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Coexpression of p-IGF-1R and MMP-7 Modulates Panitumumab and Cetuximab Efficacy in *RAS* Wild-Type Metastatic Colorectal Cancer Patients

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Abstract

INTRODUCTION: The coexpression of pIGF-1R and MMP-7 (double-positive phenotype, DP) correlates with poor overall survival (OS) in *KRAS* wild-type (WT) (exon 2) metastatic colorectal cancer (mCRC) patients treated with irinotecan-cetuximab in second/third line. *METHODS:* We analyzed two prospective biomarker design trials of newly diagnosed *RAS*-WT mCRC patients treated with panitumumab-FOLFOX6 (PULSE trial; NCT01288339) or

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cetuximab plus either FOLFOX6/FOLFIRI (POSIBA trial; NCT01276379). The main exposure was DP phenotype (DP/non-DP), as assessed by two independent pathologists. DP cases were defined by immunohistochemistry as >70% expression of moderate or strong intensity for both MMP-7 and pIGF-1R. Primary endpoint: progression-free survival (PFS); secondary endpoints: OS and response rate. PFS and OS were adjusted by baseline characteristics using multivariate Cox models. *RESULTS:* We analyzed 67 patients (30 non-DP, 37 DP) in the PULSE trial and 181 patients in the POSIBA trial (158 non-DP, 23 DP). Response rates and PFS were similar between groups in both studies. DP was associated with prolonged OS in PULSE (adjusted HR: 0.23; 95%CI: 0.11-0.52; P=.0004) and with shorter OS in POSIBA (adjusted HR: 1.67; 95%CI: 0.96-2.90; P=.07). *CONCLUSION:* A differential effect of anti-EGFRs on survival by DP phenotype was observed. Panitumumab might be more beneficial for *RAS*-WT mCRC patients with DP phenotype, whereas cetuximab might improve OS in non-DP.

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Introduction

The doublets of FOLFIRI or FOLFOX plus cetuximab or panitumumab are effective as first-line therapies for patients with *RAS* wild-type (WT) metastatic colorectal cancer (mCRC) [1–3]. However, certain patients do not fully benefit from these EGFR-targeted antibodies, requiring additional biomarkers to tailor their use.

The type 1 insulin-like growth factor receptor (IGF-1R) is a transmembrane glycoprotein composed of two extracellular and two cytoplasmic subunits acting as a receptor-tyrosine kinase [4–7]. IGF-1R is activated in colorectal cancer, mediating key processes such as cell proliferation, apoptosis resistance, and epithelial-to-mesenchymal transition (EMT) [8]. The signal transducer and activator of transcription 3 (STAT3) is also constitutively activated in colorectal cancer [9] by growth factor receptors (EGFR and IGF-1R) through AKT/mTORC/RAC1 [10], or induced by cancer-associated fibroblasts (CAFs) through IL-6-JAK1/2 [11,12]. Regardless of this intrinsic or extrinsic activation, STAT3 signaling enforces matrix metalloproteinase-7 (MMP-7) expression [13].

Recently, IGF-II was shown to activate IGF-1R and STAT3 more effectively than IGF-I and to induce SLUG transcriptional activity and EMT in CRC [14]. Feedback activation has been also demonstrated between MMP-7 and IGF-1R. MMP-7 plays a crucial role in IGF-I and IGF-II bioavailability through the insulin-like growth factor-binding protein 3 (IGFBP-3) degradation [15–17], which in turn mediates IGF-1R–dependent [18] but also IGF-1R–independent NF-kB activation [19]. The blockade of IGF-1R is also involved in the suppression of cancer cell invasion through downregulation of MMP-7 [20]. Therefore, IGF-1R and MMP-7 contribute by multiple pathways to activate the two more critical transcription factors: STAT3 and NF-kB.

Our group has previously shown that coexpression of p-IGF-1R and MMP-7 (double positivity phenotype, DP) correlates with poor prognosis in *KRAS* WT (exon 2) patients treated with irinotecan plus cetuximab as second-/third-line therapy [21]. To validate these findings, we designed two prospective, translational trials in *K-RAS* (exon-2) WT mCRC patients treated with panitumumab plus FOLFOX6 (PULSE trial) or cetuximab plus either FOLFOX6 or FOLFIRI (POSIBA trial) as a first line of therapy, with the shared objective of evaluating the prognostic role of DP in this patient population.

Methods

Trials Design

Patients were eligible in both studies if they were ≥18 years old; had histologically confirmed *KRAS* WT (exon 2) mCRC with ≥1 radiologically measurable lesion; an Eastern Cooperative Oncology Group Performance Status (ECOG-PS) of 0-1; and adequate hepatic, renal, and bone marrow functions. Patients were ineligible if they were pregnant, had a history of treatment with anti-EGFR or chemotherapy (with the exception of adjuvant therapy), or had undergone surgery of metastatic disease.

The PULSE (GEMCAD 09-03, clinicaltrials.gov id: NCT01288339) and POSIBA (GEMCAD 10-02, clinicaltrials.gov id: NCT01276379) were both single-arm prospective biomarker design trials. Patients were recruited into the PULSE trial from November 2010 to April 2013 in 24 Spanish centers and treated with FOLFOX6 plus panitumumab (6 mg/kg). Patients were recruited into the POSIBA trial from July 2011 to May 2015 in 28 Spanish centers and treated with FOLFOX6 or FOLFIRI (at investigator's choice) plus biweekly cetuximab (500 mg/m^2). In both trials, cytotoxic drugs were administered for 6 months, followed by anti-EGFR monotherapy until progressive disease or unacceptable toxicity.

Patients were classified as DP if their tumor presented moderate or strong intensity (++/+++) and >70% expression for both MMP-7 and pIGF-1R by immunohistochemistry staining (see below). The primary endpoint for both studies was progression-free survival (PFS), defined as time from enrollment to disease progression, death, or end of follow-up, whichever came first. Secondary objectives included response rate, toxicity profile, and overall survival (OS), defined as time from enrollment to death or end of follow-up. Disease status was evaluated with abdominopelvic CT scan every 2 months in the PULSE trial and every 3 months in the POSIBA trial until progressive disease. Patients without a second CT evaluation were not assessable for response rate. Patients who underwent liver resection were not censored at the time of surgical resection and were followed until progressive disease.

The safety population comprised all patients who received at least one dose of study treatment. Adverse events (AEs) were recorded according to the National Cancer Institute Common Toxicity Criteria version 2.0. The PULSE and POSIBA trials

were approved by local institutional review boards and ethics committees in accordance with national and international guidelines; all patients signed a written informed consent before study entry.

RAS and BRAF Mutational Analysis

Mutational analysis of genomic DNA of *KRAS* (exon 2) was performed by direct sequencing. In the PULSE trial, it was evaluated centrally at the Hospital Clínic (Barcelona, Spain), although analysis

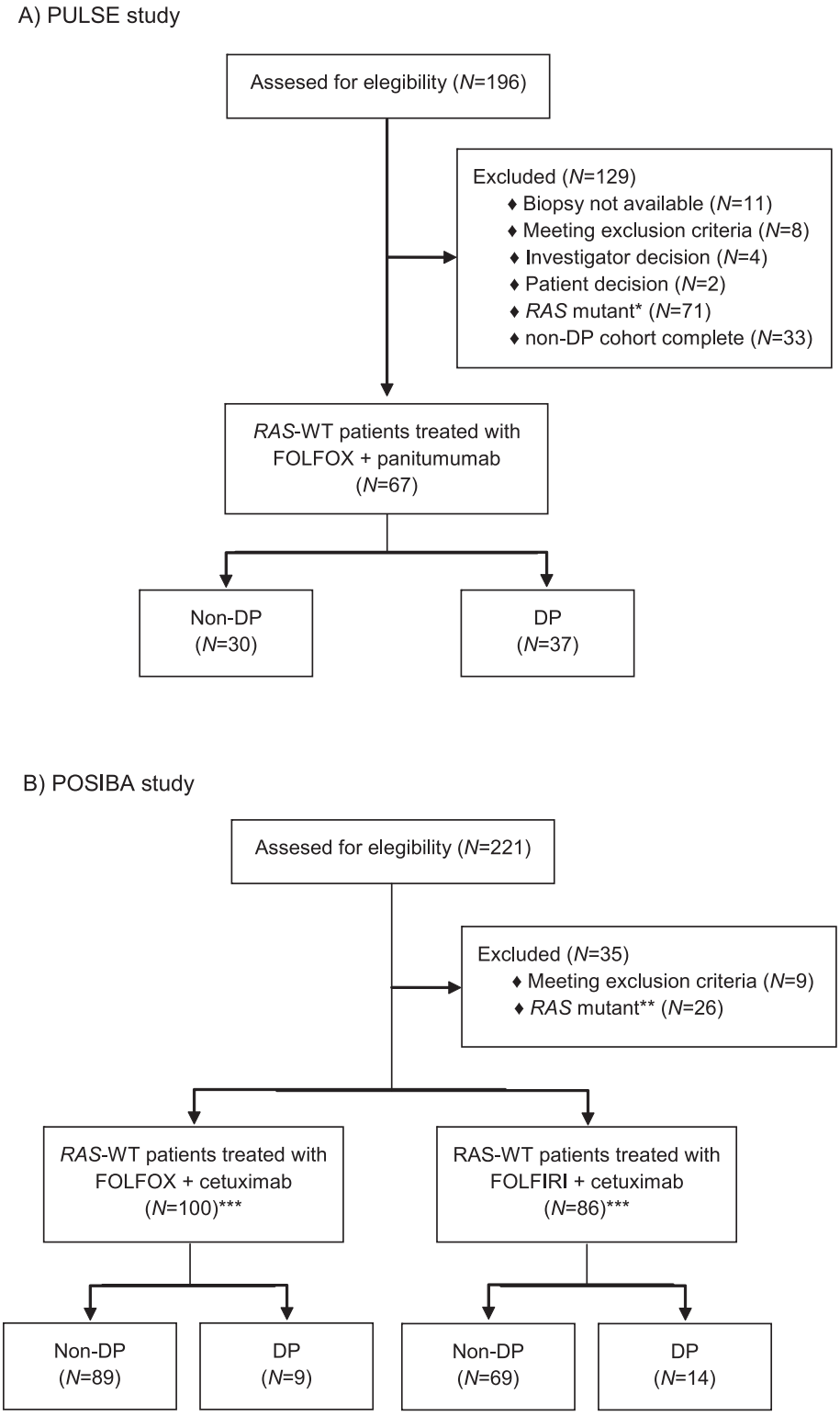

Figure 1. Patients' disposition in the (A) PULSE and (B) POSIBA trials.**RAS* mutant includes mutations in *KRAS* (exon 2) (*N*=60), and *KRAS* mutations (exons 3 and 4) and *NRAS* mutations (exons 2, 3, and 4) (*N*=11).***RAS* mutant includes mutations in *KRAS* (exons 3 and 4) and *NRAS* mutations (exons 2, 3, and 4).***The expression of p-IGF-1R and MMP-7 was not evaluable in five patients: two treated with FOLFOX +cetuximumab and three with FOLFIRI+cetuximab

at the referring Center was also allowed. In the POSIBA trial, it was evaluated at the referring center. Extended *RAS* mutational analysis (including *KRAS/NRAS* exons 2, 3 and 4) started on 10/2013 in the PULSE trial and on 10/2015 in the POSIBA trial after protocol amendments. The *BRAF* V600E mutation (exon 15) was genotyped by allelic discrimination in genomic DNA using TaqMan technology (Applied Biosystems, Foster City, CA).

Immunohistochemistry

We used hematoxylin and eosin staining to evaluate the presence and classification of the tumor specimens. Consecutive 2- to 3-μm–thick sections were used for IHC. Removal of paraffin and heat incubation in citrate (pH=6.0) were performed to achieve antigen retrieval. The primary p-IGF-1R antibody (anti-pY1316, provided by Dr. Rubini) was used at 1:100 dilution. MMP-7 (R&D System, Minneapolis, MN) was used at 1:1500 dilution. The expression was cytoplasmatic. Detection was performed using the Dako EnVision K4011 (Agilent, Santa Clara, CA). In the PULSE trial, IHC evaluation was done centrally in Hospital Clínic (Barcelona, Spain), and results were given before patient inclusion to balance the number of patients in both arms. In the POSIBA trial, IHC evaluation was performed after patients' inclusion. Thus, DP distribution represents that of the source population.

Statistical Analysis

In the PULSE trial, a recruitment of 78 patients was planned to have an 80% power to detect a difference in median PFS of 6 months between DP and non-DP patients (assuming a bilateral α error of 0.05 and the occurrence of 56 events). A screening of 270 patients was planned because only 25% of patients were expected to be DP and 40% to be *KRAS* mutant. Recruitment continued until both exposure groups (DP and non-DP) were filled in a 1:1 ratio. In the POSIBA trial, a recruitment of 170 *RAS* WT patients (after ammendent of all *RAS* WT analysis) was planned to detect, with a 80% of power and a bilateral alpha of 5%, a 20% difference in 12-month PFS. We assumed that the 12-month PFS of the non-DP patiens would be of 60%, and a 25% of DP patients in the source population.

Kaplan-Meier estimates were used to plot unadjusted survival curves. Cox proportional hazards regression was used to perform

Table 1. Baseline Characteristics by Trial and Double Positivity

	POSIBA			PULSE		
	Non-DP (*N*=158)	DP (*N*=23)	*P* Value	Non-DP (*N*=30)	DP (*N*=37)	*P* Value
BRAF mutated, *N* (%)	16 (10)	4 (17)	.29	2 (7)	5 (14)	.45
Female, *N* (%)	46 (29)	7 (30)	.99	12 (40)	10 (27)	.30
Age, mean (SD)	62 (11)	67 (7)	.031	63 (8)	64 (8)	.61
Primary tumor location, *N* (%)			.98			.47
Ascending colon	28 (18)	4 (17)		2 (7)	3 (8)	
Transverse colon	13 (8)	1 (4)		1 (3)	5 (14)	
Descending colon	12 (8)	2 (9)		3 (10)	3 (8)	
Sigma	65 (41)	11 (48)		15 (50)	12 (32)	
Rectum	40 (25)	5 (22)		9 (30)	14 (38)	
Stage (at diagnosis), *N* (%)			.77			.88
I	1 (1)	0		0	0	
II	12 (8)	1 (4)		1 (3)	2 (5)	
III	32 (20)	3 (13)		5 (17)	4 (11)	
IV	113 (72)	19 (83)		24 (80)	31 (84)	
Surgery of primary tumor, *N* (%)	89 (56)	12 (52)	.82	20 (67)	24 (65)	.99
ECOG-PS, *N* (%)			.012			.61
0	110 (70)	9 (39)		16 (53)	22 (59)	
1	45 (28)	14 (61)		13 (43)	15 (41)	
2	3 (2)	0		1 (3)	0	
Number of metastatic organs, *N* (%)			.30			.33
0	0	0		0	2 (5)	
1	79 (50)	15 (65)		13 (43)	16 (43)	
>2	79 (50)	8 (35)		17 (57)	19 (51)	
Liver metastasis, *N* (%)			.87			.93
No liver metastasis	35 (22)	5 (22)		7 (23)	10 (27)	
<=3, <=5 cm	28 (18)	5 (22)		3 (10)	4 (11)	
>3 or >5 cm	95 (60)	13 (57)		20 (67)	23 (62)	
Node metastasis, *N* (%)	50 (32)	7 (30)	.99	9 (30)	12 (32)	.99
Lung metastasis, *N* (%)	48 (30)	2 (9)	.043	11 (37)	14 (38)	.99
Peritoneal metastasis, *N* (%)	23 (15)	4 (17)	.75	9 (30.0)	8 (22)	.57
Administered therapy, *N* (%)			.18			
FOLFOX+cetuximab	89 (56)	9 (39)		NA	NA	
FOLFIRI+cetuximab	69 (44)	14 (61)		NA	NA	
FOLFOX+panitumumab	NA	NA		30 (100)	37 (100)	
Leucocytes, mean (SD)	8.3 (3.3)	8.9 (3.7)	.39	9.8 (7.1)	8.2 (2.5)	.23
Hemoglobin, mean (SD)	13.8 (9.2)	11.9 (1.6)	.023	12.9 (1.7)	12.4 (1.5)	.15
Platelets, mean (SD)	282 (104)	298 (140)	.60	298 (144)	296 (120)	.96
ALP, mean (SD)	148 (122)	179 (177)	.44	166 (208)	219 (237)	.34
LDH, mean (SD)	465 (457)	632 (1246)	.56	683 (814)	446 (415)	.16
CEA, mean (SD)	267 (732)	708 (1772)	.26	502 (1212)	838 (3609)	.61

ALP, alkaline phosphatase; *CEA*, carcinoembryonic antigen; *DP*, double positivity; *ECOG-PS*, Eastern Cooperative Oncology Group performance status; *LDH*, lactate dehydrogenase; *SD*, standard deviation.

Fisher's exact test

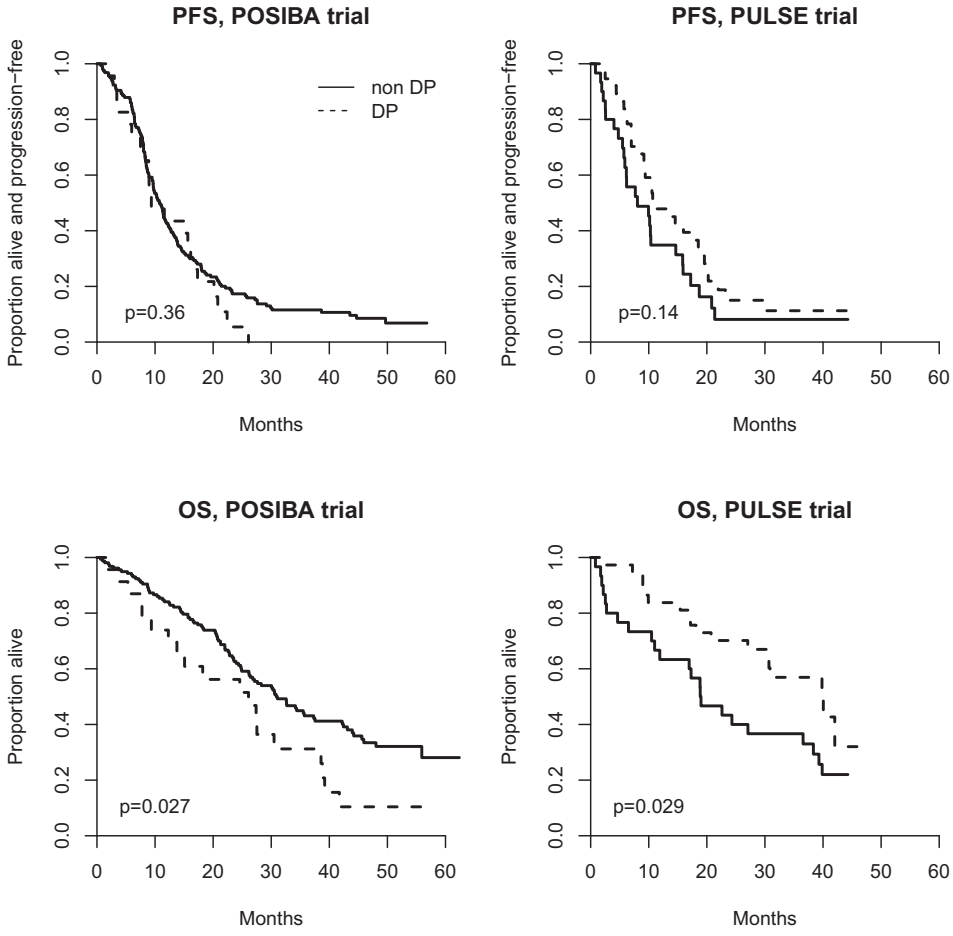

Figure 2. Kaplan-Meier estimates of progression-free survival and overall survival according to DP status in the (A) PULSE and (B) POSIBA trial.

adjusted analyses for PFS and OS. Multivariate analysis was built deciding *a priori* the variables to adjust for: age, sex, p-IGF-1R/MMP-7 expression, primary tumor location, stage at diagnosis, surgery of primary tumor, number of involved organs, type of involved organ, liver-only extension, ECOG-PS, *BRAF* mutational status, administered therapy, and baseline levels of: leucocytes, hemoglobin, platelets, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and carcinoembryonic antigen (CEA). Additionally, we performed sensitivity analyses with automated stepwise selection of variables (*P* value for variable entry into the model=.2, *P* value to stay in the model=.1) and by entering in the model those variables with a $P<.1$ in the univariate analysis. All the *P* values are

Table 2. Progression-Free Survival; Cox Regression Analysis

	POSIBA				PULSE			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
DP	1.24 (0.79-1.94)	.36	1.39 (0.84-2.31)	.20	0.68 (0.40-1.14)	.14	0.33 (0.17-0.66)	.0017
ECOG-PS >0	2.02 (1.46-2.79)	<.0001	1.77 (1.24-2.54)	.0017	1.19 (0.70-2.02)	.52	1.33 (0.71-2.509)	.37
Age >65 years	1.18 (0.87-1.61)	.30	0.99 (0.69-1.42)	.97	1.43 (1.85-2.41)	.18	1.65 (0.79-3.43)	.18
BRAF mutated	2.33 (1.44-3.79)	.0006	2.09 (1.16-3.77)	.014	1.77 (0.75-4.17)	.19	1.77 (0.34-9.03)	.49
Surgery of primary tumor	1.62 (1.19-2.22)	.0024	1.60 (1.12-2.28)	.0099	0.56 (0.32-0.98)	.041	0.45 (0.22-0.94)	.034
Left-sided primary tumor	1.02 (0.74-1.39)	.92	0.92 (0.63-1.32)	.64	0.65 (0.33-1.30)	.22	0.41 (0.14-1.18)	.10
CEA (logarithmic term)	0.55 (0.39-0.78)	.0008	0.55 (0.37-0.81)	.0029	1.10 (0.97-1.25)	.13	1.04 (0.88-1.23)	.65
LDH (logarithmic term)	1.03 (0.96-1.10)	.40	1.03 (0.94-1.14)	.49	1.14 (0.78-1.67)	.49	1.65 (0.98-2.77)	.058
Liver metastasis	1.04 (0.82-1.31)	.74	1.02 (0.78-1.33)	.88				
0					Ref.			
<=3, <=5 cm	Ref.				0.99 (0.38-2.63)	.99	0.83 (0.24-2.84)	.76
>3 or >5 cm	0.63 (0.38-1.03)	.065	0.95 (0.54-1.69)	.87	1.11 (0.59-2.09)	.74	0.86 (0.35-2.01)	.74

CEA, carcinoembryonic antigen; *CI*, confidence interval; *DP*, double positivity; *ECOG-PS*, Eastern Cooperative Oncology Group performance status; *HR*, hazard ratio; *LDH*, lactate dehydrogenase; *WT*, wild-type.

Table 3. Sensitivity Analyses for Progression-Free Survival; Cox Regression Analysis

	POSIBA				PULSE			
	Multivariate S1		Multivariate S2		Multivariate S1		Multivariate S2	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
DP	1.13 (0.71-1.81)	.61	1.13 (0.71-1.81)	.61	0.59 (0.35-1.02)	.058	0.35 (0.18-0.67)	.0015
ECOG-PS >0	1.75 (1.25-2.45)	.0011	1.75 (1.25-2.45)	.0011				
Age >65 years								
BRAF mutated	2.04 (1.24-3.34)	.0048	2.04 (1.24-3.34)	.0048				
Surgery of primary tumor	1.43 (1.04-1.97)	.029	1.43 (1.04-1.97)	.029	0.50 (0.28-0.88)	.017	0.45 (0.22-0.90)	.023
Left-sided primary tumor							0.35 (0.15-0.83)	.0165
CEA (logarithmic term)	0.58 (0.41-0.84)	.0032	0.58 (0.41-0.84)	.0032				
LDH (logarithmic term)							1.12 (0.99-2.33)	.057
Liver metastasis								
0								
<=3, <=5 cm								
>3 or >5 cm								

CEA, carcinoembryonic antigen; *CI*, confidence interval; *DP*, double positivity; *ECOG-PS*, Eastern Cooperative Oncology Group performance status; *HR*, hazard ratio; *LDH*, lactate dehydrogenase; *WT*, wild-type.
S1: multivariate model including only the variables with a *P* value <.1 in the univariate analysis.
S2: multivariate model adjusted via automated stepwise selection of variables (see text for details).

Table 4. Overall Survival; Cox Regression Analysis

	POSIBA				PULSE			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
DP	1.73 (1.06-2.85)	.029	1.67 (0.96-2.90)	.070	0.54 (0.29-0.99)	.048	0.23 (0.11-0.52)	.0004
ECOG-PS >0	2.95 (2.03-4.29)	<.0001	2.48 (1.63-3.77)	<.0001	2.20 (1.18-4.08)	.013	2.93 (1.30-6.62)	.0097
Age >65 years	1.24 (0.85-1.79)	.26	1.00 (0.65-1.53)	.99	1.37 (0.74-2.53)	.32	1.48 (0.64-3.47)	.36
BRAF mutated	3.38 (2.00-5.72)	<.0001	2.32 (1.23-4.36)	.0092	4.23 (1.17-10.48)	.0018	10.3 (1.08-58.3)	.0086
Surgery of primary tumor	1.60 (1.10-2.32)	.013	1.36 (0.90-2.07)	.15	0.35 (0.19-0.66)	.0010	0.20 (0.08-0.48)	.0003
Left-sided primary tumor	1.06 (0.73-1.53)	.78	0.82 (0.51-1.31)	.40	0.60 (0.28-1.31)	.20	0.47 (0.15-1.48)	.20
CEA (logarithmic term)	0.42 (0.28-0.62)	<.0001	0.47 (0.30-0.74)	.0012	1.09 (0.94-1.25)	.25	0.91 (0.75-1.10)	.32
LDH (logarithmic term)	0.99 (0.91-1.08)	.80	1.00 (0.89-1.12)	.97	1.30 (0.85-2.01)	.23	1.40 (0.79-2.43)	.25
Liver metastasis	0.95 (0.72-1.26)	.73	0.92 (0.66-1.27)	.61				
0	Ref.				Ref.			
<=3, <=5 cm	0.68 (0.39-1.21)	.19	1.08 (0.57-2.06)	.81	1.72 (0.54-5.46)	.35	2.49 (0.56-11.09)	.23
>3 or >5 cm	0.70 (0.45-1.10)	.12	0.95 (0.51-1.79)	.88	1.73 (0.75-3.95)	.20	1.64 (0.49-5.50)	.42

CEA, carcinoembryonic antigen; *CI*, confidence interval; *DP*, double positivity; *ECOG-PS*, Eastern Cooperative Oncology Group performance status; *HR*, hazard ratio; *LDH*, lactate dehydrogenase; *WT*, wild-type.

Table 5. Sensitivity Analysis for Overall Survival; Cox Regression Analysis

	POSIBA				PULSE			
	Multivariate S1		Multivariate S2		Multivariate S1		Multivariate S2	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
DP	1.60 (0.96-2.67)	.072	1.72 (1.01-2.94)	.048	0.36 (0.19-0.69)	.0019	0.36 (0.19-0.69)	.0019
ECOG-PS >0	2.31 (1.56-3.43)	<.0001	2.49 (1.65-3.76)	<.0001	2.16 (1.10-4.25)	.026	2.16 (1.10-4.25)	.026
Age >65 years								
BRAF mutated	2.40 (1.38-4.17)	.0019	2.57 (1.47-4.49)	.0010	3.52 (1.32-9.35)	.012	3.52 (1.32-9.35)	.012
Surgery of primary tumor	1.29 (0.88-1.90)	.19			0.33 (0.17-0.64)	.0013	0.33 (0.17-0.64)	.0013
Left-sided primary tumor								
CEA (logarithmic term)	0.50 (0.33-0.75)	.0008	0.50 (0.32-0.76)	.0014				
LDH (logarithmic term)								
Liver metastasis								
0								
<=3, <=5 cm								
>3 or >5 cm								

CEA, carcinoembryonic antigen; *CI*, confidence interval; *DP*, double positivity; *ECOG-PS*, Eastern Cooperative Oncology Group performance status; *HR*, hazard ratio; *LDH*, lactate dehydrogenase; *WT*, wild-type.
S1: multivariate model including only the variables with a *P* value <.1 in the univariate analysis.
S2: multivariate model adjusted via automated stepwise selection of variables (see text for details).

Table 6. Response Rates by Trial and Double Positivity

	POSIBA			PULSE		
	Non-DP	DP	*P* Value	Non-DP	DP	*P* Value
Complete response	13 (8.2)	4 (17.4)	.17	3 (10.0)	2 (5.4)	.28
Partial response	101 (63.9)	11 (47.8)		19 (63.3)	25 (67.6)	
Stable disease	27 (17.1)	4 (17.4)		1 (3.3)	7 (18.9)	
Progressive disease	9 (5.7)	3 (13.0)		1 (3.3)	3 (8.1)	
Not evaluable	8 (5.1)	1 (4.4)		6 (20.0)	0	

DP, double positivity.
Fisher's exact test.

two-sided. Analyses were implemented using SAS V9.3 (SAS Institute, Cary, NC).

Results

A total of 67 (PULSE) and 181 (POSIBA) *RAS*-WT mCRC patients were included in the analysis (Figure 1). In the PULSE trial, 30 patients were non-DP and 37 patients were DP, whereas in the POSIBA trial, 158 patients were non-DP and 23 patients were DP. Patients were followed for a median of 27 months in the PULSE trial and for a median of 26 months in the POSIBA trial. DP patients in the POSIBA trial were less likely to have PS 0 and lung metastasis and also have lower levels of hemoglobin than non-DP patients. There were no relevant differences in the baseline characteritics of both groups in the PULSE trial (Table 1).

Efficacy According to DP Status

Median PFS (95% CI) was 11.2 months (9.2-18.5) for DP patients and 8.0 months (5.5-14.7) for non-DP patients in the PULSE trial (*P*=.14). Median PFS (95% CI) was 9.4 months (7.5-16.1) for DP patients and 10.8 months (9.5-12.2) for non-DP patients in the POSIBA trial (*P*=.36, Figure 2). Adjusted HR for PFS was 0.33 (0.17-0.66) in the PULSE trial and 1.39 (0.84-2.31) in the POSIBA trial (Table 2). Sensitivity analysis did not change results substantially (Table 3).

Median OS (95% CI) was 39.8 months (27.0-not estimable) for DP patients and 18.9 months (11.0-36.6) for non-DP patients in the PULSE trial (*P*=.029). Median OS (95% CI) was 26.1 months (12.3-38.6) for DP patients and 31.0 months (26.2-37.5) for non-DP patients in the POSIBA trial (*P*=.027, Figure 2). DP was associated with prolonged OS in the PULSE trial (adjusted HR: 0.23: 95% CI: 0.11-0.52; *P*=.0004) and with shorter OS in the POSIBA trial (adjusted HR: 1.67; 95% CI: 0.96-2.90; *P*=.07) (Table 4). Sensitivity analysis did not change results substantially (Table 5).

Response rates were similar according to DP in both the PULSE and POSIBA studies (Table 6). There were no major differences in terms of secondary resection of metastases and second-line therapies between PULSE and POSIBA trials and between DP and non-DP groups (data not shown).

Table 7. Summary of Adverse Events in the PULSE Trial

	Any Grade	Grade 3	Grade 4
	No. of Patients (%)		
Any event	78 (100)	55 (70.5)	10 (12.8)
Skin toxicity	71 (91.0)	24 (30.8)	0 (0)
Fatigue	55 (70.5)	12 (15.4)	1 (1.3)
Mucositis	52 (66.7)	6 (7.7)	0 (0.0)
Diarrhea	48 (61.5)	11 (14.1)	1 (1.3)
Neutropenia	44 (56.4)	26 (33.3)	2 (2.6)
Nauseas/vomiting	30 (38.5)	1 (1.3)	0 (0)
Thrombocytopenia	28 (35.9)	3 (3.9)	0 (0)
Hypomagnesemia	23 (29.5)	1 (1.3)	2 (2.6)
Neurologic toxicity	16 (20.5)	1 (1.3)	0 (0)
Anaemia	10 (12.8)	1 (1.3)	0 (0)
Paronychia	8 (10.3)	1 (1.3)	0 (0)
Infusion-related reaction	7 (9.0)	0 (0)	0 (0)
Hypokalemia	6 (7.7)	2 (2.6)	1 (1.3)
Febrile neutropenia	3 (3.9)	1 (1.3)	2 (2.6)

Safety

The most common AEs (any grade) in the PULSE trial were skin toxicity (91%), fatigue (70%), and mucositis (67%) (Table 7). The most common AE (any grade) in the POSIBA trial were skin toxicity (76%), fatigue (55%), and diarrhea (50%) (Suppl. Table 1). Three patients died within 30 days of receiving protocol therapy: one patient in PULSE and two patients in POSIBA trial.

Discussion

We present data from two prospective, multicenter, translational, first-line trials in WT *RAS* mCRC patients. Our findings suggests that there is a survival benefit in the subset of DP patients treated with upfront FOLFOX plus panitumumab schedule and in non-DP patients treated upfront with FOLFOX/FOLFIRI plus cetuximab therapy. This benefit was observed after adjustment for baseline characteristics, secondary surgery of metastases, and second-line therapies.

Recent evidence shows that *RAS* WT patients with right-side primary tumors have shorter overall survival than those with left-sided tumors and that left-sided tumors obtain greater benefit when treated with chemotherapy and anti-EGFR combinations [22], although the biological reasons remain obscure. Consensus molecular subtype clasification (CMS) associates the stromal-enriched mesenchymal phenotype (CMS4) [23] with poor prognosis [24,25] and cetuximab resistance [26]. Despite data from Medema group suggesting that *BRAF* mutant CRC patients are enriched with CDX2–/ZEB1+ CMS4 phenotype [27], *BRAF* mutant mCRC patients are equally distributed between right- and left-sided, and 75% of right-sided patients treated with anti-EGFR present double WT genotype. Therefore, other CMS4 markers besides CDX2–/ZEB1+ and DP, such as CCL2 or CXCL12 (for both *BRAF* mutant and double WT genotypes), might be probably overrepresented in right-sided tumors. We could not rule out that, for currently unknown reasons, CMS4 phenotype might be induced by chemotherapy and anti-EGFR treatment [28] differently in both sides, influencing acquired resistance [29,30].

We designed the PULSE trial based on retrospective data [21] hypothesizing that DP patients treated with panitumumab-based therapy could have also poor prognosis. It's important to emphasize that the PULSE was designed in a different population (naïve) and with a different anti-EGFR exposure (panitumumab instead of cetuximab). Despite confirming our previous findings with FOLFIRI/FOLFOX plus cetuximab in the POSIBA trial, we could not confirm these results in the PULSE trial with panitumumab. In addition to inhibition of EGFR mitogenic pathways (MAPK, PI3K/AKT, and JAK/STAT), monoclonal antibodies (cetuximab and panitumumab) possess the potential advantage of recruiting immune effector mechanisms such as antibody-dependent cell mediated-cytotoxicity (ADCC) [31], although cetuximab was shown to be more effective in this mechanism than panitumumab. Although potentially cetuximab can activate ADCC also through NK cells,

these cells are almost absent in colorectal cancer, and cetuximab in M2 macrophages activates anti-inflammatory IL-10 cytokines and proangiogenic factors (IL-8 and VEGF) [24]. Taking into account that: a) DP status could increase over time after chemotherapy treatment [29] and b) IGF-1R and STAT3 activation induces T-cell tolerance through TGF-B, IL-10 and VEGF [32] and also increases chemokines and cytokines such as IL-6 and CCL2 towards macrophage M2 polarization [33], we speculate that cetuximab but not panitumumab could be influenced by DP-CMS4 acquired resistance through immune evasion.

Our study has several limitations. Firstly, PFS was evaluated differentialy (every 2 months in the PULSE trial and every 3 months in the POSIBA trial). Secondly, the percentage of DP positivity widely differs in both studies (33% in PULSE and 13% in POSIBA). Thirdly, the explanation on a potential biological reason for the contradictory results of our biomarker should be clarified.

We believe that our findings would have potential clinical importance and definitively justify a prospectively enriched-biomarker design in *RAS* WT patients with an experimental arm based on the biomarker (DP-treated with panitumumab and non–DP-treated with cetuximab) and a control arm (without this information) treated at investigator criteria (cetuximab or panitumumab).

Conclusions

Our study suggest that panitumumab is more benefitial for those *RAS* WT mCRC patients with a DP phenotype and cetuximab for those without it in terms of overall survival after adjusting for all clinical and biological confounder variables in the multivariate analysis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neo.2018.05.004.

Declaration of Interest

The authors declare no conflict of interest.

Authorship

J. M. and X. G.-A. concieved and designed the study; J. M., X. G.-A., and V. A. analyzed and interpreted the data, and drafted the manuscript; X. G.-A. performed the statistical analysis. All authors adquired the study data, revised the manuscript, and approved its final version.

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