Clinical trial results:
Estudio abierto fase II de ultra-selección de pacientes mediante tecnología de genotipado de nueva generación para el esquema FOLFIRI + Panitumumab en pacientes con cáncer colorrectal estadio IV resistentes a irinotecán sin mutaciones detectables utilizando técnicas de alta sensibilidad para la detección de mutaciones en los genes KRAS, PIK3Ca, BRAF y NRAS

Summary

EudraCT number	2012-001955-38
Trial protocol	ES
Global end of trial date	30 July 2016

Results information

Result version number	v1 (current)
This version publication date	02 July 2020
First version publication date	02 July 2020

Trial information

Trial identification

Sponsor protocol code	TTD-12-03

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT01704703
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	Grupo de Tratamiento de los Tumores Digestivos (TTD)
Sponsor organisation address	C/ Téllez Nº 30 posterior 1º oficina 4.2, Madrid, Spain, 28007
Public contact	TTD, Grupo de Tratamiento de los Tumores Digestivos (TTD), 0034 91 378 82 75, ttd@ttdgroup.org
Scientific contact	TTD, Grupo de Tratamiento de los Tumores Digestivos (TTD), 0034 91 378 82 75, ttd@ttdgroup.org

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	30 May 2018
Is this the analysis of the primary completion data?	Yes
Primary completion date	30 July 2016
Global end of trial reached?	Yes
Global end of trial date	30 July 2016
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

To estimate the effect of the combination of panitumumab with FOLFIRI on objective response rate defined as complete and partial response according RECIST criteria 1.1, in patients with metastatic colorectal cancer (mCRC) refractory to irinotecan-based chemotherapy without any mutation associated to resistance on KRAS, PIK3Ca (exon 20), BRAF and NRAS genes detected with hypersensitive techniques (Digital PCR in Fluidigm nanofluidic dPCR plataform), named as molecular ultra-selected subgroup.

Protection of trial subjects:

All patients have been treated according to GCP criteria. Patients were entitled to withdraw from the study at any time and for any reason without prejudice of their future medical care on the part of the doctor or the center.
Doses of panitumumab and FOLFIRI could be reduced/delayed in case of adverse events (AEs) as per protocol. Any medication that patients needed for their correct clinical control (except prohibited therapies), according to investigator's criteria were allowed.

Background therapy:

None.

Evidence for comparator:

Not applicable.

Actual start date of recruitment	13 November 2012
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	Spain: 72
Worldwide total number of subjects	72
EEA total number of subjects	72

Notes:

Subjects enrolled per age group

In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0

Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	38
From 65 to 84 years	34
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

Ninety-six patients were recruited from November 2012 to July 2015, 24 of whom were screening failures. Thus, 72 patients were finally included in the study. This was a national study conducted in the Departments of Medical Oncology at 12 Spanish hospitals.

Pre-assignment

Screening details:

Patients aged ≥18 years with histologically confirmed colorectal adenocarcinoma, wild-type KRAS exon 2 (KRAS and NRAS exons 2/3/4 after protocol amendment on 25 July 2013), with ≥1 initially measurable and unresectable metastatic lesion, Karnofsky performance status ≥70% and adequate bone marrow, renal, hepatic and metabolic functions.

Period 1

Period 1 title	Overall period (overall period)
Is this the baseline period?	Yes
Allocation method	Not applicable
Blinding used	Not blinded

Blinding implementation details:

Not applicable

Arms

Arm title	Panitumumab + FOLFIRI

Arm description:

Patients were treated with panitumumab plus FOLFIRI.

Arm type	Experimental
Investigational medicinal product name	Panitumumab
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Infusion
Routes of administration	Intravenous use

Dosage and administration details:

Patients received panitumumab 6mg/kg over a 60-min intravenous infusion on day 1 in 2-weeks cycles.

Investigational medicinal product name	FOLFIRI
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Infusion
Routes of administration	Intravenous use

Dosage and administration details:

FOLFIRI was intravenously administered on day 1 in 2-week cycles according to the following schema: irinotecan 180mg/m2 over 30-90-min infusion, leucovorin 400 mg/m2 over 120-min infusion, 5-fluorouracil 400mg/m2 bolus, 5-fluorouracil 2400 mg/m2 over 46-h infusion.

Number of subjects in period 1	Panitumumab + FOLFIRI
Started	72
Completed	71
Not completed	1
Consent withdrawn by subject	1

Baseline characteristics

Reporting groups	
Reporting group title	Overall period

Reporting group description: -

Reporting group values	Overall period	Total	
Number of subjects	72	72	
Age categorical Units: Subjects			
Adults (18-64 years)	38	38	
From 65-84 years	34	34	
Age continuous Units: years			
median	62		
full range (min-max)	38 to 83	-	
Gender categorical Units: Subjects			
Female	21	21	
Male	51	51	
Tumour stage at initial diagnosis Units: Subjects			
II	4	4	
III	16	16	
IV	52	52	
Karnofsky performance status Units: Subjects			
70-80	18	18	
90-100	54	54	
Primary tumour site Units: Subjects			
Right colon	10	10	
Left colon	31	31	
Rectum	31	31	
Primary tumour surgery Units: Subjects			
Yes	53	53	
No	19	19	
Number of metastatic sites Units: Subjects			
<3	49	49	
≥3	23	23	
Previous chemotherapy for colorectal cancer: adjuvant Units: Subjects			
Yes	26	26	
No	46	46	
Previous chemotherapy for colorectal cancer: palliative			

Units: Subjects			
Yes	72	72	
No	0	0	

Subject analysis sets

Subject analysis set title	RAS wild-type by qPCR
Subject analysis set type	Sub-group analysis

Subject analysis set description:

RAS wild-type population by qPCR. This analysis set will be used for reporting efficacy results.

Reporting group values	RAS wild-type by qPCR		
Number of subjects	65		
Age categorical Units: Subjects			
Adults (18-64 years) From 65-84 years			
Age continuous Units: years median full range (min-max)	 62 38 to 83		
Gender categorical Units: Subjects			
Female Male	19 46		
Tumour stage at initial diagnosis Units: Subjects			
II III IV	4 14 47		
Karnofsky performance status Units: Subjects			
70-80 90-100	14 51		
Primary tumour site Units: Subjects			
Right colon Left colon Rectum	9 31 25		
Primary tumour surgery Units: Subjects			
Yes No	49 16		
Number of metastatic sites Units: Subjects			
<3 ≥3	45 20		
Previous chemotherapy for colorectal cancer: adjuvant Units: Subjects			
Yes	25		

No	40		
Previous chemotherapy for colorectal cancer: palliative Units: Subjects			
Yes	65		
No	0		

End points

End points reporting groups

Reporting group title	Panitumumab + FOLFIRI
Reporting group description:	
Patients were treated with panitumumab plus FOLFIRI.	
Subject analysis set title	RAS wild-type by qPCR
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
RAS wild-type population by qPCR. This analysis set will be used for reporting efficacy results.	

Primary: Tumor response in the RAS wild-type population by q-PCR (N=65): RAS (KRAS + NRAS)

End point title	Tumor response in the RAS wild-type population by q-PCR (N=65): RAS (KRAS + NRAS)[1]
End point description:	
CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.	
End point type	Primary
End point timeframe:	
Tumor response at the end of the study	
Notes:	
[1] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point. Justification: Statistical analyses (Fisher exact) within a single analysis set (N=65) for Nanofluidic dPCR results: Cut-off 0%, p=0.843; Cut-off 0.1%, p=0.745; Cut-off 1%, p=0.624; Cut-off 2%, p= 0.362; Cut-off 3%, p= 0.850; Cut-off 4%, p= 0.850; Cut-off 5%, p= 0.549.	

End point values	RAS wild-type by qPCR			
Subject group type	Subject analysis set			
Number of subjects analysed	65			
Units: percent				
number (not applicable)				
CR: Conventional qPCR, wild-type	0.0			
CR: Conventional qPCR, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 0%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 0%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 0.1%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 0.1%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 1%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 1%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 2%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 2%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 3%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 3%, mutation	0.0			

CR: Nanofluidic dPCR, Cut-off 4%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 4%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 5%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 5%, mutation	0.0			
PR: Conventional qPCR, wild-type	47.7			
PR: Conventional qPCR, mutation	0.0			
PR: Nanofluidic dPCR, Cut-off 0%, wild-type	49.0			
PR: Nanofluidic dPCR, Cut-off 0%, mutation	43.8			
PR: Nanofluidic dPCR, Cut-off 0.1%, wild-type	50.0			
PR: Nanofluidic dPCR, Cut-off 0.1%, mutation	40.0			
PR: Nanofluidic dPCR, Cut-off 1%, wild-type	50.0			
PR: Nanofluidic dPCR, Cut-off 1%, mutation	33.3			
PR: Nanofluidic dPCR, Cut-off 2%, wild-type	50.0			
PR: Nanofluidic dPCR, Cut-off 2%, mutation	28.6			
PR: Nanofluidic dPCR, Cut-off 3%, wild-type	48.3			
PR: Nanofluidic dPCR, Cut-off 3%, mutation	40.0			
PR: Nanofluidic dPCR, Cut-off 4%, wild-type	48.3			
PR: Nanofluidic dPCR, Cut-off 4%, mutation	40.0			
PR: Nanofluidic dPCR, Cut-off 5%, wild-type	48.4			
PR: Nanofluidic dPCR, Cut-off 5%, mutation	33.3			
SD: Conventional qPCR, wild-type	36.9			
SD: Conventional qPCR, mutation	0.0			
SD: Nanofluidic dPCR, Cut-off 0%, wild-type	36.7			
SD: Nanofluidic dPCR, Cut-off 0%, mutation	37.5			
SD: Nanofluidic dPCR, Cut-off 0.1%, wild-type	36.0			
SD: Nanofluidic dPCR, Cut-off 0.1%, mutation	40.0			
SD: Nanofluidic dPCR, Cut-off 1%, wild-type	35.7			
SD: Nanofluidic dPCR, Cut-off 1%, mutation	44.4			
SD: Nanofluidic dPCR, Cut-off 2%, wild-type	36.2			
SD: Nanofluidic dPCR, Cut-off 2%, mutation	42.9			
SD: Nanofluidic dPCR, Cut-off 3%, wild-type	36.7			
SD: Nanofluidic dPCR, Cut-off 3%, mutation	40.0			

SD: Nanofluidic dPCR, Cut-off 4%, wild-type	36.7			
SD: Nanofluidic dPCR, Cut-off 4%, mutation	40.0			
SD: Nanofluidic dPCR, Cut-off 5%, wild-type	37.1			
SD: Nanofluidic dPCR, Cut-off 5%, mutation	33.3			
PD: Conventional qPCR, wild-type	13.8			
PD: Conventional qPCR, mutation	0.0			
PD: Nanofluidic dPCR, Cut-off 0%, wild-type	12.2			
PD: Nanofluidic dPCR, Cut-off 0%, mutation	18.8			
PD: Nanofluidic dPCR, Cut-off 0.1%, wild-type	12.0			
PD: Nanofluidic dPCR, Cut-off 0.1%, mutation	20.0			
PD: Nanofluidic dPCR, Cut-off 1%, wild-type	12.5			
PD: Nanofluidic dPCR, Cut-off 1%, mutation	22.2			
PD: Nanofluidic dPCR, Cut-off 2%, wild-type	12.1			
PD: Nanofluidic dPCR, Cut-off 2%, mutation	28.6			
PD: Nanofluidic dPCR, Cut-off 3%, wild-type	13.3			
PD: Nanofluidic dPCR, Cut-off 3%, mutation	20.0			
PD: Nanofluidic dPCR, Cut-off 4%, wild-type	13.3			
PD: Nanofluidic dPCR, Cut-off 4%, mutation	20.0			
PD: Nanofluidic dPCR, Cut-off 5%, wild-type	12.9			
PD: Nanofluidic dPCR, Cut-off 5%, mutation	33.3			

Statistical analyses

No statistical analyses for this end point

Primary: Tumor response in the RAS wild-type population by q-PCR (N=65): RAS + BRAF

End point title	Tumor response in the RAS wild-type population by q-PCR (N=65): RAS + BRAF[2]

End point description:

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

End point type	Primary

End point timeframe:

Tumor response at the end of the study

Notes:

[2] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: Statistical analyses (Fisher exact) within a single analysis set (N=65): Conventional qPCR,

p= 0.067; Nanofluidic dPCR Cut-off 0%, p=0.773; Cut-off 0.1%, p=0.604; Cut-off 1%, p=0.241; Cut-off 2%, p= 0.130; Cut-off 3%, p= 0.164; Cut-off 4%, p= 0.164; Cut-off 5%, p= 0.076.

End point values	RAS wild-type by qPCR			
Subject group type	Subject analysis set			
Number of subjects analysed	65			
Units: percent				
number (not applicable)				
CR: Conventional qPCR, wild-type	0.0			
CR: Conventional qPCR, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 0%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 0%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 0.1%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 0.1%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 1%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 1%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 2%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 2%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 3%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 3%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 4%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 4%, mutation	0.0			
CR: Nanofluidic dPCR, Cut-off 5%, wild-type	0.0			
CR: Nanofluidic dPCR, Cut-off 5%, mutation	0.0			
PR: Conventional qPCR, wild-type	50.8			
PR: Conventional qPCR, mutation	0.0			
PR: Nanofluidic dPCR, Cut-off 0%, wild-type	51.1			
PR: Nanofluidic dPCR, Cut-off 0%, mutation	38.9			
PR: Nanofluidic dPCR, Cut-off 0.1%, wild-type	52.1			
PR: Nanofluidic dPCR, Cut-off 0.1%, mutation	35.3			
PR: Nanofluidic dPCR, Cut-off 1%, wild-type	52.8			
PR: Nanofluidic dPCR, Cut-off 1%, mutation	25.0			
PR: Nanofluidic dPCR, Cut-off 2%, wild-type	52.7			
PR: Nanofluidic dPCR, Cut-off 2%, mutation	20.0			
PR: Nanofluidic dPCR, Cut-off 3%, wild-type	51.8			

PR: Nanofluidic dPCR, Cut-off 3%, mutation	22.2			
PR: Nanofluidic dPCR, Cut-off 4%, wild-type	51.8			
PR: Nanofluidic dPCR, Cut-off 4%, mutation	22.2			
PR: Nanofluidic dPCR, Cut-off 5%, wild-type	51.7			
PR: Nanofluidic dPCR, Cut-off 5%, mutation	14.3			
SD: Conventional qPCR, wild-type	36.1			
SD: Conventional qPCR, mutation	50.0			
SD: Nanofluidic dPCR, Cut-off 0%, wild-type	34.0			
SD: Nanofluidic dPCR, Cut-off 0%, mutation	44.4			
SD: Nanofluidic dPCR, Cut-off 0.1%, wild-type	33.3			
SD: Nanofluidic dPCR, Cut-off 0.1%, mutation	47.1			
SD: Nanofluidic dPCR, Cut-off 1%, wild-type	34.0			
SD: Nanofluidic dPCR, Cut-off 1%, mutation	50.0			
SD: Nanofluidic dPCR, Cut-off 2%, wild-type	34.5			
SD: Nanofluidic dPCR, Cut-off 2%, mutation	50.0			
SD: Nanofluidic dPCR, Cut-off 3%, wild-type	35.7			
SD: Nanofluidic dPCR, Cut-off 3%, mutation	44.4			
SD: Nanofluidic dPCR, Cut-off 4%, wild-type	35.7			
SD: Nanofluidic dPCR, Cut-off 4%, mutation	44.4			
SD: Nanofluidic dPCR, Cut-off 5%, wild-type	36.2			
SD: Nanofluidic dPCR, Cut-off 5%, mutation	42.9			
PD: Conventional qPCR, wild-type	11.5			
PD: Conventional qPCR, mutation	50.0			
PD: Nanofluidic dPCR, Cut-off 0%, wild-type	12.8			
PD: Nanofluidic dPCR, Cut-off 0%, mutation	16.7			
PD: Nanofluidic dPCR, Cut-off 0.1%, wild-type	12.5			
PD: Nanofluidic dPCR, Cut-off 0.1%, mutation	17.6			
PD: Nanofluidic dPCR, Cut-off 1%, wild-type	11.3			
PD: Nanofluidic dPCR, Cut-off 1%, mutation	25.0			
PD: Nanofluidic dPCR, Cut-off 2%, wild-type	10.9			
PD: Nanofluidic dPCR, Cut-off 2%, mutation	30.0			
PD: Nanofluidic dPCR, Cut-off 3%, wild-type	10.7			

PD: Nanofluidic dPCR, Cut-off 3%, mutation	33.3			
PD: Nanofluidic dPCR, Cut-off 4%, wild-type	10.7			
PD: Nanofluidic dPCR, Cut-off 4%, mutation	33.3			
PD: Nanofluidic dPCR, Cut-off 5%, wild-type	10.3			
PD: Nanofluidic dPCR, Cut-off 5%, mutation	42.9			

Statistical analyses

No statistical analyses for this end point

Secondary: KRAS mutation distribution

End point title	KRAS mutation distribution
End point description:	
End point type	Secondary
End point timeframe:	
Mutations calculated in the population of patients included in the study	

End point values	Panitumumab + FOLFIRI			
Subject group type	Reporting group			
Number of subjects analysed	72			
Units: Subjects				
KRAS: Conventional qPCR, wild-type	67			
KRAS: Conventional qPCR, mutation	5			
KRAS: Nanofluidic dPCR, wild-type	52			
KRAS: Nanofluidic dPCR, mutation	20			
Exon 2-codon 12–13: Conventional qPCR, wild-type	71			
Exon 2-codon 12–13: Conventional qPCR, mutation	1			
Exon 2-codon 12–13: Nanofluidic dPCR, wild-type	62			
Exon 2-codon 12–13: Nanofluidic dPCR, mutation	10			
Exon 3-codon 58–61: Conventional qPCR, wild-type	70			
Exon 3-codon 58–61: Conventional qPCR, mutatiion	2			
Exon 3-codon 58–61: Nanofluidic dPCR, wild-type	65			
Exon 3-codon 58–61: Nanofluidic dPCR, mutation	7			
Exon 4-codon 117: Conventional qPCR, wild-type	72			
Exon 4-codon 117: Conventional qPCR, mutation	0			

Exon 4-codon 117: Nanofluidic dPCR, wild-type	71			
Exon 4-codon 117: Nanofluidic dPCR, mutation	1			
Exon 4-codon 146: Conventional qPCR, wild-type	70			
Exon 4-codon 146: Conventional qPCR, mutation	2			
Exon 4-codon 146: Nanofluidic dPCR, wild-type	69			
Exon 4-codon 146: Nanofluidic dPCR, mutation	3			

Statistical analyses

No statistical analyses for this end point

Secondary: NRAS mutation distribution

End point title	NRAS mutation distribution

End point description:

End point type	Secondary

End point timeframe:

Mutations calculated in the population of patients included in the study

End point values	Panitumumab + FOLFIRI			
Subject group type	Reporting group			
Number of subjects analysed	72			
Units: Subjects				
NRAS: Conventional qPCR, wild-type	70			
NRAS: Conventional qPCR, mutation	2			
NRAS: Nanofluidic dPCR, wild-type	67			
NRAS: Nanofluidic dPCR, mutation	5			
Exon 2-codon 12–13: Conventional qPCR, wild-type	71			
Exon 2-codon 12–13: Conventional qPCR, mutation	1			
Exon 2-codon 12–13: Nanofluidic dPCR, wild-type	69			
Exon 2-codon 12–13: Nanofluidic dPCR, mutation	3			
Exon 3-codon 59–61: Conventional qPCR, wild-type	71			
Exon 3-codon 59–61: Conventional qPCR, mutation	1			
Exon 3-codon 59–61: Nanofluidic dPCR, wild-type	69			
Exon 3-codon 59–61: Nanofluidic dPCR, mutation	3			
Exon 4-codon 117: Conventional qPCR, wild-type	72			

Exon 4-codon 117: Conventional qPCR, mutation	0			
Exon 4-codon 117: Nanofluidic dPCR, wild-type	72			
Exon 4-codon 117: Nanofluidic dPCR, mutation	0			
Exon 4-codon 146: Conventional qPCR, wild-type	72			
Exon 4-codon 146: Conventional qPCR, mutation	0			
Exon 4-codon 146: Nanofluidic dPCR, wild-type	71			
Exon 4-codon 146: Nanofluidic dPCR, mutation	1			

Statistical analyses

No statistical analyses for this end point

Secondary: BRAF mutation distribution

End point title	BRAF mutation distribution

End point description:

BRAF mutational status according to Exon 15-codon 600.

End point type	Secondary

End point timeframe:

Mutations calculated in the population of patients included in the study

End point values	Panitumumab + FOLFIRI			
Subject group type	Reporting group			
Number of subjects analysed	72			
Units: Subjects				
BRAF: Conventional qPCR, wild-type	68			
BRAF: Conventional qPCR, mutation	4			
BRAF: Nanofluidic dPCR, wild-type	68			
BRAF: Nanofluidic dPCR, mutation	4			

Statistical analyses

No statistical analyses for this end point

Secondary: PIK3CA mutation distribution

End point title	PIK3CA mutation distribution

End point description:

PIK3CA mutational status according to Exon 20-codon 1043–1047

End point type	Secondary

End point timeframe:

Mutations calculated in the population of patients included in the study

End point values	Panitumumab + FOLFIRI			
Subject group type	Reporting group			
Number of subjects analysed	72			
Units: Subjects				
PIK3CA: Conventional qPCR, wild-type	71			
PIK3CA: Conventional qPCR, mutation	1			
PIK3CA: Nanofluidic dPCR, wild-type	70			
PIK3CA: Nanofluidic dPCR, mutation	2			

Statistical analyses

No statistical analyses for this end point

Secondary: Progression-free survival (PFS, months) in the RAS wild-type population: RAS (KRAS/NRAS)

End point title	Progression-free survival (PFS, months) in the RAS wild-type population: RAS (KRAS/NRAS)

End point description:

Statistical analysis (Log-rank test) was performed within a single analysis set (N=65). Find below the HR (95% CIs), p-value for each Nanofluidic dPCR Cut-off: Cut-off 0%, 0.9 (0.5–1.6), p=0.741; Cut-off 0.1%, 0.9 (0.5–1.7), p=0.818; Cut-off 1%, 0.8 (0.4–1.8), p=0.657; Cut-off 2%, 1.3 (0.6–2.9), p= 0.513; Cut-off 3%, 1.0 (0.4–2.5), p= 0.996; Cut-off 4%, 1.0 (0.4–2.5), p= 0.996; Cut-off 5%, 3.3 (1.0–11.0), p= 0.048.

End point type	Secondary

End point timeframe:

Progression-free survival (PFS) was measured from study inclusion to progression or death.

End point values	RAS wild-type by qPCR			
Subject group type	Subject analysis set			
Number of subjects analysed	65[3]			
Units: median				
number (not applicable)				
Conventional qPCR, wild-type	7.4			
Nanofluidic dPCR, Cut-off 0%, wild-type	7.2			
Nanofluidic dPCR, Cut-off 0%, mutation	7.4			
Nanofluidic dPCR, Cut-off 0.1%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 0.1%, mutation	7.4			
Nanofluidic dPCR, Cut-off 1%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 1%, mutation	7.4			

Nanofluidic dPCR, Cut-off 2%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 2%, mutation	6.7			
Nanofluidic dPCR, Cut-off 3%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 3%, mutation	7.4			
Nanofluidic dPCR, Cut-off 4%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 4%, mutation	7.4			
Nanofluidic dPCR, Cut-off 5%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 5%, mutation	4.0			

Notes:

[3] - dPCR Cut-off, n (wt/mut): 0%, 49/16; 0.1%, 50/15; 1%, 56/9; 2%, 58/7; 3%, 60/5; 4% 60/5; 5% 62/3

Statistical analyses

No statistical analyses for this end point

Secondary: Progression-free survival (PFS, months) in the RAS wild-type population: RAS/BRAF

End point title	Progression-free survival (PFS, months) in the RAS wild-type population: RAS/BRAF

End point description:

Statistical analysis (Log-rank test) was performed within a single analysis set (N=65). Find below the HR (95% CIs), p-value: Conventional qPCR, 6.0 (2.0–17.7), p= 0.001; Nanofluidic dPCR Cut-off 0%, 1.0 (0.6–1.8), p=0.965; Nanofluidic dPCR Cut-off 0.1%, 1.0 (0.6–1.9), p=0.879; Nanofluidic dPCR Cut-off 1%, 1.1 (0.6–2.2), p=0.732; Nanofluidic dPCR Cut-off 2%, 1.7 (0.9–3.4), p= 0.123; Nanofluidic dPCR Cut-off 3%, 1.7 (0.8–3.4), p= 0.160; Nanofluidic dPCR Cut-off 4%, 1.7 (0.8–3.4), p= 0.160; Nanofluidic dPCR Cut-off 5%, 5 (2.1–11.7), p<0.001.

End point type	Secondary

End point timeframe:

Progression-free survival (PFS) was measured from study inclusion to progression or death

End point values	RAS wild-type by qPCR			
Subject group type	Subject analysis set			
Number of subjects analysed	65[4]			
Units: median				
number (not applicable)				
Conventional qPCR, wild-type	7.6			
Conventional qPCR, mutation	1.8			
Nanofluidic dPCR, Cut-off 0%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 0%, mutation	6.7			
Nanofluidic dPCR, Cut-off 0.1%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 0.1%, mutation	6.7			
Nanofluidic dPCR, Cut-off 1%, wild-type	7.6			
Nanofluidic dPCR, Cut-off 1%, mutation	5.5			
Nanofluidic dPCR, Cut-off 2%, wild-type	8.1			
Nanofluidic dPCR, Cut-off 2%, mutation	4.6			
Nanofluidic dPCR, Cut-off 3%, wild-type	8.1			
Nanofluidic dPCR, Cut-off 3%, mutation	4.6			
Nanofluidic dPCR, Cut-off 4%, wild-type	8.1			

Nanofluidic dPCR, Cut-off 4%, mutation	4.6			
Nanofluidic dPCR, Cut-off 5%, wild-type	8.8			
Nanofluidic dPCR, Cut-off 5%, mutation	4.0			

Notes:

[4] - q/dPCR Cut-off, n(wt/mut): 61/4; 0%, 47/18; 0.1%, 48/17; 1%, 53/12; 2%, 55/10; 3&4%, 56/9; 5% 58/7

Statistical analyses

No statistical analyses for this end point

Secondary: Overall survival (OS, months) in the RAS wild-type population: RAS (KRAS/NRAS)

End point title	Overall survival (OS, months) in the RAS wild-type population: RAS (KRAS/NRAS)

End point description:

Statistical analysis (Log-rank test) was performed within a single analysis set (N=65). Find below the HR (95% CIs), p-value for each Nanofluidic dPCR Cut-off: Cut-off 0%, 0.6 (0.3–1.2), p=0.142; Cut-off 0.1%, 0.7 (0.3–1.4), p=0.294; Cut-off 1%, 0.6 (0.3–1.7), p=0.367; Cut-off 2%, 0.8 (0.3–2.1), p= 0.689; Cut-off 3%, 0.8 (0.3–2.2), p= 0.620; Cut-off 4%, 0.8 (0.3–2.2), p= 0.620; Cut-off 5%, 1.5 (0.4–4.7), p= 0.534.

End point type	Secondary

End point timeframe:

Overall survival (OS) was measured from enrolment to death

End point values	RAS wild-type by qPCR			
Subject group type	Subject analysis set			
Number of subjects analysed	65[5]			
Units: median				
number (not applicable)				
Conventional qPCR, wild-type	13.9			
Nanofluidic dPCR, Cut-off 0%, wild-type	11.7			
Nanofluidic dPCR, Cut-off 0%, mutation	17.4			
Nanofluidic dPCR, Cut-off 0.1%, wild-type	11.8			
Nanofluidic dPCR, Cut-off 0.1%, mutation	16.1			
Nanofluidic dPCR, Cut-off 1%, wild-type	12.5			
Nanofluidic dPCR, Cut-off 1%, mutation	16.1			
Nanofluidic dPCR, Cut-off 2%, wild-type	13.9			
Nanofluidic dPCR, Cut-off 2%, mutation	16.1			
Nanofluidic dPCR, Cut-off 3%, wild-type	13.9			
Nanofluidic dPCR, Cut-off 3%, mutation	16.1			
Nanofluidic dPCR, Cut-off 4%, wild-type	13.9			
Nanofluidic dPCR, Cut-off 4%, mutation	16.1			
Nanofluidic dPCR, Cut-off 5%, wild-type	13.9			
Nanofluidic dPCR, Cut-off 5%, mutation	16.1			

Notes:

[5] - dPCR Cut-off, n (wt/mut): 0%, 49/16; 0.1%, 50/15; 1%, 56/9; 2%, 58/7; 3%, 60/5; 4% 60/5; 5% 62/3

Statistical analyses

No statistical analyses for this end point

Secondary: Overall survival (OS, months) in the RAS wild-type population: RAS/BRAF

End point title	Overall survival (OS, months) in the RAS wild-type population: RAS/BRAF

End point description:

Statistical analysis (Log-rank test) was performed within a single analysis set (N=65). Find below the HR (95% CIs), p-value: Conventional qPCR, 8.1 (2.6–25.5), p< 0.001; Nanofluidic dPCR Cut-off 0%, 0.7 (0.4–1.4), p=0.349; Nanofluidic dPCR Cut-off 0.1%, 0.8 (0.4–1.7), p=0.619; Nanofluidic dPCR Cut-off 1%, 1.0 (0.5–2.2), p=0.951; Nanofluidic dPCR Cut-off 2%, 1.3 (0.6–2.8), p= 0.528; Nanofluidic dPCR Cut-off 3%, 1.5 (0.7–3.3), p= 0.290; Nanofluidic dPCR Cut-off 4%, 1.5 (0.7–3.3), p= 0.290; Nanofluidic dPCR Cut-off 5%, 2.8 (1.3–6.4), p= 0.012.

End point type	Secondary

End point timeframe:

Overall survival (OS) was measured from enrolment to death

End point values	RAS wild-type by qPCR			
Subject group type	Subject analysis set			
Number of subjects analysed	65[6]			
Units: median				
number (not applicable)				
Conventional qPCR, wild-type	16.1			
Conventional qPCR, mutation	6.2			
Nanofluidic dPCR, Cut-off 0%, wild-type	11.8			
Nanofluidic dPCR, Cut-off 0%, mutation	16.1			
Nanofluidic dPCR, Cut-off 0.1%, wild-type	12.5			
Nanofluidic dPCR, Cut-off 0.1%, mutation	16.1			
Nanofluidic dPCR, Cut-off 1%, wild-type	13.9			
Nanofluidic dPCR, Cut-off 1%, mutation	13.7			
Nanofluidic dPCR, Cut-off 2%, wild-type	15.6			
Nanofluidic dPCR, Cut-off 2%, mutation	8.4			
Nanofluidic dPCR, Cut-off 3%, wild-type	16.2			
Nanofluidic dPCR, Cut-off 3%, mutation	8.4			
Nanofluidic dPCR, Cut-off 4%, wild-type	16.2			
Nanofluidic dPCR, Cut-off 4%, mutation	8.4			
Nanofluidic dPCR, Cut-off 5%, wild-type	16.2			
Nanofluidic dPCR, Cut-off 5%, mutation	7.3			

Notes:

[6] - q/dPCR Cut-off, n(wt/mut): 61/4; 0%, 47/18; 0.1%, 48/17; 1%, 53/12; 2%, 55/10; 3&4%, 56/9; 5% 58/7

Statistical analyses

No statistical analyses for this end point

Adverse events

Adverse events information

Timeframe for reporting adverse events:

Toxicity was assessed at every study visit according to the Common Toxicity Criteria for Adverse Events version 4.0

Adverse event reporting additional description:

If a patient had more than one event classified with the same preferred term, then the worst case was used

Assessment type	Systematic

Dictionary used

Dictionary name	MedDRA
Dictionary version	16.0

Reporting groups

Reporting group title	Safety population

Reporting group description: -

Serious adverse events	Safety population		
Total subjects affected by serious adverse events			
subjects affected / exposed	28 / 72 (38.89%)		
number of deaths (all causes)	52		
number of deaths resulting from adverse events	4		
Vascular disorders			
Ictus minor			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	1 / 1		
deaths causally related to treatment / all	0 / 0		
General disorders and administration site conditions			
Fever			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Confusional syndrome			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Lumbar pain			

subjects affected / exposed	1 / 72 (1.39%)			
occurrences causally related to treatment / all	0 / 1			
deaths causally related to treatment / all	0 / 1			
Febrile syndrome				
subjects affected / exposed	1 / 72 (1.39%)			
occurrences causally related to treatment / all	0 / 1			
deaths causally related to treatment / all	0 / 0			
Blood and lymphatic system disorders				
Neutropenia				
subjects affected / exposed	1 / 72 (1.39%)			
occurrences causally related to treatment / all	1 / 1			
deaths causally related to treatment / all	0 / 0			
Gastrointestinal disorders				
Intestinal subocclusion				
subjects affected / exposed	5 / 72 (6.94%)			
occurrences causally related to treatment / all	0 / 9			
deaths causally related to treatment / all	0 / 1			
Gastrointestinal toxicity				
subjects affected / exposed	2 / 72 (2.78%)			
occurrences causally related to treatment / all	2 / 2			
deaths causally related to treatment / all	0 / 0			
Diarrhoea				
subjects affected / exposed	4 / 72 (5.56%)			
occurrences causally related to treatment / all	5 / 5			
deaths causally related to treatment / all	0 / 0			
Digestive toxicity				
subjects affected / exposed	1 / 72 (1.39%)			
occurrences causally related to treatment / all	1 / 1			
deaths causally related to treatment / all	0 / 0			
Mucositis				
subjects affected / exposed	1 / 72 (1.39%)			
occurrences causally related to treatment / all	1 / 1			
deaths causally related to treatment / all	0 / 0			
Enteritis				

subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	1 / 1		
deaths causally related to treatment / all	0 / 0		
Respiratory, thoracic and mediastinal disorders			
Dyspnoea			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Pulmonary thromboembolism			
subjects affected / exposed	2 / 72 (2.78%)		
occurrences causally related to treatment / all	1 / 2		
deaths causally related to treatment / all	0 / 0		
Bilateral pneumonia			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 1		
Bronchoaspiration			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 1		
Hepatobiliary disorders			
Cholecystitis acute			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Obstructive jaundice			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Skin and subcutaneous tissue disorders			
Rash			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	1 / 1		
deaths causally related to treatment / all	0 / 0		

Renal and urinary disorders			
Urinary sepsis			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 2		
deaths causally related to treatment / all	0 / 0		
Pyelonephritis			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Musculoskeletal and connective tissue disorders			
Muscle deterioration			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Infections and infestations			
Urine infection			
subjects affected / exposed	2 / 72 (2.78%)		
occurrences causally related to treatment / all	0 / 2		
deaths causally related to treatment / all	0 / 0		
E.Coli sepsis			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Catheter infection			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Respiratory infection			
subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Febrile neutropenia			

subjects affected / exposed	1 / 72 (1.39%)		
occurrences causally related to treatment / all	1 / 1		
deaths causally related to treatment / all	0 / 0		

Frequency threshold for reporting non-serious adverse events: 5 %

Non-serious adverse events	Safety population		
Total subjects affected by non-serious adverse events			
subjects affected / exposed	72 / 72 (100.00%)		
Vascular disorders			
Epistaxis			
subjects affected / exposed	4 / 72 (5.56%)		
occurrences (all)	4		
Thrombosis			
subjects affected / exposed	4 / 72 (5.56%)		
occurrences (all)	5		
General disorders and administration site conditions			
Xerosis			
subjects affected / exposed	8 / 72 (11.11%)		
occurrences (all)	11		
Asthenia			
subjects affected / exposed	47 / 72 (65.28%)		
occurrences (all)	129		
Fever			
subjects affected / exposed	8 / 72 (11.11%)		
occurrences (all)	10		
Blood and lymphatic system disorders			
Edema of lower extremities			
subjects affected / exposed	4 / 72 (5.56%)		
occurrences (all)	4		
Anaemia			
subjects affected / exposed	17 / 72 (23.61%)		
occurrences (all)	32		
Leukopenia			
subjects affected / exposed	7 / 72 (9.72%)		
occurrences (all)	22		

Neutropenia			
subjects affected / exposed	27 / 72 (37.50%)		
occurrences (all)	64		
Thrombocytopenia			
subjects affected / exposed	5 / 72 (6.94%)		
occurrences (all)	13		
Gastrointestinal disorders			
Abdominal pain			
subjects affected / exposed	15 / 72 (20.83%)		
occurrences (all)	23		
Diarrhoea			
subjects affected / exposed	53 / 72 (73.61%)		
occurrences (all)	165		
Dysphagia			
subjects affected / exposed	5 / 72 (6.94%)		
occurrences (all)	6		
Stomatitis			
subjects affected / exposed	4 / 72 (5.56%)		
occurrences (all)	8		
Constipation			
subjects affected / exposed	17 / 72 (23.61%)		
occurrences (all)	24		
Mucositis			
subjects affected / exposed	30 / 72 (41.67%)		
occurrences (all)	114		
Nausea			
subjects affected / exposed	30 / 72 (41.67%)		
occurrences (all)	59		
Intestinal subocclusion			
subjects affected / exposed	5 / 72 (6.94%)		
occurrences (all)	9		
Vomiting			
subjects affected / exposed	18 / 72 (25.00%)		
occurrences (all)	28		
Xerostomia			

subjects affected / exposed	8 / 72 (11.11%)		
occurrences (all)	14		
Respiratory, thoracic and mediastinal disorders			
Tracheal dryness			
subjects affected / exposed	7 / 72 (9.72%)		
occurrences (all)	14		
Common cold			
subjects affected / exposed	5 / 72 (6.94%)		
occurrences (all)	5		
Dyspnoea			
subjects affected / exposed	8 / 72 (11.11%)		
occurrences (all)	12		
Skin and subcutaneous tissue disorders			
Acne			
subjects affected / exposed	12 / 72 (16.67%)		
occurrences (all)	33		
Alopecia			
subjects affected / exposed	9 / 72 (12.50%)		
occurrences (all)	12		
Cutaneous			
subjects affected / exposed	26 / 72 (36.11%)		
occurrences (all)	53		
Dermatitis			
subjects affected / exposed	6 / 72 (8.33%)		
occurrences (all)	16		
Erythema			
subjects affected / exposed	7 / 72 (9.72%)		
occurrences (all)	8		
Finger fissure			
subjects affected / exposed	11 / 72 (15.28%)		
occurrences (all)	16		
Skin hyperpigmentation			
subjects affected / exposed	5 / 72 (6.94%)		
occurrences (all)	5		
Hypertrichosis			

subjects affected / exposed	6 / 72 (8.33%)		
occurrences (all)	9		
Paronychia			
subjects affected / exposed	16 / 72 (22.22%)		
occurrences (all)	27		
Pruritus			
subjects affected / exposed	7 / 72 (9.72%)		
occurrences (all)	12		
Rash			
subjects affected / exposed	43 / 72 (59.72%)		
occurrences (all)	141		
Hand and foot syndrome			
subjects affected / exposed	5 / 72 (6.94%)		
occurrences (all)	7		
Cutaneous xerosis			
subjects affected / exposed	7 / 72 (9.72%)		
occurrences (all)	8		
Periungual fissure			
subjects affected / exposed	4 / 72 (5.56%)		
occurrences (all)	6		
Onycholysis			
subjects affected / exposed	4 / 72 (5.56%)		
occurrences (all)	4		
Trichomalacia			
subjects affected / exposed	4 / 72 (5.56%)		
occurrences (all)	8		
Psychiatric disorders			
Anxiety			
subjects affected / exposed	6 / 72 (8.33%)		
occurrences (all)	9		
Musculoskeletal and connective tissue disorders			
Lumbar pain			
subjects affected / exposed	8 / 72 (11.11%)		
occurrences (all)	10		
Infections and infestations			

Conjunctivitis subjects affected / exposed occurrences (all) Urine infection subjects affected / exposed occurrences (all) Respiratory infection subjects affected / exposed occurrences (all)	 20 / 72 (27.78%) 26 6 / 72 (8.33%) 8 4 / 72 (5.56%) 5		
Metabolism and nutrition disorders Anorexia subjects affected / exposed occurrences (all) Iron deficiency subjects affected / exposed occurrences (all) Hypomagnesaemia subjects affected / exposed occurrences (all)	 20 / 72 (27.78%) 33 5 / 72 (6.94%) 8 18 / 72 (25.00%) 42		

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
29 January 2013	Through this amendment the following changes were implemented: 1- Incorporation of a new exploratory objective (identification of mutation through high-sensitivity methodology in biologic fluids, i.e. blood); 2- Protocol changes in order to enhance text comprehension; 3- Update of the principal investigators and study sites.
21 June 2013	The approval of this protocol amendment was on 25th July 2013. Through this amendment the following changes were implemented: 1- Modification of a selection criteria, extension of the mutation panel for KRAS and NRAS genes (initially patients may have had no mutation in KRAS exons 2 and 3, and after protocol amendment may have had no mutation in KRAS exons 2, 3 and 4 and NRAS exons 2, 3 and 4) and review of the sample size justification; 2- Protocol changes in order to enhance text comprehension; 3- Update of the principal investigators and study sites.

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

Limitations of the trial such as small numbers of subjects analysed or technical problems leading to unreliable data.

The impossibility to assess the role of PIK3CA status due to the low number of mutations. In addition, initially enrolled patients may have had mutation in KRAS 3/4 exons and NRAS 2/3/4 exons.

Notes:

Online references

http://www.ncbi.nlm.nih.gov/pubmed/30840064