Clinical Cancer Research

Phase II Study of the Dual EGFR/HER3 Inhibitor Duligotuzumab (MEHD7945A) versus Cetuximab in Combination with FOLFIRI in Second-Line *RAS* Wild-Type Metastatic Colorectal Cancer

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Abstract

Purpose: Duligotuzumab is a dual-action antibody directed against EGFR and HER3.

Experimental Design: Metastatic colorectal cancer (mCRC) patients with *KRAS* ex2 wild-type received duligotuzumab or cetuximab and FOLFIRI until progression or intolerable toxicity. Mandatory tumor samples underwent mutation and biomarker analysis. Efficacy analysis was conducted in patients with *RAS* exon 2/3 wild-type tumors.

Results: Of 134 randomly assigned patients, 98 had *RAS* ex2/3 wild-type. Duligotuzumab provided no progression-free survival (PFS) or overall survival (OS) benefit compared with cetuximab, although there was a trend for a lower objective response rate (ORR) in the duligotuzumab arm. No relationship was seen between PFS or ORR and ERBB3, NRG1, or AREG expression. There were fewer skin rash events for duligotuzumab but more diarrhea. Although the incidence of grade $\geq$3 AEs was similar, the frequency of serious AEs was higher for duligotuzumab.

Conclusions: Duligotuzumab plus FOLFIRI did not appear to improve the outcomes in patients with *RAS* exon 2/3 wild-type mCRC compared with cetuximab + FOLFIRI. *Clin Cancer Res; 24(10); 2276–84. ©2018 AACR.*

Introduction

EGFR is a growth factor receptor with tyrosine kinase activity implicated in both colorectal tumorigenesis and tumor progression, and its overexpression (found in 65% to 70% of human colorectal cancer) has been associated with advanced disease (1). Anti-EGFR monoclonal antibodies (mAb) are established in the treatment of metastatic colorectal cancer (mCRC), either as single agent or in combination with chemotherapy (2–4). Although the benefit was initially thought to be restricted to patients lacking hotspot mutations in *KRAS* exon 2, codons 12 and 13, a more recent retrospective analysis of phase III studies with anti-EGFR mAbs either in first-line or second-line mCRC identified additional mutations in *KRAS* or *NRAS* exons 2, 3, and 4 as negative predictive biomarkers for EGFR inhibition (5–9). Consequently, the European Society for Medical Oncology clinical practice guidelines for the treatment of mCRC were updated to recommend additional testing for *KRAS* mutations in exons 3 and 4, and *NRAS* mutations in exons 2 to 4 as a prerequisite for anti-EGFR antibody therapy (10). Similarly, National Comprehensive Cancer Network guidelines now recommend against treating patients with known *KRAS* (exon 2 or non-exon 2) or *NRAS* mutation with either cetuximab or panitumumab (11).

Nonclinical and preliminary clinical data suggest a role for HER3 in acquired resistance to EGFR inhibitors (12–14). Yonesaka and colleagues (15) reported amplification of the HER2 gene and/or increased concentrations of neuregulin (NRG1) the ligand for HER3 in cetuximab-resistant clones of colorectal and lung cancers. Further analysis suggested that aberrant HER2 signaling, through either HER2/HER2 dimers associated with gene amplification or HER2/HER3 dimer activation through autocrine expression of neuregulin, led to persistent ERK signaling

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Translational Relevance

Duligotuzumab, a dual-action antibody to EGFR and HER3, shows preclinical efficacy in EGFR-dependent colorectal cancer cell lines refractory to EGFR inhibition. We evaluated duligotuzumab compared with cetuximab in combination with FOLFIRI as second-line therapy in patients with *RAS* exon 2/3 wild-type metastatic colorectal cancer and found no advantage to duligotuzumab.

and consequently to cetuximab resistance. Interestingly, a retrospective review of data from mCRC patients treated with cetuximab as a single agent or in combination with irinotecan (also reported in ref. 15) found that those patients with a partial response had significantly lower baseline expression of NRG1 in plasma or tumor samples than patients with a best response of stable or progressive disease. Furthermore, patients with lower baseline plasma NRG1 levels had significantly longer progression-free survival (PFS) and overall survival (OS) when compared with those with higher levels.

Clinical trial reports on the evaluation of therapeutics that inhibit HER3-dependent signaling are relatively rare, as are HER3-related biomarker studies that aim to identify clinically meaningful patient subsets. This is in part due to the attenuated enzymatic activity of HER3 that renders HER3 activity dependent on critical protein–protein interactions. Innovative methods for inhibiting activity often focus on these interactions, and, moreover, biomarkers of HER3 activity often include these interaction partners independent of HER3 expression. HER3 activity can be enhanced by elevated ligand or heterodimer expression or, in some cases, activity is driven by multiple binding sites for signaling partners (i.e., PI3K). In addition, there is evidence for controlled HER3 feedback including altered transcription of HER3 itself. This was most clearly shown in a randomized phase II trial of pertuzumab (an mAb that targets HER2 and therefore inhibits heterodimerization of HER2 and HER3) in patients with platinum refractory ovarian cancer. Those patients with low expression of HER3 in tumors (measured by qRT-PCR) had a statistically significant improvement in PFS and numerically improved response rate and OS (16). These results, together with preclinical observations that activation of the HER2/HER3 signaling pathway by ligand stimulation leads to downregulation of HER3 through negative feedback modulation (17), as well as the cumulative mechanisms of HER3 activation independent of enhanced HER3 expression, may suggest that even low tumor expression of HER3 may be indicative of pathway activation and consequently sensitive to HER3 blocking agents.

Duligotuzumab (MEHD7945A) is a novel humanized phage-derived, dual-action antibody that blocks ligand binding to EGFR and HER3, with either antigen-binding fragment (Fab). When bound to these receptors, duligotuzumab blocks ligand binding (Kd huHER3 = 0.39 nmol/L; Kd hu EGFR = 1.9 nmol/L), resulting in inhibition of ligand-driven signaling from EGFR/EGFR, EGFR/HER2, EGFR/HER3, and HER2/HER3 dimer pairs. As an IgG1 antibody, duligotuzumab is also able to bind to Fcγ receptors and has demonstrated antibody-dependent cell-mediated cytotoxicity (ADCC) in *in vitro* models (18). *In vivo*, duligotuzumab shows activity in colorectal cancer *KRAS* wild-type xenograft models equal or superior to cetuximab, no effect in *KRAS*-mutated models and is additive in combination with chemotherapy (19). In colorectal cancer cell lines, standard chemotherapy may modulate the HER3/NRG network. These properties provided the rationale for investigating duligotuzumab for the treatment of patients with mCRC.

Materials and Methods

Study design

This open-label, randomized phase II study enrolled patients with *KRAS* exon 2 wild-type mCRC who progressed on/after oxaliplatin-containing chemotherapy. The primary objectives of this study were to (i) evaluate the efficacy of duligotuzumab + FOLFIRI versus cetuximab + FOLFIRI in *KRAS* wild-type mCRC patients, and (ii) evaluate the efficacy of duligotuzumab + FOLFIRI versus cetuximab + FOLFIRI in *KRAS* wild-type mCRC patients whose tumors express low levels of HER3. The secondary objectives included evaluating the safety and tolerability of duligotuzumab versus cetuximab in combination with FOLFIRI in *KRAS* exon 2 wild-type mCRC patients, assessing the effect of concomitant FOLFIRI on the pharmacokinetics (PK) of duligotuzumab and vice versa, and evaluating the incidence and impact of anti-duligotuzumab antibodies.

Eligible patients were randomly assigned in a 1:1 ratio and received duligotuzumab at a fixed dose of 1,100 mg i.v. every 2 weeks (q2w; arm A). Patients in arm B received cetuximab administered according to the prescribing label, with a loading dose of 400 mg/m^2 i.v. on day 1 of cycle 1, followed by weekly doses of 250 mg/m^2 i.v. (2 × per cycle). No dose reductions were allowed for duligotuzumab, and for cetuximab doses were limited to reductions for rash in accordance with its prescribing information. FOLFIRI chemotherapy was administered q2w (every cycle) starting on day 1 of cycle 1. FOLFIRI consisted of irinotecan (180 mg/m^2), 5-FU (bolus and 46-hour infusional doses of 400 mg/m^2 and 2,400 mg/m^2, respectively), and leucovorin (racemic, 400 mg/m^2 or L-isomer form, 200 mg/m^2). Recommendations for chemotherapy dose reductions were in accordance with standard clinical practice. Dosing continued until progression or intolerable toxicity. An early per-protocol interim safety analysis occurred after an initial 6 and then 20 patients in each treatment arm received two cycles of treatment.

Patients

Eligible patients aged 18 years and older with histologically or cytologically confirmed adenocarcinoma of the colon and/or rectum, *KRAS* exon 2 wild-type status based on local assessment (EGFR expression status was not required for enrollment) and progressive disease on or after a first-line oxaliplatin-containing chemotherapy regimen for mCRC were enrolled into the study (Supplementary Fig. S1). Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, adequate hematologic and end-organ function, and evaluable or measurable disease per modified RECIST v1.1 was required. Main exclusion criteria included prior treatment with irinotecan, HER-targeted agents, dihydropyrimidine dehydrogenase deficiency or current severe uncontrolled systemic disease.

The protocol was approved by Institutional Review Boards prior to patient recruitment and was conducted in accordance with International Conference on Harmonization E6 Guidelines for Good Clinical Practice. Written informed consent was obtained for all patients prior to performing study-

related procedures in accordance with federal and institutional guidelines.

Safety assessments

Safety assessments consisted of recording protocol-defined adverse events (AE) and serious AEs (SAE); measurement of protocol specified hematology, clinical chemistry, and urinalysis variables; measurement of protocol specified vital signs; and other tests deemed critical to the safety evaluation of the study drug(s). Safety was assessed by the incidence, nature, severity, and relatedness of AEs, which were graded for severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events, v4.0. All patients who received ≥ 1 dose of study treatment were included in the safety evaluation. Protocol-defined Adverse Events of Special Interest included grade ≥ 3 events associated with infusion-related reactions (defined as AEs occurring within 24 hours of infusion and attributed to treatment), grade ≥ 3 rash, grade ≥ 3 diarrhea, and grade ≥ 2 gastrointestinal (GI) hemorrhage.

Pharmacokinetic assessments

Serum samples for PK analysis were collected on day 1 of each cycle. PK parameters were derived from noncompartmental analysis (Phoenix WinNonlin version 6.2) from the plasma concentration–time profile of duligotuzumab. A validated ELISA with a lower limit of quantitation of 150 ng/mL was used to measure the concentration of duligotuzumab in serum samples. All study samples were analyzed at Genentech. Plasma concentrations of 5-FU, irinotecan, and SN-38 were measured using validated liquid chromatography tandem mass spectrometry methods. Serum samples were assayed for the presence of anti-therapeutic antibodies (ATA) to duligotuzumab using a validated bridging ELISA.

Activity outcomes

Per protocol, the primary efficacy outcome measure for this study was PFS in all patients with *KRAS* exon 2 wild-type mCRC and later restricted to *RAS* wild-type mCRC, and among patients whose tumors expressed low levels of HER3. PFS was defined as the time from study treatment initiation to the first occurrence of disease progression and was determined by investigator review of tumor assessments with use of the modified RECIST v1.1, or death, whichever occurred first. Objective response by investigator assessment, duration of response, and overall survival were secondary efficacy outcome measures. Objective response was defined as a complete or partial response according to modified RECIST v1.1, confirmed ≥ 4 weeks after the initial response. Duration of objective response was defined as the time from first occurrence of a documented objective response until the time of relapse or death from any cause. OS was defined as the time from study treatment initiation to death from any cause. Time to treatment failure was defined as time from randomization to discontinuation of treatment for any reason, including disease progression, treatment toxicity, and death.

Biomarker assessments

Tumor samples were mandatory and biomarker expression analysis focused on ERBB3 and its ligand NRG1, as well as on EGFR and its ligands AREG and EREG, by qRT-PCR. IHC was used to determine protein expression and localization of HER3. Precut tissue sections were stained for HER3 for analysis by IHC using the Ventana BenchMark XT staining platform (Ventana Medical Systems, Inc.). Hematoxylin and eosin previously stained slides or images were reviewed to assess tissue quality and presence of tumor. IHC was performed in the TDx CAP/CLIA laboratory of VMSI using assays developed and validated in the Translational Diagnostics Laboratories of VMSI. Immunostaining was assessed by a board-certified pathologist.

qRT-PCR was assessed using the Fluidigm platform using an allele-specific PCR mutation panel that detects mutations in *KRAS* and *NRAS* in exon 2 (G12 and G13), exon 3 (Q61), and exon 4 (K117 and A146) as previously described (20).

Statistical methods

This phase II trial was designed to make a preliminary comparison of the safety and efficacy of FOLFIRI + duligotuzumab versus FOLFIRI + cetuximab in patients with *KRAS* wild-type mCRC and in patients with low HER3 levels in their tumors. In particular, it was designed to obtain informative estimates of the PFS hazard ratios in the overall patient population and the HER3-low patient population to enable further decision making. This trial is hypothesis generating and was not intended to detect the minimal clinically meaningful benefit.

Results

Patient characteristics

A total of 68 patients were enrolled in the duligotuzumab + FOLFIRI arm (78% *RAS* wild-type), and 66 in the cetuximab + FOLFIRI arm (68% *RAS* wild-type), from October 22, 2012, to December 24, 2013, at 43 sites. The last patient's final visit was completed on November 26, 2014; this date served as the clinical data cutoff for the analyses. The baseline characteristics of the patient population are shown in Table 1 and were well balanced between treatment arms with the exception of a slight imbalance in sex, ECOG PS, *RAS* wild-type, and *PIK3CA* mutation status. Of 134 randomized patients, 98 were *RAS* exon 2/3 wild-type ($n = 53$ in the duligotuzumab arm); *BRAF* and *PIK3CA* mutations were present in 15% and 12% of all patients enrolled. Sixty-five percent of patients were triple wild-type (*RAS*, *BRAF*, and *PIK3CA*). Most patients (77%) had progressed on first-line oxaliplatin within 6 months.

Safety and tolerability

There were 67 and 63 patients in the duligotuzumab and cetuximab arms, respectively, who were evaluable for safety (Table 2). The most common AEs of any grade were rash (84%), diarrhea (79%), fatigue (62%), and nausea (50%). There were fewer rash events of any grade in the duligotuzumab arm but more diarrhea.

The incidence of grade ≥ 3 AEs was similar between arms (All AEs: 85% and 89%; related AEs 46% and 60%); overall, neutropenia (23%) was the most common AE of grade ≥ 3 intensity, regardless of attribution. The frequency of SAEs was higher in the cetuximab arm (56% vs. 48% for duligotuzumab). AEs of special interest included grade ≥ 3 diarrhea that was higher in the duligotuzumab arm (18%) compared with the cetuximab arm (14%), and grade ≥ 3 infusion-related reactions (8% vs. 2%) and grade ≥ 3 rash (22% vs. 8%) that were higher in the cetuximab arm.

AEs that led to a fatal outcome were reported in 3 patients on the duligotuzumab + FOLFIRI arm (pneumonia, respiratory failure, and one unknown cause of death), and 2 patients on the cetuximab arm (*Campylobacter* infection and lung infection).

Table 1. Patient baseline and disease characteristics

	Duligotuzumab + FOLFIRI		Cetuximab + FOLFIRI		All patients	
	All ($n = 68$)	*RAS* WT ($n = 53$, 78%)	All ($n = 66$)	*RAS* WT ($n = 45$, 68%)	All ($N = 134$)	*RAS* WT ($N = 98$)
Age (years), median (range)	61 (21–85)	62 (21–85)	62 (26–82)	65 (39–82)	62 (21–85)	63 (21–85)
Sex (male)	36 (53%)	27 (51%)	46 (70%)	34 (76%)	82 (61%)	61 (62%)
Race (white)	60 (88%)	46 (87%)	59 (89%)	42 (93%)	119 (89%)	88 (90%)
ECOG PS (0)	30 (45%)	24 (45%)	39 (62%)	25 (57%)	69 (53%)	49 (51%)
BRAF mutation[a]	8 (12%)	8 (15%)	8 (12%)	8 (18%)	16 (12%)	16 (16%)
PIK3CA mutation[a]	11 (20%)	9 (17%)	6 (12%)	4 (9%)	17 (16%)	13 (13%)
Triple WT (*RAS*, *BRAF*, and *PIK3CA*)[a]	36 (64%)	36 (68%)	33 (65%)	33 (73%)	69 (65%)	69 (70%)
Received prior bevacizumab	32 (47%)	23 (44%)	31 (48%)	18 (40%)	63 (47%)	41 (42%)
Time to PD on first-line oxaliplatin-based chemo (≤6 months)	53 (78%)	38 (72%)	50 (76%)	34 (76%)	103 (77%)	72 (74%)
Primary tumor location						
Left colon	22 (42%)	—	18 (40%)	—	40 (41%)	—
Right colon	12 (23%)	—	11 (24%)	—	23 (24%)	—
Rectum	19 (36%)	—	14 (31%)	—	33 (34%)	—
Unknown	0	—	2 (4%)	—	2 (2%)	—

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PD, progressive disease; WT, wild-type.
[a]The denominator for the all-randomized population is the number of patients with mutation data.

SAEs occurred in 54% and 56% of patients on duligotuzumab versus cetuximab arm. The SAEs, regardless of attribution, occurring in ≥ 5% of patients were pyrexia (7%), diarrhea, and pulmonary embolism (5% each).

Treatment discontinuation of mAbs due to AEs was comparable in the duligotuzumab (14%) and cetuximab (16%) arms. However, cumulative dose intensity of FOLFIRI chemotherapy components was lower on the duligotuzumab versus cetuximab arm [irinotecan: 66 (24–100) vs. 75 (32–100); 5-FU infusion 67 (24–99) vs. 74 (32–107)]. In the duligotuzumab arm, select GI toxicities (diarrhea, mucosal inflammation, and stomatitis) occurring at relatively high frequency led to disproportionately more frequent dose changes for irinotecan and 5-FU (Supplementary Table S2). For example, 52% of the stomatitis events occurring in the duligotuzumab arm led to chemotherapy dose modifications, while in the cetuximab arm, 8% of stomatitis events led to chemotherapy modifications. Furthermore, AEs leading to chemotherapy dose modifications, as a whole, occurred earlier in the duligotuzumab arm compared with the cetuximab arm. The median time of onset to first AEs for which irinotecan or 5-FU was modified (dose reduced, drug interrupted, or drug withdrawn) was 22 days in the duligotuzumab arm versus 35 days in the cetuximab arm, of safety-evaluable patients.

Pharmacokinetic analysis

In the duligotuzumab arm, mean peak and trough (±SD) serum concentrations of duligotuzumab were 299 μg/mL (±66.3) and 39.5 μg/mL (±43.7), respectively, in cycle 1, and the trough serum concentration was 76.0 (±42.6) μg/mL in cycle 9 [cycle 10, day 1 (predose)]. The data were comparable with previously reported serum duligotuzumab concentration data (equivalent dose of 14 mg/kg q2w i.v.) in the phase I study (21).

There was no apparent effect of 5-FU and irinotecan coadministration on duligotuzumab PK. From an assessment of mean observed serum duligotuzumab trough concentration (69.4 μg/mL) from cycle 3 [cycle 4, day 1 (predose)] onward, there appeared to be minimal accumulation of duligotuzumab during the treatment period.

Table 2. All AEs regardless of attribution in ≥20% of patients

	Duligotuzumab + FOLFIRI ($n = 67$)		Cetuximab + FOLFIRI ($n = 63$)	
MedDRA-preferred term	**Any grade**	**≥Grade 3**	**Any grade**	**≥Grade 3**
All	**67 (100%)**	**57 (85%)**	**63 (100%)**	**56 (89%)**
Rash and related terms[a]	53 (79%)	5 (8%)	56 (89%)	14 (22%)
Diarrhea	61 (91%)	12 (18%)	42 (67%)	9 (14%)
Fatigue	43 (64%)	6 (9%)	37 (59%)	10 (16%)
Nausea	33 (49%)	3 (5%)	32 (50%)	2 (3%)
Mucosal inflammation	26 (38%)	7 (10%)	23 (37%)	3 (5%)
Stomatitis	21 (31%)	6 (9%)	26 (41%)	4 (6%)
Alopecia	18 (27%)	—	22 (35%)	1 (2%)
Neutropenia	18 (27%)	14 (21%)	21 (33%)	16 (25%)
Paronychia	21 (31%)	2 (3%)	18 (29%)	1 (2%)
Hypokalemia	25 (37%)	4 (6%)	13 (21%)	4 (6%)
Abdominal pain	13 (19%)	1 (2%)	22 (35%)	4 (6%)
Decreased appetite	16 (24%)	2 (3%)	14 (22%)	2 (3%)
Dry skin	17 (25%)	—	13 (21%)	—
Infusion-related reaction[b]	13 (19%)	1 (2%)	17 (27%)	5 (8%)
Vomiting	17 (25%)	4 (6%)	12 (19%)	3 (5%)
Hand–foot syndrome	13 (19%)	1 (2%)	13 (21%)	2 (3%)

[a]Rash and related MedDRA terms = rash dermatitis acneiform, rash maculopapular, acne, dermatitis, rash macular, rash erythematous, rash pruritic, dermatitis atopic, dermatitis bullous, dermatitis exfoliative, rash generalized, rash papular, and rash pustular.
[b]Any AE occurring during infusion or within 24 hours and suspected to be caused by duligotuzumab or cetuximab.

Table 3. Summary outcomes in (A) all randomly assigned *RAS* WT patients and (B) all randomly assigned *KRAS* exon 2 WT patients

	A.		
All randomly assigned *RAS* WT patients	**Duligotuzumab + FOLFIRI (*n* = 53)**	**Cetuximab + FOLFIRI (*n* = 45)**	**HR[a] or OR (90% CI)**
PFS events	41 (77%)	35 (78%)	1.21 (0.81–1.81)
Median PFS, mo (90% CI)	7.3 (5.3–8.1)	5.7 (5.5–7.7)	
OS events	24 (45%)	22 (49%)	1.00 (0.61–1.66)
Median OS, mo (90% CI)	14.0 (12.0–NE)	13.1 (10.2–NE)	
ORR, % (90% CI)	10 (19%; 11–29)	15 (33%; 22–46)	0.47 (0.21–1.01)
Complete response	—	2 (4%)	
Partial response	12 (23%)	19 (42%)	
Stable disease	27 (51%)	19 (42%)	
Progressive disease	8 (15%)	4 (9%)	
Missing/unevaluable	6 (11%)	1 (2%)	
	B.		
All randomly assigned *KRAS exon 2* WT patients	**Duligotuzumab + FOLFIRI (*n* = 68)**	**Cetuximab + FOLFIRI (*n* = 66)**	**HR[a] or OR (90% CI)**
PFS events	54 (79%)	50 (76%)	1.30 (0.93–1.82)
Median PFS, mo (90% CI)	5.4 (3.8–7.5)	5.6 (5.3–7.5)	
OS events	34 (50%)	33 (50%)	0.97 (0.64–1.46)
Median OS, mo (90% CI)	14.0 (11.0–20.3)	12.4 (10.2–NE)	
ORR, % (90% CI)	11 (16%; 10–24)	21 (32%; 22–42)	0.41 (0.21–0.83)
Complete response	—	2 (3%)	
Partial response	15 (22%)	25 (38%)	
Stable disease	34 (50%)	27 (41%)	
Progressive disease	9 (13%)	6 (9%)	
Missing/unevaluable	10 (15%)	6 (9%)	

Abbreviations: CI, confidence interval; NE, not estimated; ORR, objective response rate (note that not all responses were confirmed); OS, overall survival; PFS, progression-free survival; WT, wild-type.
[a]Stratified hazard ratio. OR indicates overall response (odds ratio >1 indicates benefit in the active arm).

There was no evidence of trends in PFS or OS with duligotuzumab exposure, based on exposure–response analyses, indicating that dose was close to or at the top of the exposure–response curve (Supplementary Table S1).

The baseline prevalence of ATAs was 0% in the overall study population (0/70 patients). None of the 59 post-baseline evaluable patients treated with duligotuzumab had positive ATA results.

Clinical activity

Efficacy results (Table 3A) show no benefit of duligotuzumab + FOLFIRI compared with cetuximab + FOLFIRI in *RAS* wild-type patients. Patients in the duligotuzumab arm did not show improved PFS by investigator assessment (Fig. 1). In the *RAS* wild-type subgroup, median PFS was 7.3 versus 5.7 months for duligotuzumab versus cetuximab (stratified HR 1.21, 90% CI 0.81–1.81). In HER3-low *RAS* wild-type randomized patients (based on the median ERBB3 qRT-PCR expression; $n = 54$), the HR for PFS was 1.34 (90% CI, 0.80–2.25).

OS data were immature with 45% of OS events having occurred on the duligotuzumab and 49% on the cetuximab arms at the time to data cutoff (HR 1.00, 90% CI, 0.61–1.66). Median OS was 14.0 months for duligotuzumab, and 13.1 months for cetuximab. Time-to-treatment failure was longer in the cetuximab arm compared with the duligotuzumab arm.

The objective response rate (ORR) was numerically lower in duligotuzumab-receiving patients (19%) compared with cetuximab (33%; OR 0.47, 90% CI 0.21–1.01). Best overall response rates in the duligotuzumab arm consisted of 12 (23%) PR, as compared with 2 (4%) CR and 19 (42%) PR in the cetuximab arm. The waterfall plot for best CT response in Fig. 2A shows the corresponding degree of tumor shrinkage in cetuximab versus the duligotuzumab arm. The overall time on study treatment was lower on duligotuzumab versus cetuximab (Fig. 3).

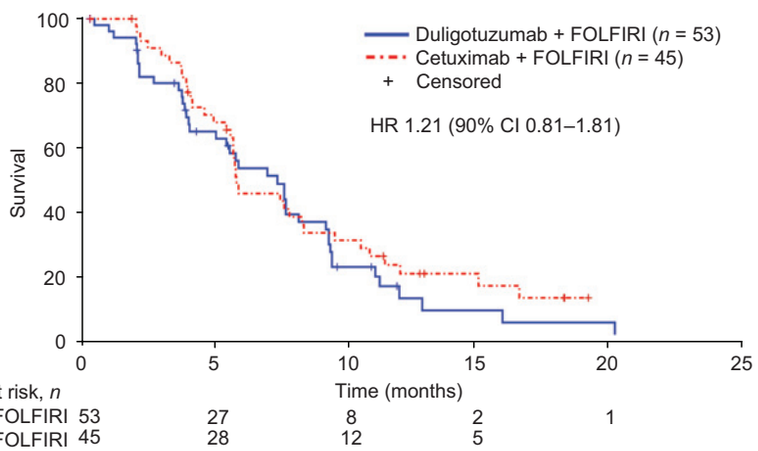

Figure 1.
Progression-free survival and number of patients at risk by duligotuzumab and cetuximab treatment arms.

Biomarker analysis

By qRT-PCR, ERBB3 expression was in the range of 0.75 to 9.96 relative expression units (2^{-dCT}), and membranous staining of HER3 protein measured by IHC was observed with H-scores ranging from 100 to 245. Neither HER3 protein levels nor relative gene expression based on RNA (ERBB3) showed a relationship with tumor shrinkage (Fig. 2A) or PFS (Fig. 2B). Although no nuclear staining was observed, there was evidence for cytoplasmic

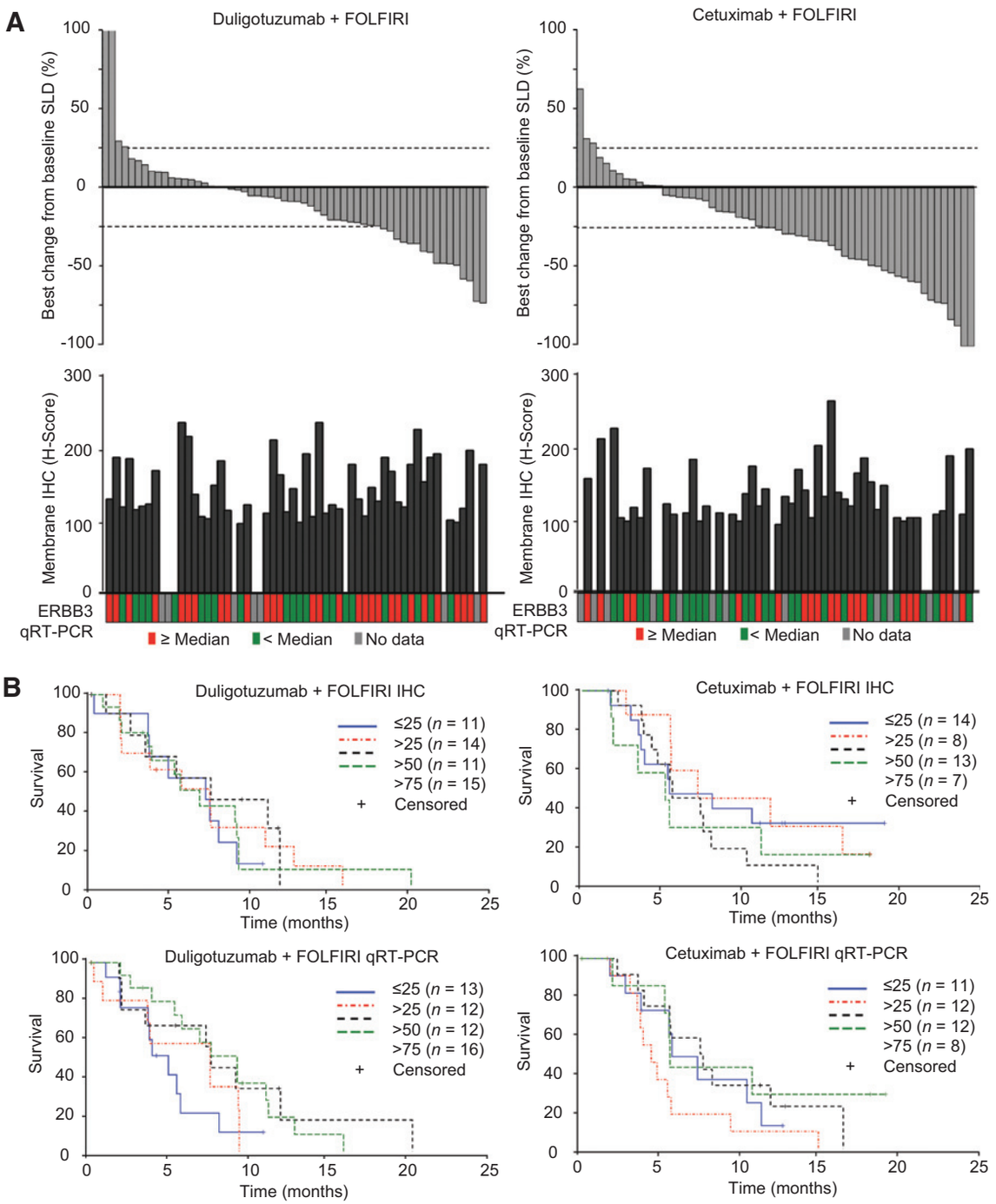

Figure 2.

A, Best response in the duligotuzumab and cetuximab treatment arms in all randomly assigned patients with RAS wild-type tumors with corresponding HER expression by membrane IHC H-score and ERBB3 qRT-PCR. **B,** PFS by the duligotuzumab or cetuximab arm by membranous H-score and ERBB3 qRT-PCR quartile.

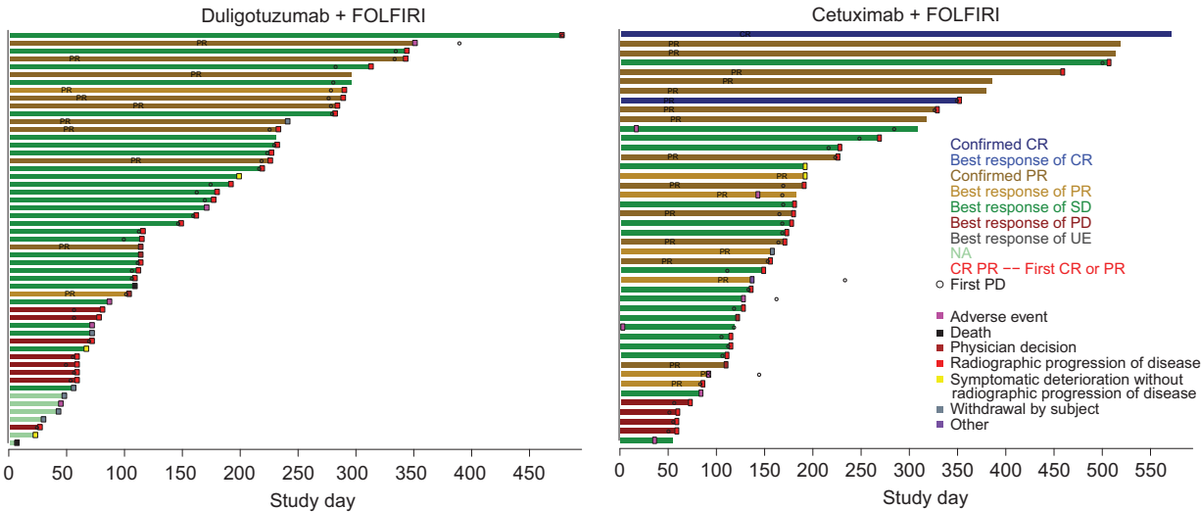

Figure 3.
Time on study treatment for the duligotuzumab and cetuximab treatment arms.

staining. Comprehensive H-scores including both membranous and cytoplasmic staining patterns did not show benefit based on tumor response.

HER3 and EGFR ligand expression was also evaluated. NRG1 expression was in the range of 0.008 to 17.53 relative expression units (2^{-dCT}) in biopsy specimens but also failed to discriminate for response based on tumor shrinkage (22). Similarly, there was no clear relationship between AREG and EREG and best CT response or degree of tumor shrinkage. However, AREG and EREG, which were significantly correlated, showed a trend toward benefit in the cetuximab arm. EGFR levels showed no difference (22).

Evaluation of *KRAS* mutation status showed that 3.7% of samples exhibited mutations at codons 12 and 13 (exon 2, possibly undetected at screening), 0% with mutations at codon 61 in exon 3, and 117 (0.9%) and 146 (2.8%) in exon 4. Mutations in *NRAS* were detected at codons 12 and 13 (1.9%) in exon 2, and 61 (1.9%) in exon 3; no mutations were detected in exon 4 at codons 117 and 146. Additionally, mutations in *BRAF* were observed in 15% of patients at codon 600 (exon 15), but not in exon 11. Both *NRAS* and *BRAF* mutations were balanced between treatment arms. The majority of patients harboring mutations in *KRAS* exon 2 or 4, or *BRAF*, did not respond to either treatment arm (Table 3B); however, limited responses were observed in patients with mutations in *NRAS* (3 PRs, 2 cetuximab, and 1 duligotuzumab arm).

Discussion

HER3 is thought to have a functional role in colorectal cancer tumorigenesis (23). Although normal colonic tissue has little to no HER3 expression, HER3 is expressed in a significant proportion of colorectal tumors (24–29). Indeed, colorectal cancer tumors express high levels of NRG1 and several EGFR ligands, suggesting that these tumors use both the EGFR and HER3 pathways to sustain proliferation (22).

Given the limited sample size no definitive conclusions can be drawn with respect to efficacy; however, a large clinical benefit was excluded. Furthermore, HER3 protein or gene expression levels did not select for benefit with duligotuzumab, nor did NRG1 or AREG expression. The mean peak and trough serum concentrations of duligotuzumab in the duligotuzumab + FOLFIRI arm were comparable with those previously reported (equivalent dose of 14 mg/kg q2w i.v.) in the phase I study (21), indicating that there was no apparent effect of 5-FU and irinotecan coadministration on the duligotuzumab PK.

Duligotuzumab in combination with FOLFIRI has an acceptable safety profile. The combination of duligotuzumab was overall well tolerated with no unexpected safety findings. The overall incidence of AEs, SAEs, deaths, and withdrawal from the study due to AEs, dose modification/interruption due to AEs was comparable between the duligotuzumab and cetuximab arms. In the duligotuzumab arm, there was less rash, consistent with the phase I study (21), and immune-related reactions. However, selected GI toxicities, including diarrhea, of all grades were more frequent.

A number of factors could have contributed to lower ORR in the duligotuzumab arm. For one, select GI toxicities (diarrhea, mucosal inflammation, and stomatitis) occurred at relatively high frequency and led to disproportionately more frequent and earlier dose changes for irinotecan and 5-FU (Supplementary Table S2). The more frequent occurrences of diarrhea and mucosal inflammation were associated with shorter duration and reduced dose intensity of the chemo backbone. The 5-FU bolus was preferentially reduced to the lowest dose intensity in an attempt to counter these AEs. In addition, no severe imbalances were seen in overall incidence of grade 3–5 AEs, and the overall percentage of patients in whom irinotecan or 5-FU was modified due to a grade 3–5 AE was similar between the arms. Therefore, it was mainly, and often multiple, grade 1–2 AEs accounting for the disproportionally more frequent irinotecan and 5-FU dose modifications in the duligotuzumab arm.

The role of HER3 appears limited in the mCRC EGFR inhibitor–naïve setting because there was no additional benefit of inhibiting HER3 in addition to EGFR. Expression of HER3 measured by RNA or protein did not correlate with response suggesting a minor role

for HER3 in this disease. Conversely, the EGFR ligands AREG and EREG did show a trend toward improved survival in the cetuximab arm consistent, with published data demonstrating stratified responses to antibody-based EGFR inhibitors (30). In the context of chemotherapy, it is difficult to distinguish between specific differences between the two regimens, but these data may suggest more potent EGFR clinical inhibitory activity by cetuximab (Kd = 0.2 nmol/L, ref. 31). The affinity of duligotuzumab is almost 1 log higher for HER3 than that for EGFR (Kd huHER3 = 0.39 nmol/L; Kd huEGFR = 1.9 nmol/L; ref. 32), which may in part explain the lack of additional benefit of duligotuzumab compared with cetuximab in this setting. Abrogation of dual HER3/EGFR signaling may explain the higher incidence of diarrhea and mucosal inflammation in the duligotuzumab arm, which has been seen with other HER3 inhibitors, and the lower affinity for EGFR could translate in a lower EGFR-signaling down regulation that may be the cause of the lower frequency of cutaneous toxicities observed in the duligotuzumab-containing arm given the binding affinity of each compound.

Whether dual EGFR/HER3 inhibition could restore sensitivity once tumors have failed initial EGFR inhibition is something that we cannot rule out due to the design of this study. Receptor expression based on HER3 protein and gene expression analysis did not select for benefit with duligotuzumab. Similarly, the expression of the ligands NRG1 and AREG also does not select for benefit. Unlike SCCHN, no relationship between NRG1 and EGFR ligands was noted (33–34).

In light of this and another randomized phase II study in SCCHN showing no benefit for the dual inhibition of EGFR and HER3 over EGFR alone, neither in all randomized patients nor in biomarker selected subsets, we conclude that the role of dual HER3/HER1 inhibition remains not well understood in patients not previously treated with EGFR inhibitors. Further development of duligotuzumab has been stopped.

Disclosure of Potential Conflicts of Interest

M.E. Burge reports receiving commercial research grants from Amgen, and is a consultant/advisory board member for Amgen, Roche, Merck, Sirtex, and Merck, Sharpe & Dohme. L. Samuel is a consultant/advisory board member for Servier. J. Tabernero is a consultant/advisory board member for Bayer, Boehringer Ingelheim, Genentech/Roche, Lilly, Merck, Sharpe & Dohme, Merck Soreno, Novartis, Roche, Sanofi, Symphogen, and Taiho. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The authors take full responsibility for the design of the study, the collection of the data, the analysis and interpretation of the data, the decision to submit the article for publication, and the writing of the article.

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References

1. Lockhart AC, Berlin JD. The epidermal growth factor receptor as a target for colorectal cancer therapy. Semin Oncol 2005;32:52–60.
2. Troiani T, Zappavigna S, Martinelli E, Addeo SR, Stiuso P, Ciardiello F, et al. Optimizing treatment of metastatic colorectal cancer patients with anti-EGFR antibodies: overcoming the mechanisms of cancer cell resistance. Expert Opin Biol Ther 2013;13:241–55.
3. Pietrantonio F, Cremolini C, Petrelli F, Di Bartolomeo M, Loupakis F, Maggi C, et al. First-line anti-EGFR monoclonal antibodies in panRAS wild-type metastatic colorectal cancer: A systematic review and meta-analysis. Crit Rev Oncol Hematol 2015;96:156–66.
4. Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Ann Oncol 2015;26:13–21.
5. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013;369:1023–34.
6. Stintzing S, Jung A, Rossius L, Modest DP, von Weikersthal LF, Decker T, et al. Mutations within the EGFR signaling pathway: Influence on efficacy in FIRE-3—A randomized phase III study of FOLFIRI plus cetuximab or bevacizumab as first-line treatment for wild-type (WT) KRAS (exon 2) metastatic colorectal cancer (mCRC) patients. J Clin Oncol 2014;32 suppl 3; abstr 445.
7. Bokemeyer C, Kohne C-H, Ciardiello F, Lenz H-J, Heinemann V, Klinkhardt U, et al. Treatment outcome according to tumor RAS mutation status in OPUS study patients with metastatic colorectal cancer (mCRC) randomized to FOLFOX4 with/without cetuximab. J Clin Oncol 2014;32:suppl 5; abstr 3505.
8. Ciardiello F, Lenz H-J, Kohne C-H, Heinemann V, Tejpar S, Melezinek I, et al. Treatment outcome according to tumor RAS mutation status in CRYSTAL study patients with metastatic colorectal cancer (mCRC) randomized to FOLFIRI with/without cetuximab. J Clin Oncol 2014;32:suppl 5; abstr 3506.
9. Peeters M, Oliner KS, Price TJ, Cervantes A, Sobrero AF, Ducreux M, et al. Analysis of KRAS/NRAS mutations in phase 3 study 20050181 of panitumumab (pmab) plus FOLFIRI versus FOLFIRI for second-line treatment (tx) of metastatic colorectal cancer (mCRC). J Clin Oncol 2014;32:suppl 3; abstr LBA387.
10. Van Cutsem E, Cervantes A, Nordlinger B, Arnold D. Metastatic colorectal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014;25:suppl 3, iii1-iii9.

11. NCCN guidelines for detection, prevention, and risk reduction, colorectal cancer screening Version 2.2016. https://www.nccn.org/professionals/physician_gls/pdf/colorectal_screening.pdf.
12. Sergina NV, Rausch M, Wang D, Blair J, Hann B, Shokat KM, et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. Nature 2007;445:437–41.
13. Wheeler DL, Huang S, Kruser TJ, Nechrebecki MM, Armstrong EA, Benavente S, et al. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. Oncogene 2008;27:3944–56.
14. Iida M, Brand TM, Starr MM, Huppert EJ, Luthar N, Bahrar H, et al. Overcoming acquired resistance to cetuximab by dual targeting HER family receptors with antibody-based therapy. Mol Cancer 2014;13:242.
15. Yonesaka K, Zejnullahu K, Okamoto I, Satoh T, Cappuzzo F, Souglakos J, et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci Transl Med 2011;3,99ra86.
16. Makhija S, Amler LC, Glenn D, Ueland FR, Gold MA, Dizon DS, et al. Clinical activity of gemcitabine plus pertuzumab in platinum-resistant ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. J Clin Oncol 2010;28:1215–23.
17. Amler LC. HER3 mRNA as a predictive biomarker in anticancer therapy [review]. Expert Opin Biol Ther 2010;10:1343–55.
18. Schaefer G, Haber L, Crocker LM, Shia S, Shao L, Dowbenko D, et al. A two-in-one antibody against HER3 and EGFR has superior inhibitory activity compared with monospecific antibodies. Cancer Cell 2011;20:472–86.
19. Crocker M, Fields C, Shao L, Sliwkowski MX, Lewis Phillips GD, Schaefer G. The dual action antibody MEHD7945A targeting EGFR and HER3 enhances chemotherapy induced cytotoxicity in vitro and in vivo. Proc Am Assoc Cancer Res 2012; abstract nr 1212.
20. Schleifman EB, Tam R, Patel R, Tsan A, Sumiyoshi T, Fu L, et al. Next generation MUT-MAP, a high-sensitivity high-throughput microfluidics chip-based mutation analysis panel. PLoS One 2014;9:e90761.
21. Juric D, Dienstmann R, Cervantes A, Hidalgo M, Messersmith W Blumenschein GR Jr, et al. Safety and pharmacokinetics/pharmacodynamics of the first-in-class dual action HER3/EGFR antibody MEHD7945A in locally advanced or metastatic epithelial tumors. Clin Cancer Res 2015; 21:2462–70.
22. Qu X, Sandmann T, Frierson H Jr, Fu L, Fuentes E, Walter K, et al. Integrated genomic analysis of colorectal cancer progression reveals activation of EGFR through demethylation of the EREG promoter. Oncogene 2016; 35:6403–15.
23. Amin DN, Campbell MR, Moasser MM. The role of HER3, the unpretentious member of the HER family, in cancer biology and cancer therapeutics. Semin Cell Dev Biol 2010;21:944–50.
24. Ciardiello F, Kim N, Saeki T, Dono R, Persico MG, Plowman GD, et al. Differential expression of epidermal growth factor-related proteins in human colorectal tumors. Proc Natl Acad Sci U S A 1991; 88:7792–6.
25. Rajkumar T, Gooden CS, Lemoine NR, Gullick WJ, Goden CS. Expression of the c-erbB-3 protein in gastrointestinal tract tumours determined by monoclonal antibody RTJ1. J Pathol 1993;170:271–8.
26. Maurer CA, Friess H, Kretschmann B, Zimmermann A, Stauffer A, Baer HU, et al. Increased expression of erbB3 in colorectal cancer is associated with concomitant increase in the level of erbB2. Hum Pathol 1998;29:771–7.
27. Kountourakis P, Pavlakis K, Psyrri A, Rontogianni D, Xiros N, Patsouris E, et al. Prognostic significance of HER3 and HER4 protein expression in colorectal adenocarcinomas. BMC Cancer 2006;6:46.
28. Kapitanović S, Radosević S, Slade N, Kapitanović M, Andelinović S, Ferencić Z, et al. Expression of erbB-3 protein in colorectal adenocarcinoma: correlation with poor survival. J Cancer Res Clin Oncol 2000;126:205–11.
29. Beji A, Horst D, Engel J, Kirchner T, Ullrich A. Toward the prognostic significance and therapeutic potential of HER3 receptor tyrosine kinase in human colon cancer. Clin Cancer Res 2012;18:956–68.
30. Licitra L, Perrone F, Tamborini E, Bertola L, Ghirelli C, Negri T, et al. Role of EGFR family receptors in proliferation of squamous carcinoma cells induced by wound healing fluids of head and neck cancer patients. Ann Oncol 2011;22:1886–93.
31. Erbitux (cetuximab) product monograph, Eli Lilly, 2016.
32. Fuh G. Two-in-one antibody: from proof-of-concept to therapeutic candidate. Society for Immunotherapy of Cancer 25th Annual Scientific Meeting, 2010.
33. Fayette J, Wirth LJ, Oprean C, Hitt R, Udrea A, Jimeno A, et al. Randomized phase II study of MEHD7945A vs cetuximab in 2nd-line recurrent/metastatic squamous cell carcinoma of the head and neck progressive on/after platinum-based chemotherapy. Annals of Oncology 2014;25(suppl_4).
34. Hill AG, Findlay M, Burge M, Jackson C, Alfonso PG, Samuel L, et al. Randomized phase II study of MEHD7945A + FOLFIRI vs cetuximab + FOLFIRI in 2nd-line patients with KRAS exon 2 wild-type metastatic colorectal cancer. AACR annual meeting, 2015.