CONFIDENTIAL

ABBREVIATED CLINICAL STUDY REPORT

ANALYSIS OF 2nd LINE PANITUMUMAB + FOLFIRI EFFICACY IN WILD TYPE *RAS* CONVERTED SUBJECTS FROM INITIALLY MUTATED *RAS* SUBJECTS WITH METASTATIC COLORECTAL CANCER TREATED IN 1ST LINE WITH STANDARD FOLFOX/CAPOX+BEVACIZUMAB TREATMENT. STUDY CONVERTIX

Study code: GITuD-20172019

Study development phase: Phase II

EudraCT number: 2017-003242-25

Product: Panitumumab

Indication: Wild-type *RAS* metastatic colorectal cancer (mCRC)

First patient included: Not applicable (no patients were included in the study)

Last patient completed/last visit: Not applicable (no patients were included in the study)

Version: Final 1.0

Date: 17 May 2021

Coordinating/Principal Investigator: Dr Ana Fernández Montes

Sponsor signatory: Asociación GITuD

This study was performed in compliance with Good Clinical Practice (E6).

2 SYNOPSIS

Name of the Sponsor/Company: GITuD	**Individual Study Table Referring to Module 5 of the Dossier** **Volume:** **Page:** **Study No.:**	**(For National Authority Use only)**
Name of Finished Product: NA		
Name of Active Ingredient: NA		

STUDY CODE:
GITuD-20172019

TITLE OF STUDY:
Analysis of 2nd line panitumumab + FOLFIRI efficacy in wild-type *RAS* converted subjects from initially mutated *RAS* subjects with metastatic colorectal cancer treated in 1st line with standard FOLFOX/CAPOX + bevacizumab treatment. Study CONVERTIX

INVESTIGATOR(S):
Dr Ana Fernández Montes

STUDY CENTRE(S):
Eight centres were planned to participate in the study, but finally 6 centres screened patients: H. Ourense, H. A Coruña, H. Arquitecto Marcide, H. Lucus Augusti, H. Alvaro Cunqueiro, H. Povisa and H. Reina Sofia

PUBLICATION (REFERENCE):
None

STUDY PERIOD (YEARS):
Not applicable as no patients were included in the study

PHASE OF DEVELOPMENT:
Phase II

OBJECTIVES:
The original study objectives as stated in the protocol were not accomplished

METHODOLOGY:
Phase II, multi-center, single-arm study. A liquid biopsy was performed before second-line initiation in patients with mutated *RAS* metastatic colorectal cancer (mCRC) confirmed as per standard of care according to international guidelines before first-line initiation and who received 5-fluorouracil/leucovorin+oxaliplatin (FOLFOX) treatment in first line. Patients with *RAS* wild-type (WT) mCRC according to the liquid biopsy results were planned to enter the study to receive panitumumab plus 5-fluorouracil/leucovorin+irinotecan (FOLFIRI) until disease progression, unacceptable toxicity, investigator's decision or withdrawal of consent.

Since there was no information regarding the conversion to *RAS* WT in patients initially mutated, it was initially planned to evaluate *RAS* mutational status in 20 patients. If less than 2 patients had *RAS* WT mCRC according to the results of the liquid biopsy, the study would be stopped early due to lack of eligible patients and no more would be screened. In case that 2 or more patients presented *RAS* WT mCRC, a total of 40 patients with *RAS* WT mCRC were planned to enter the study at approximately 8 centers.

Analyses of *RAS* genes in solid and liquid biopsy (before first- and second-line treatment) using the

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Name of Active Ingredient: NA		

Idylla™ system were performed. Discordant results between solid and liquid biopsies were assessed by an independent NGS test (in both solid/liquid biopsies).

NUMBER OF PATIENTS (planned and analysed):

No. planned: 40
No. screened: 23
Males/females: 16/7
Mean age (SD):66.1 (9.8)
No. included: 0
No. analysed for efficacy: 0
No. analysed for safety: 0
No. completed the study: 0

A total of 23 patients were screened, but none of them met the selection criteria. Although the study could not be done, the results of *RAS* mutation analysis before first-line and second-line treatment, respectively, have provided new evidence relevant to clinical practice (see summary and conclusions in the synopsis).

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION:

Please see section 9.3

DURATION OF TREATMENT:

No patients were included in the study, so the duration of the treatment could not be assessed

CRITERIA FOR EVALUATION (EFFICACY/ SAFETY):

The original study criteria for evaluation of efficacy and safety as stated in the protocol could not be assessed

STATISTICAL METHODS:

Not applicable

SUMMARY AND CONCLUSION(S):

RESULTS:

As this study could not be performed because no patient met the selection criteria, efficacy and safety data are not reported.

Nevertheless, the genetic analysis of *RAS* mutations in solid biopsy (before first-line treatment) and liquid biopsy (before second-line treatment) showed that most patients (16/23, 69.6%) preserved the *RAS* mutation observed before first-line treatment. In 7 out of 23 patients the *RAS* mutation presented some discrepancies between the solid biopsy and the liquid biopsy using the Idylla™ system.

The genetic results of these 7 patients were confirmed using a more sensitive technique, NGS (in solid and liquid biopsies). In 4 out of 23 patients (1-003, 1-005, 1-007 and 4-001) the *RAS* mutation observed in solid biopsy was not detected in liquid biopsy using the Idylla™ system. However, the genetic analysis in liquid biopsy using NGS allowed to detect a *KRAS* mutation in patient 1-003. Therefore, in 3 out of 23 patients (13.0%) (1-005, 1-007 and 4-001) undetectable ctDNA in liquid biopsy was verified by NGS as previously observed using the Idylla™ system. In addition, in 3 patients

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Name of Finished Product: NA	**Volume:** **Page:** **Study No.:**	
Name of Active Ingredient: NA		

(4-001, 3-002 and 2-002) some discrepancies in mutated *RAS* genes (*KRAS* and *NRAS*) (Idylla™ system) were reported between solid and liquid biopsies. In these three patients, NGS confirmed the results in liquid biopsy but in solid biopsy, some discrepancies were noted when comparing the results of the Idylla™ system with NGS. The analysis of these three samples in solid biopsies using both techniques were repeated with Idylla™ system in the original tissue, and the results of NGS were confirmed. The initial data of solid biopsies with Idylla™ system showed some inconsistencies found in each corresponding centre. Specifically, patient 4-001 had an *NRAS* mutation in solid biopsy using Idylla™ system while only a *BRAF* mutation was confirmed by NGS. Patient 3-002 had a *KRAS* mutation in solid biopsy but an *NRAS* mutation by NGS. Patient 2-002 had an *NRAS* mutation in solid biopsy but it was confirmed by NGS that this was a *KRAS* mutation instead.

CONCLUSION(S):

Most patients (70%) preserved the *RAS* mutation observed before first-line treatment and a small percentage of patients (nearly 13%) had undetectable *RAS* mutant clones in liquid biopsy after first-line treatment with FOLFOX/CAPOX+bevacizumab.

Genotyping mCRC is crucial for personalised treatments. The results of the genetic analyses suggest that a clinical improvement in the accuracy of genotyping is needed, especially in solid biopsies. As there is clonal selection, this approach should be implemented as part of the routine care of these patientsto allow for an adequate follow-up. In addition, this approach may not only be implemented in mCRC but also in localized disease.

3 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

4.1 List of Abbreviations

5FU	5-fluorouracil
5FU/LV	5-fluorouracil/leucovorin
BRAF	B-Raf proto-oncogene
CAPOX	Capecitabine+oxaliplatin
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	circulating cell-free tumour DNA
ECOG	Eastern Cooperative Oncology Group
EEA	European Economic Area
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
FOLFIRI	5-fluorouracil/leucovorin+irinotecan
FOLFOX	5-fluorouracil/leucovorin+oxaliplatin
GITuD	Grupo Gallego de Investigación en Tumores Digestivos
GCP	Good Clinical Practice
IEC	Independent Ethics Committee
IRB	Institutional Review Board
KRAS	Kirsten Rat Sarcoma
MAF	Minor Allele Fraction
Max	Maximum
mCRC	Metastatic Colorectal Cancer
Min	Minimum
NRAS	Neuroblastoma *RAS*
NGS	Next Generation Sequencing
Q1	First Quartile
Q3	Third Quartile
RECIST	Response Evaluation Criteria in Solid Tumors

SD	Standard Deviation
TFS	Trial Form Support
TGFα	Transforming growth factor α
ULN	Upper Limit of Normal
VHIO	Vall d'Hebron Institute of Oncology
WT	Wild-type

4.2 Definition of Terms

Competent Authority	A government body or government appointed body that has legal authority to approve or disapprove clinical studies.
Eligible Participant	Any potential participant who upon entrance into the study meets all of the inclusion criteria and none of the exclusion criteria set forth in the protocol and had signed a valid independent ethics committee (IEC) approved informed consent form. The form prepared in conformance with the regulations (as hereinafter defined), in consultation with the Sponsor, and the IEC (as hereinafter defined), approved by the IEC and signed and personally dated by all patients before any study related procedures or assessments are performed.
Study	The clinical study known as Study Convertix to be conducted according to the protocol.
Study Center	The location where study-related activities are conducted.

5 ETHICS

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

The final study protocol, including the substantial amendment and the final version of the patient information and consent form, were reviewed and approved by an IEC or IRB prior to inclusion of patients.

5.2 Ethical Conduct of the Study

The study was conducted in compliance with the protocol, regulatory requirements, good clinical practice (GCP) and the ethical principles of the latest revision of the Declaration of Helsinki as adopted by the World Medical Association.

5.3 Patient Information and Consent

All patients received written and verbal information regarding the study. The given information emphasized that participation in the study was voluntary and that the patient could withdraw from the study at any time and for any reason. All patients were given the opportunity to ask questions about the study and were given sufficient time to decide whether to participate in the study.

Before any study-related procedures, the informed consent form was signed and personally dated by the patient (or their legally acceptable representative and/or witness, as applicable) and by the person who conducted the informed consent discussion.

The consent included information that data was recorded, collected, processed and could be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC), the data did not identify any persons taking part in the study.

6 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

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7 INTRODUCTION

Panitumumab is a high-affinity fully human IgG2 monoclonal antibody against human epidermal growth factor receptor (EGFR). It blocks epidermal growth factor (EGF) and Transforming growth factor (TGF)α ligand binding to EGFR, inhibits tumor growth and causes tumor regression and eradication of established tumors in xenograft mouse models.

The results of a prospective-retrospective analysis of biomarkers in subjects included in a study of panitumumab + 5-fluorouracil/leucovorin+oxaliplatin (FOLFOX) in first-line (PRIME) allowed to confirm the clinical relevance of the analysis of mutations in exons 3 and 4 of Kirsten Rat Sarcoma (*KRAS)* and exons 2, 3 and 4 of neuroblastoma *RAS* (*NRAS)*, in addition to mutations in exon 2 of *KRAS* in subjects candidates for anti-EGFR therapy.

Product specifications of panitumumab have been modified and only subjects with wild-type (WT) *RAS* tumors are now candidates for anti-EGFR therapy.

In a study conducted among subjects with mCRC treated with panitumumab, mutations in *KRAS* were detected by analysis of circulating cell-free tumour DNA (ctDNA) in 9 of 24 (38%) subjects who initially had WT *KRAS* tumors. These mutations were detected between 5 and 6 months after initiation of treatment with panitumumab. Further studies have shown a 50% *RAS* WT conversion after only 3 months of first-line anti-angiogenic drug treatments and a 38% *RAS* WT conversion in mCRC patients pretreated with irinotecan- and oxaliplatin- or oxaliplatin-based chemotherapy which progressed after 5-fluorouracil/leucovorin+irinotecan (FOLFIRI) + panitumumab second-line treatment.

However, we cannot predict which are going to be the changes in the mutational status of subjects that are initially mutated *RAS* (before first-line initiation). Therefore, it is important to investigate whether these subjects can become *RAS* WT after first-line treatment. The analysis of ctDNA in liquid biopsies can provide information on the genetic status of the tumor before initiating a second-line treatment.

In addition, studying the *RAS* mutation status at the time of disease progression may help investigators understand the mechanisms involved in the onset of drug resistance during treatment with panitumumab. All mCRC patients who initially respond to anti-EGFR therapy eventually develop resistance, which in approximately 50% of cases is due to the emergence of *RAS* mutations, indicating that clonal evolution of drug-resistant cells is associated with the clinical outcome of CRC patients treated with anti-EGFR antibodies.

An understanding of the mechanisms involved in this secondary resistance to panitumumab therapy is critical for monitoring, preventing and/or overcoming the onset of drug resistance.

A more detailed background and rationale is provided in Protocol-Section 2.

8 STUDY OBJECTIVES

The original study objectives as stated in the protocol were not accomplished.

Detailed study objectives are described in Protocol-Section 1.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan-Description

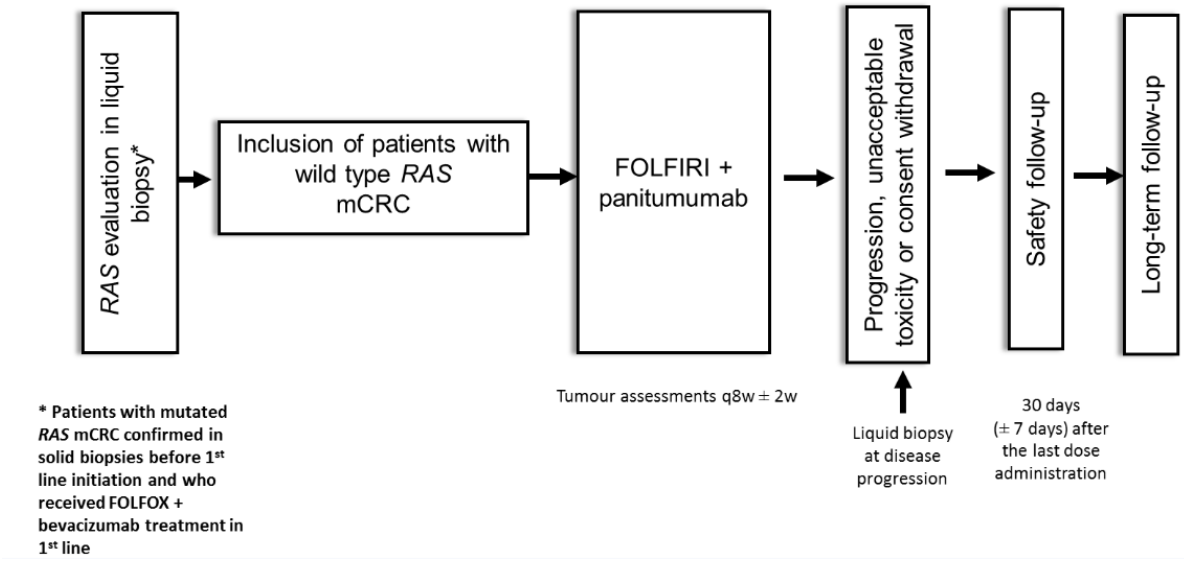
Phase II, multi-center, single-arm study. A liquid biopsy was performed before second line initiation in patients with mutated *RAS* mCRC confirmed as per standard of care according to international guidelines before first-line initiation and who received FOLFOX treatment in first line. Patients with *RAS* WT mCRC according to the liquid biopsy results were planned to enter the study to receive panitumumab plus FOLFIRI until disease progression, unacceptable toxicity, investigator decision or withdrawal of consent.

Following disease progression, it was planned to collect information on the subsequent lines of treatment chosen by the investigator and survival at follow-up visits hold every 12 weeks (± 4 weeks), until completion of the trial (approximately 18 months after inclusion of the last patient).

Since there was no information regarding the conversion to *RAS* WT in patients initially mutated, it was initially planned to evaluate *RAS* mutational status in 20 patients. If less than 2 patients had *RAS* WT mCRC according to the results of the liquid biopsy, the study would be stopped early due to lack of eligible patients and no more would be screened. In case that 2 or more patients presented *RAS* WT mCRC, a total of 40 patients with *RAS* WT mCRC were planned to enter the study at approximately 8 centers. Finally, 23 patients were screened at 8 centers), but any of them were included in the study because they did not meet the selection criteria.

Although the study could not finally be done, *KRAS* results of these samples (solid and liquid biopsy, before first- and second-line treatment, respectively) using Idylla™ system were compared and, in some, discordant results between solid and liquid biopsies were observed. A letter to the IEC was sent informing about these discordances and asking permission to analyze these samples using next-generation sequencing (NGS) (Vall d'Hebron Institute of Oncology [VHIO] Custom Amplicon-seq panel). The IEC approved this request.

Figure 1 Overall Study Design



Abbreviations: FOLFORI, 5-fluorouracil/leucovorin+irinotecan; FOLFOX, 5-fluorouracil/leucovorin+oxaliplatin; mCRC, metastatic colorectal cancer; q8w, every 4 weeks; w, weeks

9.2 Discussion of Study Design, including the Choice of Control Groups

The study was designed to evaluate the efficacy and safety of panitumumab + FOLFIRI as second-line treatment in patients with mCRC that are *RAS* WT according to the results of a liquid biopsy at treatment initiation and who were mutated *RAS* at first-line initiation (standard FOLFOX+ bevacizumab treatment).

The *RAS* mutational status was initially planned to be evaluated in 20 patients. If less than 2 patients had *RAS* WT mCRC according to the results of the liquid biopsy, the study would be stopped early due to lack of eligible patients and no more would be screened. In case that 2 or more patients presented *RAS* WT mCRC, a total of 40 patients with *RAS* WT mCRC would enter the study at approximately 8 centers.

9.3 Selection of Study Population

9.3.1 Inclusion criteria

1) Man or woman at least 18 years old
2) Capable of understand, sign and date an informed consent approved by an IEC
3) Histologically confirmed adenocarcinoma of the colon or rectum in subjects with unresectable metastatic (M1) disease
4) At least one unidimensionally measurable lesion of at least 10 mm per Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.1)
5) Patients who received only one prior chemotherapy regimen for mCRC consisting of first-line FOLFOX+ bevacizumab.
6) Radiographically confirmed disease progression after first-line FOLFOX + bevacizumab.

7) Patients who had mutated *RAS* status confirmed as per standard of care according to international guidelines prior to first-line initiation

8) Patients candidate to second-line treatment and with *RAS* WT status in liquid biopsy confirmed prior to second-line initiation

9) Eastern Cooperative Oncology Group (ECOG) performance status 0-2

10) Adequate bone marrow function: neutrophils ≥1.5 $x10^9$/L; platelets ≥100 $x10^9$/L; hemoglobin ≥9 g/dL

11) Hepatic, renal and metabolic function as follows:

    - Total bilirubin count ≤1.5 x upper limit of normal (ULN), alanine aminotransferase and aspartate aminotransferase <5 x ULN

    - Renal function, calculated as creatinine clearance or 24-hour creatinine clearance ≥ 50 mL/min

    - Magnesium > lower limit of normal

9.3.2 Exclusion criteria

1) History of prior or concurrent central nervous system metastases

2) History of another primary cancer, except curatively treated *in situ* cervical cancer, or curatively resected non-melanoma skin cancer, or other primary solid tumor curatively treated with no known active disease present and no treatment administered for ≥ 5 years before the inclusion in the study

3) Unresolved toxicities of a previous systemic treatment that, in the opinion of the investigator, cause the subject unfit for inclusion

4) Prior EGFR antibody therapy (eg, cetuximab) or prior irinotecan therapy

5) Significant cardiovascular disease including unstable angina or myocardial infarction within 12 months before initiating study treatment or a history of ventricular arrhythmia

6) History of interstitial pneumonitis or pulmonary fibrosis or evidence of interstitial pneumonitis or pulmonary fibrosis on baseline chest computerized tomography

7) Acute or subacute intestinal occlusion and/or active inflammatory bowel disease or other bowel disease that causes chronic diarrhea (defined as grade ≥ 2 diarrhea according to Common Terminology Criteria for Adverse Events (CTCAE) v 4.03)

8) Evidence of previous acute hypersensitivity reaction, of any grade, to any component of the treatment

9) History of Gilbert disease or known dihydropyrimidine deficiency syndrome

10) History of any disease that may increase the risks associated with study participation or may interfere with the interpretation of study results.

11) Known positive test for human immunodeficiency virus infection, hepatitis C virus, chronic active hepatitis B infection

12) Any disorder that compromises the subject's ability to provide written informed consent and/or comply with study procedures

13) Any investigational agent within 30 days prior to inclusion

14) Pregnant or breastfeeding woman

15) Major surgery (excluding diagnostic biopsy or placement of a central venous catheter) and/or radiotherapy within 28 days prior to inclusion in the study.

16) Male or female of childbearing age who do not agree with taking adequate contraceptive precautions, i.e. use contraception double barrier (e.g. diaphragm plus condoms) or abstinence during the course of the study and for 6 months after the last administration of study drug for women and 1 month for men

17) The subject is unwilling or unable to meet the requirements of the study

18) Psychological, geographical, familial or sociological conditions that potentially prevent compliance with the study protocol and follow-up schedule. These conditions should be discussed with the subject before inclusion in the trial.

9.3.3 Removal of Patients from Therapy or Assessment

Withdrawal of patients is described in Protocol-Section 8.

9.4 Treatments

Treatments are described in Protocol-Section 6.

9.5 Efficacy and Safety Variables

Efficacy and Safety variables are described in Protocol-Section 13.2.

9.6 Data Quality Assurance

Details are provided in Protocol-Section 17.

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

Details are provided in Protocol-Section 13.

9.8 Changes in the Conduct of the Study or Planned Analyses

An amendment was done in January 2019. It included:

- Inclusion of CAPOX as first-line treatment option
- Elimination of efficacy analyses according to prior administration of bevacizumab
- Clarification of the number of patients *RAS* WT needed to decide the continuity of the study (2 patients out of 20 should be *RAS* WT after first-line treatment in order to proceed with the study)
- Clarification on the possibility to include patients in whom oxaliplatin had been discontinued during first-line treatment
- The exclusion criteria “Treatment for systemic infection within 14 days before the start of study treatment” was eliminated
- Correction of folinic acid dose according to dose marketed in Spain (isomer L +D)
- Patient information sheet/Consent form update

Since there was no information regarding the conversion to *RAS* WT in patients initially mutated, it was initially planned to evaluate *RA*S mutational status in 20 patients.

If less than 2 patients had *RAS* WT mCRC according to the results of the liquid biopsy, the study would be stopped early due to lack of eligible patients and no more would be screened. In case than 2 or more patients presented *RAS* WT mCRC, a total of 40 patients with *RAS* WT mCRC would enter the study at approximately 8 centers.

A total of 23 patients were screened at 8 centers. None were included in the study because they did not meet the selection criteria detailed in Section 9.3. Therefore, the study was not performed. Nevertheless, among these 23 patients the genetic results of *KRAS* (solid and liquid biopsy, before first- and second-line treatment, respectively) using Idylla™ system were compared. In some patients, discordant results between solid and liquid biopsies were observed. After IEC permission (see Section 9.1), discordant results were checked using NGS (in both solid and liquid biopsies).

10 STUDY PATIENTS

10.1 Disposition of Patients

A total of 8 centers in Spain participated in the study (H. Ourense, H. A Coruña, H. Arquitecto Marcide, H. Lucus Augusti, H. Alvaro Cunqueiro, H. Povisa and H. Reina Sofia) and a total of 23 patients were screened. None of these 23 patients met the selection criteria, mainly (n=19, 82.6%) confirmation of *RAS* WT status in liquid biopsy prior to second-line initiation (inclusion criteria 8). Therefore, the study could not be performed (Table 1).

Table 1 Inclusion/Exclusion Criteria. Screened Population

		Screened patients (N=23)
Is the subject eligible for study enrollment based on inclusion/exclusion criteria		
Yes	n (%)	0 (0.0%)
No	n (%)	23 (100.0%)
Inclusion/exclusion criteria numbers not met		
Exclusion criteria 6	n (%)	1 (4.3%)
Inclusion criteria 5	n (%)	1 (4.3%)
Inclusion criteria 6	n (%)	1 (4.3%)
Inclusion criteria 7	n (%)	1 (4.3%)
Inclusion criteria 8	n (%)	15 (65.2%)
Inclusion criteria 8/Inclusion criteria 10/Inclusion criteria 11	n (%)	1 (4.3%)
Inclusion criteria 8/Inclusion criteria 11	n (%)	1 (4.3%)
Inclusion criteria 8/Inclusion criteria 4	n (%)	2 (8.7%)

Source data 14.1.1 and Table 14.1.2. See definition of inclusion/exclusion criteria in section 9.3

Most of the 23 screened patients were male (69.6%), with a mean (standard deviation [SD]) age of 66.1 (9.8) years. ECOG 1 was reported in 65.2% of patients and a median (interquartile range [Q1,Q3] of 11.1 (7.5, 14.9) months since metastatic disease (Table 2, Table 3, Table 4).

A 56.5% of patients had undergone a prior surgical resection (Table 5 and Table 6), and only one patient (4.3%) had received radiotherapy (Table 7). Location of metastases is described in Table E.1 (see appendix).

In most patients, the first-line treatment was FOLFOX+bevacizumab (87.0%) and in three patients (13.0%) was CAPOX+bevacizumab. Further details about first-line treatment are given in Table 8.

Table 2 Demographic Data. Screened Population

			Screened patients (N=23)
Age (years)			
		n	23
		N missing	0
		Mean (SD)	66.1 (9.79)
		(Min, Max)	(48.0, 83.0)
		Median	68.0
		(Q1, Q3)	(57.0, 73.0)
Gender			
	Male	n (%)	16 (69.6%)
	Female	n (%)	7 (30.4%)
Race			
	Caucasian	n (%)	23 (100.0%)
	Asian	n (%)	0 (0.0%)
	Black	n (%)	0 (0.0%)
	Other	n (%)	0 (0.0%)

Source data Table 14.1.3

Abbreviations: n, number; min, minimum; max, maximum; Q1-Q3, first and third quartile; SD, standard deviation

Table 3 Performance Status: ECOG (Baseline). Screened Population

		Screened patients (N=23)
ECOG		
0: Fully active, able to carry on all pre-disease performance without restriction	n (%)	4 (17.4%)
1: Restricted in physically strenuous activity but ambulatory and able to carry out work	n (%)	15 (65.2%)
2: Ambulatory and capable of all self-care but unable to carry out any work activities	n (%)	4 (17.4%)
3: Capable of only limited self-care; confined to bed or chair more than 50% of waking hours	n (%)	0 (0.0%)
4: Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair	n (%)	0 (0.0%)
5: Dead	n (%)	0 (0.0%)

Source data Table 14.1.4

Abbreviations: n, number

Table 4 Cancer History. Screened Population

		Screened patients (N=23)
Time from diagnosis to baseline* (months)		
	n	23
	N missing	0
	Mean (SD)	13.8 (9.75)
	(Min, Max)	(3.9, 42.5)
	Median	11.1
	(Q1, Q3)	(7.5, 14.9)
Primary tumor site		
Colon	n (%)	21 (91.3%)
Rectum	n (%)	2 (8.7%)
Tumor sidedness (*)		
Left	n (%)	14 (60.9%)
Right	n (%)	8 (34.8%)
Missing	n (%)	1 (4.3%)
Histology type		
Adenocarcinoma	n (%)	23 (100.0%)
Other	n (%)	0 (0.0%)
Histology grade		
Well differentiated	n (%)	3 (13.0%)
Mildly differentiated	n (%)	8 (34.8%)
Poorly differentiated	n (%)	6 (26.1%)
Undifferentiated	n (%)	0 (0.0%)
Not evaluable	n (%)	6 (26.1%)
Time from metastatic disease diagnostic to baseline* (months)		
	n	23
	N missing	0
	Mean (SD)	12.8 (9.00)
	(Min, Max)	(3.9, 40.3)
	Median	11.1
	(Q1, Q3)	(6.3, 13.7)
TNM stage at diagnosis		
Missing	n (%)	1 (4.3%)
T2 N2 M1	n (%)	1 (4.3%)
T3 N1 M0	n (%)	1 (4.3%)
T3 N1 M1	n (%)	1 (4.3%)
T3 N1c M1	n (%)	1 (4.3%)
T3 N2 M1	n (%)	1 (4.3%)
T3 N2a M1	n (%)	1 (4.3%)
T3 N2a M1b	n (%)	1 (4.3%)

		Screened patients (N=23)
T4 N0 M1	n (%)	1 (4.3%)
T4 N1 M1	n (%)	1 (4.3%)
T4a N1b M1b	n (%)	1 (4.3%)
T4a N2 M1	n (%)	1 (4.3%)
T4a N2a M1	n (%)	1 (4.3%)
T4a N2b M1b	n (%)	1 (4.3%)
T4b N2 M1	n (%)	1 (4.3%)
T4b N2a M1	n (%)	2 (8.7%)
TX NX M1	n (%)	2 (8.7%)
TX NX M1b	n (%)	4 (17.4%)
Primary tumour pathological staging at diagnosis		
0	n (%)	0 (0.0%)
I	n (%)	0 (0.0%)
IIA	n (%)	0 (0.0%)
IIB	n (%)	0 (0.0%)
IIC	n (%)	0 (0.0%)
IIIA	n (%)	0 (0.0%)
IIIB	n (%)	1 (4.3%)
IIIC	n (%)	0 (0.0%)
IVA	n (%)	14 (60.9%)
IVB	n (%)	7 (30.4%)
Missing	n (%)	1 (4.3%)
Time from last radiologically confirmed progression after 1st line treatment to baseline (days)		
	n	23
	N missing	0
	Mean (SD)	7.7 (4.63)
	(Min, Max)	(1.0, 17.0)
	Median	7.0
	(Q1, Q3)	(5.0, 11.0)

(*) Patient with missing tumor sidedness: 001-007
Baseline=Screening visit.

Source data Table 14.1.5

*Baseline=Screening visit. Abbreviations: n, number; min, minimum; max, maximum; Q1-Q3, first and third quartile; SD, standard deviation

Table 5 Prior Anticancer Treatment: Surgery. Screened Population

		Screened patients (N=23)
Has patient undergone surgery to treat the disease?		
Yes	n (%)	13 (56.5%)
No	n (%)	10 (43.5%)

Source data Table 14.1.6.1

Abbreviations: n, number

Table 6 Prior Anticancer Treatment: Surgery by Center and Procedure. Screened Population

Center and procedure			Time from onset to baseline (months)			
	N	%	N	Mean		
RIGHT COLECTOMY + OMENTECTOMY	1	7.69%	1	11.24	Palliative	Macroscopical
ILEOSTOMY	1	7.69%	1	12.72	Palliative	Macroscopical
RECTOSIGMOID RESECTION	1	7.69%	1	10.62	Palliative	Macroscopical
SIGMOIDECTMY	1	7.69%	1	10.16	Radical	Microscopical
RIGHT HEMICOLECTOMY	1	7.69%	1	14.43	Radical	Macroscopical
	1	7.69%	1	41.36		No evidence of disease
SIGMA	1	7.69%	1	5.52	Palliative	Macroscopical
	1	7.69%	1	7.76		No evidence of disease
HARTMANN	1	7.69%	1	22.03	Palliative	Macroscopical
LIVER METASTASES	1	7.69%	1	30.66	Radical	No evidence of disease
COLOSTOMY	1	7.69%	1	5.29	Palliative	Macroscopical
HARTMANN SIGMOIDECTOMY	1	7.69%	1	10.13	Palliative	Macroscopical
ANTERIOR RESECTION OF THE RECT	1	7.69%	1	15.42	Radical	Macroscopical

Source data Table 14.1.6.2

Abbreviations: n, number

Table 7 Prior Anticancer Treatment: Radiotherapy. Screened Population

		Screened patients (N=23)
Has patient received radiotherapy to treat the disease?		
Yes	n (%)	1 (4.3%)
No	n (%)	22 (95.7%)

Source data Table 14.1.7.1 (for more details about the patient who received radiotherapy see Table 14.1.7.2 in the appendix)

Table 8 Prior Anticancer Treatment: First-line FOLFOX/CAPOX+Bevacizumab Treatment. Screened Population

		Screened patients (N=23)
Schema		
FOLFOX+bevacizumab	n (%)	20 (87.0%)
CAPOX+bevacizumab	n (%)	3 (13.0%)
Number of 5FU/LV or capecitabin cycles		
	n	23
	N missing	0
	Mean (SD)	13.3 (8.62)
	(Min, Max)	(5.0, 42.0)
	Median	12.0
	(Q1, Q3)	(8.0, 17.0)
Number of oxaliplatin cycles		
	n	23
	N missing	0
	Mean (SD)	9.9 (3.90)
	(Min, Max)	(5.0, 18.0)
	Median	11.0
	(Q1, Q3)	(5.0, 12.0)
Number of bevacizumab cycles		
	n	23
	N missing	0
	Mean (SD)	13.0 (8.76)
	(Min, Max)	(5.0, 42.0)
	Median	12.0
	(Q1, Q3)	(7.0, 16.0)
Number of patients receiving each number of drugs at the end of the treatment		
1	n (%)	2 (8.7%)
2	n (%)	10 (43.5%)
3	n (%)	11 (47.8%)
Did patient receive maintenance treatment?		
Yes	n (%)	5 (21.7%)
No	n (%)	18 (78.3%)
Type of maintenance treatment		
5FU	n (%)	0 (0.0%)
5FU-Bevacizumab	n (%)	4 (80.0%)
Bevacizumab	n (%)	1 (20.0%)
Time from first-line treatment start date to baseline (months)		

		Screened patients (N=23)
	n	23
	N missing	0
	Mean (SD)	8.9 (4.22)
	(Min, Max)	(2.3, 20.7)
	Median	9.4
	(Q1, Q3)	(5.6, 10.4)
Treatment duration (months)		
	n	23
	N missing	0
	Mean (SD)	7.9 (5.00)
	(Min, Max)	(1.9, 20.9)
	Median	6.0
	(Q1, Q3)	(5.0, 9.2)
Best response		
Complete Response	n (%)	0 (0.0%)
Partial Response	n (%)	12 (52.2%)
Stable disease	n (%)	6 (26.1%)
Progressive disease	n (%)	5 (21.7%)
Not evaluable	n (%)	0 (0.0%)
Unknown	n (%)	0 (0.0%)
Time from first-line treatment start date to progression (months)		
	n	20
	N missing	1
	Mean (SD)	8.7 (4.43)
	(Min, Max)	(1.9, 20.5)
	Median	9.0
	(Q1, Q3)	(5.6, 11.1)
Time from progression to baseline (weeks)		
	n	20
	N missing	1
	Mean (SD)	1.0 (0.71)
	(Min, Max)	(0.0, 2.4)
	Median	0.9
	(Q1, Q3)	(0.4, 1.5)

Baseline=Screening visit.

Source data Table 14.1.8

*Baseline=Screening visit. Abbreviations: n, number; min, minimum; max, maximum; Q1-Q3, first and third quartile; SD, standard deviation

The results of *RAS* mutational status were analyzed in all these 23 patients in solid and liquid biopsies (before first- and second-line treatment, respectively) using the Idylla™ system. Among the 23 patients screened, before initiation of second-line treatment most (16/23, 69.6%) preserved the *RAS* mutation observed before first-line treatment. However, in 7 out of 23 patients there were discrepancies in *RAS* mutations between solid biopsy (analized before first-line treatment) and liquid biopsy (analized before second-line treatment) by using Idylla™ system (see Table 9). The results of these 7 patients were confirmed using a more sensitive technique, NGS (in both solid and liquid biopsies). In 4 out of 23 patients (1-003, 1-005, 1-007 and 4-001) the *RAS* mutation observed in the solid biopsy was not detected in the liquid biopsy using the Idylla™ system. The use of NGS on the liquid biopsy (before second-line treatment) allowed to detect a *KRAS* mutation in patient 1-003. Therefore, in 3 out of 23 patients (13.0%) (1-005, 1-007 and 4-001) undetectable of ctDNA in liquid biopsy was verified by NGS as previously observed using Idylla™ system. In addition, in 3 patients (4-001, 3-002 and 2-002) some discrepancies in mutated *RAS* genes (*KRAS* and *NRAS* genes) (Idylla™ system) were reported between solid and liquid biopsies. In these three patients, the results of NGS confirmed the results in liquid biopsy but some discrepancies in solid biopsy using Idylla™ system compared with NGS were reported. The analysis of these three patients with discordant results in solid biopsies using both techniques were repeated with Idylla™ system in the original tissue. The results obtained showed that the initial results of solid biopsies with Idylla™ system had some inconsistencies/mistakes found in each corresponding center. Specifically, patient 4-001 presented an *NRAS* mutation in solid biopsy using Idylla™ system while only a *BRAF* mutation was confirmed by NGS. Patient 3-002 had a *KRAS* mutation in solid biopsy but an NRAS mutation by NGS. Patient 2-002 reported an *NRAS* mutation in solid biopsy, but it was confirmed by NGS that this was a *KRAS* mutation instead. The result of NGS was later reconfirmed using Idylla™ system in solid biopsy.

Table 9 Changes in *KRAS*, *NRAS* and *BRAF* Biomarkers Mutation Status. Screened Population

Patients with first-line FOLFOX/CAPOX+bevacizumab treatments (N=23)

		Drugs at the end of first-line treatment								Result of biopsies				Codon Exon Nucleotide position/change					
			No of cycles of each drug			Drugs administered				*RAS*		*BRAF*		*KRAS*		*NRAS*		*BRAF*	
Center ID	Schema	Number of drugs	5FU/LV or Capecitabin	Oxaliplatin	Bevacizumab	5FU/LV	Capecitabin	Oxaliplatin	Bevacizumab	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy
001/001	FOLFOX+ Bevacizumab	2	22	5	22	Yes	No	No	Yes	Mutant	Mutant	NA	NA	Codon 12 (exon 2) - G12C (c.34G>T)	Codon 12 (exon 2) - G12C (c.34G>T)				
001/002	FOLFOX+ Bevacizumab	3	18	18	18	Yes	No	Yes	Yes	Mutant	Mutant	NA	Wild type		Codon 12 (exon 2) - G12D (c.35G>A)				
001/003	CAPOX+ Bevacizumab	3	5	5	5	No	Yes	Yes	Yes	Mutant	Wild type	NA	NA	Codon 59 (exon 3) - A59E (c.176C>A)					
001/004	FOLFOX+ Bevacizumab	2	15	13	15	Yes	No	No	Yes	Mutant	Mutant	Wild type	NA	Codon 12 (exon 2) - G12C (c.34G>T)					
															Codon 12 (exon 2) - G12S (c.34G>A)				

Patients with first-line FOLFOX/CAPOX+bevacizumab treatments (N=23)

		Drugs at the end of first-line treatment								Result of biopsies				Codon Exon Nucleotide position/change					
			No of cycles of each drug			Drugs administered				*RAS*		*BRAF*		*KRAS*		*NRAS*		*BRAF*	
Center ID	Schema	Number of drugs	5FU/LV or Capecitabin	Oxaliplatin	Bevacizumab	5FU/LV	Capecitabin	Oxaliplatin	Bevacizumab	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy
001/005	FOLFOX+ Bevacizumab	3	13	13	13	Yes	No	Yes	Yes	Mutant	Wild type	NA	NA	Codon 12 (exon 2) - G12C (c.34G>T)					
001/006	FOLFOX+ Bevacizumab	3	7	7	7	Yes	No	Yes	Yes	Mutant	Mutant	Wild type	Wild type	Codon 12 (exon 2) - G12C (c.34G>T)					
															Codon 12 (exon 2) - G12A (c.35G>C)				
001/007	FOLFOX+ Bevacizumab	2	8	9	9	No	No	Yes	Yes	Mutant	Wild type	NA	NA	Codon 12 (exon 2) - G12C (c.34G>T)					
002/001	FOLFOX+ Bevacizumab	3	13	13	13	Yes	No	Yes	Yes	Mutant	Mutant	NA	NA	Codon 61 (exon 3) - Q61H (c.183A>C; C.183A>T)	Codon 61 (exon 3) - Q61H (c.183A>C; C.183A>T)				
002/002	FOLFOX+ Bevacizumab	2	10	10	8	Yes	No	Yes	No	Mutant	Mutant	NA	NA		Codon 12 (exon 2) - G12D (c.35G>A)				

Patients with first-line FOLFOX/CAPOX+bevacizumab treatments (N=23)

		Drugs at the end of first-line treatment								Result of biopsies				Codon Exon Nucleotide position/change					
			No of cycles of each drug			Drugs administered				*RAS*		*BRAF*		*KRAS*		*NRAS*		*BRAF*	
Center ID	Schema	Number of drugs	5FU/LV or Capecitabin	Oxaliplatin	Bevacizumab	5FU/LV	Capecitabin	Oxaliplatin	Bevacizumab	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy
																Codon 12 (exon 2) - G12D (c.35G>A)			
002/003	FOLFOX+ Bevacizumab	3	5	5	5	Yes	No	Yes	Yes	Mutant	Mutant	NA	NA	Codon 13 (exon 2) - G13D (c.38G>A)	Codon 13 (exon 2) - G13D (c.38G>A)				
002/004	CAPOX+ Bevacizumab	3	5	5	5	No	Yes	Yes	Yes	Mutant	Mutant	NA	NA	Codon 12 (exon 2) - G12C (c.34G>T)	Codon 12 (exon 2) - G12C (c.34G>T)				
002/005	CAPOX+ Bevacizumab	1	9	5	10	No	No	No	Yes	Mutant	Mutant	NA	NA		Codon 61 (exon 3) - Q61K (c.181C>A; c.180_181delinsAA)				
														Codon 61 (exon 3) - Q61R (c.182A>G)	Codon 61 (exon 3) - Q61R (c.182A>G)				
003/001	FOLFOX+ Bevacizumab	2	29	12	29	Yes	No	No	Yes	Mutant	Mutant	NA	NA	Codon 12 (exon 2) - G12C (c.34G>T)					

Patients with first-line FOLFOX/CAPOX+bevacizumab treatments (N=23)

		Drugs at the end of first-line treatment								Result of biopsies				Codon Exon Nucleotide position/change					
			No of cycles of each drug			Drugs administered				*RAS*		*BRAF*		*KRAS*		*NRAS*		*BRAF*	
Center ID	Schema	Number of drugs	5FU/LV or Capecitabin	Oxaliplatin	Bevacizumab	5FU/LV	Capecitabin	Oxaliplatin	Bevacizumab	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy
003/002	FOLFOX+ Bevacizumab	2	42	12	42	Yes	No	No	Yes	Mutant	Mutant	NA	NA	Codon 13 (exon 2) - G13D (c.38G>A) Codon 59 (exon 3) - A59E (c.176C>A) Codon 61 (exon 3) - Q61L (c.182A>T)	Codon 13 (exon 2) - G13D (c.38G>A)		Codon 61 (exon 3) - Q61K (c.181C>A) Codon 61 (exon 3) - Q61R (c.182A>G)		
004/001	FOLFOX+ Bevacizumab	1	9	7	5	Yes	No	No	No	Mutant	Wild type	NA	Mutant						Codon 600 BRAF V600E (c.1799T>A; c.1799_1800 delinsAA)

Patients with first-line FOLFOX/CAPOX+bevacizumab treatments (N=23)

		Drugs at the end of first-line treatment								Result of biopsies						Codon Exon Nucleotide position/change			
			No of cycles of each drug			Drugs administered				*RAS*		*BRAF*		*KRAS*		*NRAS*		*BRAF*	
Center ID	Schema	Number of drugs	5FU/LV or Capecitabin	Oxaliplatin	Bevacizumab	5FU/LV	Capecitabin	Oxaliplatin	Bevacizumab	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy
																			Codon 600 BRAF V600D (c.1799_1800 delinsAC)
																Codon 12 (exon 2) - G12D (c.35G>A)			
004/002	FOLFOX+ Bevacizumab	3	11	11	11	Yes	No	Yes	Yes	Mutant	Mutant	NA	NA	Codon 12 (exon 2) - G12D (c.35G>A)	Codon 12 (exon 2) - G12D (c.35G>A)				
005/001	FOLFOX+ Bevacizumab	3	5	5	5	Yes	No	Yes	Yes	Mutant	Mutant	Wild type	Wild type	Codon 12 (exon 2) - G12V (c.35G>T)	Codon 12 (exon 2) - G12V (c.35G>T)				
005/002	FOLFOX+ Bevacizumab	2	18	12	18	Yes	No	No	Yes	Mutant	Mutant	Wild type	NA	Codon 13 (exon 2) - G13D (c.38G>A)	Codon 13 (exon 2) - G13D (c.38G>A)				
005/003	FOLFOX+ Bevacizumab	2	8	8	7	Yes	No	Yes	No	Mutant	Mutant	Wild type	NA	Codon 13 (exon 2) - G13D (c.38G>A)	Codon 13 (exon 2) - G13D (c.38G>A)				
005/004	FOLFOX+ Bevacizumab	2	17	17	16	Yes	No	Yes	No	Mutant	Mutant	Wild type	NA			Codon 61 (exon 3) - Q61R (c.182A>G)	Codon 61 (exon 3) - Q61R (c.182A>G)		

Patients with first-line FOLFOX/CAPOX+bevacizumab treatments (N=23)

		Drugs at the end of first-line treatment								Result of biopsies				Codon Exon Nucleotide position/change					
			No of cycles of each drug			Drugs administered				*RAS*		*BRAF*		*KRAS*		*NRAS*		*BRAF*	
Center ID	Schema	Number of drugs	5FU/LV or Capecitabin	Oxaliplatin	Bevacizumab	5FU/LV	Capecitabin	Oxaliplatin	Bevacizumab	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy
005/005	FOLFOX+ Bevacizumab	2	12	11	12	Yes	No	No	Yes	Mutant	Mutant	Wild type	NA	Codon 12 (exon 2) - G12D (c.35G>A)	Codon 12 (exon 2) - G12D (c.35G>A)				
006/001	FOLFOX+ Bevacizumab	3	12	12	12	Yes	No	Yes	Yes	Mutant	Mutant	NA	NA	Codon 12 (exon 2) - G12C (c.34G>T)					
															Codon 13 (exon 2) - G13D (c.38G>A)				
006/002	FOLFOX+ Bevacizumab	3	12	12	12	Yes	No	Yes	Yes	Mutant	Mutant	Wild type	NA	Codon 117 (exon 4) - K117N (c.351A>C; C.351A>T)					
															Codon 146 (exon 4) - A146P (c.436G>C)				
															Codon 146 (exon 4) - A146T (c.436G>A)				

Patients with first-line FOLFOX/CAPOX+bevacizumab treatments (N=23)

			Drugs at the end of first-line treatment							Result of biopsies					Codon Exon Nucleotide position/change				
			No of cycles of each drug			Drugs administered				*RAS*		*BRAF*		*KRAS*	*NRAS*				*BRAF*
Center ID	Schema	Number of drugs	5FU/LV or Capecitabin	Oxaliplatin	Bevacizumab	5FU/LV	Capecitabin	Oxaliplatin	Bevacizumab	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy	Solid biopsy	Liquid biopsy
															Codon 146 (exon 4) - A146V (c.437C>T)				

Baseline=Screening visit.

Source Table 14.1.9

Abbreviations: BRAF, B-Raf proto-oncogene; ID, identification; KRAS, Kirsten Rat Sarcoma; n, number; NRAS, Neuroblastoma RAS

11 EFFICACY EVALUATION

Not applicable as no patients met the selection criteria, and thus no patients were included in the study.

12 SAFETY EVALUATION

Not applicable as no patients were included in the study.

13 DISCUSSION AND OVERALL CONCLUSIONS

Although this clinical study (EudraCT number 2017-003242-25) could not be performed as no patients met the selection criteria, the results of *RAS* mutation analysis before first-line and second-line treatment, have provided new knowledge. In particular, the analysis of *RAS* genes of those patients that presented discordant results between solid and liquid biopsies (before first and second-line treatment, respectively) using the Idylla™ system has provided new evidence relevant for clinical practice.

RAS mutations in solid and liquid biopsies were analyzed to determine if the treatment pressure could confer any selection of *RAS* mutations at progression of disease as reported previously[1,2]. Most patients (70%) had concordant solid and liquid biopsy results in mutated *RAS* genes. However, 7 patients presented discordant results between their solid and liquid biopsy using the Idylla™ system. NGS was used to confirm these results in both solid and liquid biopsies.

The discordances found among solid and liquid biopsies with the Idylla™ system revealed that in 4 patients *RAS* mutations were undetectable in plasma before initiation of second-line treatment. In 3 of them, *RAS* mutations were undetectable in plasma by NGS as well. However, in the remaining patient (1-003) a *KRAS* mutation could be detected in plasma by NGS with a low minor allele fraction (MAF). Therefore, it is relevant to use a sensitive method, as patients getting false negative *RAS* mutation results could inappropriately receive anti-EGFR therapy.

On the other hand, in the remaining 3 patients some differences were observed in *RAS* mutation results between solid biopsy using the Idylla™ system and NGS. Genetic analyses in solid biopsy were repeated using the Idylla™ system. The results obtained matched those obtained with NGS confirming that the first results obtained with Idylla™ were most likely due to technical issues during routine testing. With the development of *KRAS* inhibitors, it is crucial to accurately genotype patients, as knowing which codon and exon are affected would allow for a better therapeutic approach.

This study has some limitations. Firstly, it was not designed to assess the genetic analysis of *RAS* genes in mCRC patients and the number of patients in whom this analysis was undertaken was very small. Moreover, the limited access to liquid biopsies performed before first-line treatment is also an important limitation. In addition, the absence of detectable *RAS* mutations in plasma before second-line treatment certainly does not exclude that a *RAS* mutation might be present in the sample below the assay limit of detection. In this sense, the sensitivity of techniques for genetic analyses is relevant[3] , as for example for MAFs <1% the capacity of the Idylla™ system to detect to detect *KRAS* mutation in plasma is reduced. Although NGS is more sensitive than the Idylla™ system, standardization of diagnostic NGS still limits its implementation in clinical practice, and the correct detection of mutations at low MAF is still needed[4].

Genotyping mCRC is crucial for personalizing treatments. These results presented herein suggest a clinical improvement in the accuracy of genotyping is needed, especially in solid biopsies. As there is clonal selection, this approach should be implemented as part of the routine care of these patients, to allow for an adequate follow-up. In addition, this approach may not only be implemented in mCRC but also in localized disease.

14 TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

Tables are attached in an appendix.

15 REFERENCE LIST

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