDeoxyGlucose-Positron Emission Tomography-Computed Tomography; FFPE, Formalin Fixed Paraffin Embedded; FGFR, Fibroblast Growth Factor Receptor; HIV, Human Immunodeficiency Virus; HR, Hazard Ratio; IC, Informed Consent; IEC, Independent Ethic Committee; INR, International normalized ratio; mCRC, metastatic ColoRectal Cancer; MRI, Magnetic Resonance Imaging; NCI-CTCAE, National Cancer Institute – Common Terminology Criteria for Adverse Event; NGS, Next generation sequencing; NYHA, New York Heart Association; OS, Overall Survival; PDGFR, Platelet-Derived Growth Factor Receptor; PFS, Progression Free Survival; PIC, Patient Information and Consent forms; PR, Partial Response; PFS, Progression-free Survival; PT, Prothrombin time; RECIST, Response Evaluation Criteria in Solid Tumors; Response Rate; RTKs, Receptor Tyrosine Kinases; SD, Stable Disease; TIE, Tyrosine kinase with Immunoglobulin and EGF homology domains; ULN, Upper Limit of Normal; US, UltraSonography; VEGFR, Vascular Endothelial Growth Factor Receptor.

Competing interests

The authors report no conflicts of interest.

Authors' contributions

1. Alain Hendlisz, MD (Corresponding author) (A, B, C, D)
2. Amélie Deleporte, MD (A, B, C, D)
3. Caroline Vandeputte, PhD (A, B, D)
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6. Thomas Guiot, PhD (A, C, E)
7. Camilo Garcia, MD (A, B, C, D, E)
8. Patrick Flamen, MD, PhD (A, B, C, D, E)

A) Contribution to protocol writing
B) Manuscript design
C) Trial set-up  
D) Manuscript writing  
E) Coordination of PET imaging network  

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Figure legends

Figure 1. Study design. FDG PET = FluoroDeoxyGlucose-Positron Emission Tomography, FFPE = Formalin Fixed Paraffin Embedded, OS = Overall survival, PD = Disease progression.

Figure 2. Schedule of assessments. FDG PET = FluoroDeoxyGlucose-Positron Emission Tomography, FFPE = Formalin Fixed Paraffin Embedded.
In case of treatment interruption, the second PET scan can be delayed till maximum Q21 of first cycle. It must be performed while the patient is under the study medication since at least 4 days. Plasma sample will be taken simultaneously with the second PET scan.

1057x793mm (72 x 72 DPI)
Arched frozen (preferentially) or FFPE primary (preferentially) or metastatic tumor tissue previously collected.

Frozen tumor samples obtained (from a FDG-PET targetable lesion) prior to treatment start (2-4 cores of the metastasis or ≥10 fresh frozen cores of the primary tumor), and/or at disease progression

Two blood samples (2x9mL) → x aliquots plasma

One whole blood sample (1x9mL)
Regorafenib assessment in refractory advanced colorectal cancer: RegARd-C study protocol

Alain Hendlisz, Amélie Deleporte, Caroline Vandeputte, Nicolas Charette, Marianne Paesmans, Thomas Guiot, Camilo Garcia and Patrick Flamen

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