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Inherent Resistance to Epidermal Growth Factor Receptor Antibodies in Refractory Metastatic Colorectal Cancer

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Frequency of Epidermal Growth Factor Receptors (EGFR) tyrosine kinase mutations is very low in metastatic colorectal cancer. Mutations in other genes in EGFR pathway, such as PI3K, K-ras and B-raf are more frequent in colorectal cancer but their relationship with response to EGFR-targeted antibodies is less well studied. Thirty-five patients with metastatic colorectal cancer were randomized to receive cetuximab with or without oral sorafenib. Patients were stratified according to tumor K-ras status. Patients received cetuximab IV weekly for 4 week and oral sorafenib twice daily on days 1-28, with recycling every 4 weeks. Primary end point was response rate (partial and complete), while secondary end points were adverse effects, time to progression and overall survival. Wild K-ras cases constituted 64.7 and 61.1% of cetuximab (E) and cetuximab-sorafenib (EN) groups, respectively. Partial response was higher in (EN) that constituted 33.3% compared to 17.6% in cetuximab group ($p = 0.44$). Multivariate analysis revealed that K-ras status had statistically significant effect on progression-free survival. Progression-free survival had higher statistically significant difference in wild K-ras compared to mutant K-ras cases ($p = 0.0001$). Median overall survival was 7 and 5 months in (EN) and (E) groups respectively ($p = 0.49$). The study reflects that mutation status of molecular markers such as K-ras and B-raf is a predictor of response, so genotyping of tumors is needed for defining the patient population that is likely to benefit from targeted therapy. Combination of therapy that simultaneously targets K-ras and B-raf could be a useful approach to increase number of patients who may benefit from anti-EGFR therapy, however, large scale prospective randomized trials are needed to properly determine which patients are best candidates for these targeted agents.

Key words: K-ras, cetuximab, sorafenib, metastatic colorectal carcinoma

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INTRODUCTION

Colorectal cancer is a major cause of morbidity and mortality worldwide. While early stage colorectal cancer is frequently curable with surgery, unresectable metastatic disease is uniformly fatal. Palliative treatment for metastatic colorectal cancer depends on the use of chemotherapy with the antineoplastic agents as fluorouracil (FU), irinotecan and oxaliplatin. Once, a patient's cancer becomes refractory to chemotherapy agents, however, there are essentially no established treatment options with demonstrated efficacy. Clearly, there is a desperate need for new and improved therapies for this lethal disease (Saltz *et al*., 2004). Recent advances in the identification of key tumorigenesis signaling pathways and protein kinases have led to the development of novel targeted anticancer therapies. These agents have activity in a variety of solid tumors and are better tolerated than standard chemotherapy (Gollob *et al*., 2006). Epidermal Growth Factor Receptor (EGFR), a member of the ErbB family of receptors, is relevant in colorectal cancer because expression or up-regulation of the EGFR gene occurs in 60 to 80%of cases (Messa *et al*., 1998; Porebska *et al*., 2000; Salomon *et al*., 1995). When inactive, EGFR is a monomer, but when bound by epidermal growth factor or transforming growth factor α (TGF-α), it forms homodimers or heterodimers with another member of the ErbB family of receptors. Dimerization activates the intracellular tyrosine kinase region of EGFR, resulting in autophosphorylation and initiating a cascade of intracellular events (Klapper *et al*., 2000). The EGFR signaling pathway regulates cell differentiation, proliferation, migration, angiogenesis and apoptosis, all of which become deregulated in cancer cells (Ciardiello and Tortora, 2001).

Cetuximab (Erbitux) is a chimeric IgG1 monoclonal antibody that binds to extracellular domain of EGFR with high specificity and with a higher affinity than either epidermal growth factor or TGF-α thus blocking ligand-induced phosphorylation of EGFR (Fan *et al*., 1993). *In vitro* assays and *in vivo* animal studies have shown that binding of erbitux to the EGFR blocks phosphorylation and activation of receptor-associated kinases, resulting in inhibition of cell growth, induction of apoptosis and decreased matrix metalloproteinase and vascular endothelial growth factor production. Cetuximab was approved in patients with EGFR-expressing metastatic colorectal cancer refractory to irinotecan-based chemotherapy, either in combination with irinotecan (for irinotecan-refractory patients) or as monotherapy (for irinotecan-intolerant patients). Approval was based on data from previous randomized phase II studies (Saltz *et al*., 2004; Cunningham *et al*., 2004) showing that cetuximab monotherapy produced objective responses.

Multiple cellular pathways influence the growth and metastatic potential of tumors. This creates heterogeneity, redundancy and the potential for tumors to bypass signaling pathway blockade, resulting in primary or acquired resistance. Constitutive activation of signaling pathways such as Ras, Raf and Src can initiate downstream signaling that leads to cell proliferation and inhibition of apoptosis. Therefore, if a tumor cell possesses one or more of these receptors, targeting a single cell surface protein tyrosine kinase such as EGFR may not be enough to inhibit growth or survival signals. Thus, tumors may be primarily resistant or could become resistant to therapies targeting a specific pathway. Combining therapies that inhibit different signaling pathways has the potential to be more effective than inhibition of a single pathway and to overcome tumor resistance. Sorafenib (Nexavar), an oral biaryl urea multikinase inhibitor has been shown to inhibit tumor growth and tumor angiogenesis by targeting Raf kinase, vascular endothelial growth factor receptor and platelet-derived growth factor receptor. Sorafenib also inhibits several receptor tyrosine kinases, Fms-like tyrosine kinase-3 (Flt3), stem cell growth factor receptor (c-KIT) and p38α, a member of the MAP kinase family (Wilhelm *et al*., 2004, 2006; Sridhar *et al*., 2005). The multiple molecular targets of sorafenib may explain its broad preclinical and clinical activity. In phase I studies, sorafenib demonstrated single-agent activity in patients with advanced solid tumors (Kupsch *et al*., 2005).

Ratain *et al*. (2006) had a trial on sorafenib originally focused on patients with colorectal cancer. However, because many patients with Renal Cell Carcinoma (RCC) showed evidence of tumor regression, the protocol was amended during its course to extend enrollment to patients with RCC. Subsequently, enrollment of patients with colorectal cancer was terminated.

MATERIALS AND METHODS

Rationale of the study depends on that monoclonal antibodies, such as cetuximab, can block the ability of tumor cells to grow and spread. Sorafenib may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. Giving sorafenib together with cetuximab may kill more tumor cells. This phase II trial is studying how well giving sorafenib together with cetuximab works in treating patients with metastatic colorectal cancer.

This randomized trial was conducted for 35 patients with metastatic colorectal cancer referred to Saudi German Hospital-oncology center in Kingdom of Saudi Arabia from October, 2007 to June, 2009. All the patients signed a consent form. The study was approved by the hospital ethics committees. The objective of the study was to determine the response rate (partial and complete) as primary end point for metastatic colorectal cancer patients with wild or mutant K-RAS tumors treated with sorafenib and cetuximab, while secondary end points included incidence of adverse effects, time to progression and overall survival time.

Inclusion criteria:

- Age > 18 years
- ECOG performance status 0-2
- Life expectancy > 3 months
- Histologically or cytologically confirmed metastatic colorectal cancer
- Evidence of disease recurrence or progression after ≥1 prior chemotherapy regimen administered for the treatment of metastatic disease
- Measurable disease, defined as ≥1 unidimensionally measurable lesion, ≥20 mm by conventional techniques or ≥10 mm by spiral CT scan
- Must have tumor blocks or unstained slides from an archival pathological specimen suitable for isolation of genomic DNA
- Not eligible for or refused tumor resection
- No known brain metastases unless stable for ≥6 months without anticonvulsant or steroid therapy.
- Adequate blood count; platelet count ≥100,000 mm^{-3}; absolute neutrophil count ≥1,500 mm^{-3}
- Adequate hepatic and renal functions; AST and ALT ≤2.5 times Upper Limit of Normal (ULN); bilirubin ≤1.5 times ULN; creatinine ≤ 1.5 times ULN or creatinine clearance ≥60 mL min^{-1}
- PT/PTT ≤1.5 times ULN
- Not pregnant (negative pregnancy test) or nursing
- Systolic blood pressure ≤150 mmHg and diastolic blood pressure ≤90 mmHg
- Recovered from prior therapy, with at least 4 weeks since prior chemotherapy, radiotherapy, or surgery.
- No prior sorafenib or cetuximab
- No concurrent therapeutic anticoagulation

Exclusion criteria:

- Evidence of bleeding diathesis
- Uncontrolled illness including: active serious infection, symptomatic congestive heart failure, unstable angina pectoris and uncontrolled cardiac arrhythmia or hypertension
- Known HIV positivity
- Other malignancy within the past 5 years

Study design: Patients were randomized to receive cetuximab with or without oral sorafenib. Patients received cetuximab IV over 1-2 h on days 1, 8, 15 and 22 and oral sorafenib twice daily on days 1-28. Treatment was repeated every 4 weeks in the absence of disease progression or unacceptable toxicity. Cetuximab was given at an initial dose of 400 mg per square meter, followed by weekly infusions of 250 mg per square meter. This loading dose was preceded by a 20 mg test dose to observe for evidence of allergic reactions. A histamine-receptor antagonist diphenhydramine 50 mg was given intravenously as premedication before at least the first infusion. Sorafenib is available as 200 mg, film-coated tablets. The recommended dosage is 400 mg twice/day, at least 1 h before or 2 h after food intake. Patients were stratified according to tumor K-ras status (wild type vs. codon 12/13 mutation in K-ras) by DNA sequencing (Lopez-Crapez *et al*., 2004). All patients were to be treated until disease progression or unacceptable toxic effects occurred. In case of disease progression, patients assigned to the monotherapy group could continue to receive combined cetuximab and sorafenib.

Evaluation of patients: Blood samples were collected periodically for blood counts, hepatic and renal functions. Tumor tissue collected prior to and during study treatment was analyzed for K-ras mutations. Patients underwent weekly blood counts and physical examinations were performed at every 4 weeks while on study. Patients should have their blood pressure checked weekly for the first 8 weeks of therapy. Any hypertension should be treated appropriately. Tumor response was evaluated every 8 weeks for the first 24 weeks and thereafter every 3 months with the use of consistent imaging techniques (CT or MRI). Assessment was performed with the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*., 2000). Toxic effects were assessed according to the National Cancer Institute Common Toxicity Criteria, version 2 (National Cancer Institute, Cancer Therapy Evaluation Program, 2003). Modifications of the dose of cetuximab were made only in cases of toxic effects to the skin, with 25% dose reduction or discontinuation of cetuximab if more than grade II toxicity.

Statistical analysis: Primary end point was the rate of confirmed radiological tumor response in the intention-to-

treat population. Differences in response rates between the two groups were evaluated by means of a two-sided Fisher's exact test. The p-value of less than 0.05 was considered to indicate statistical significance. Secondary end points included time to progression, overall survival time and incidence of adverse effects. The time to progression was calculated as the period from the date of randomization to the first observation of disease progression or to death from any cause within 60 days after randomization or the most recent tumor assessment. The overall survival time was calculated as the period from the date of randomization until death from any cause or until the date of last follow-up, at which point data were censored. The statistical analysis was made using the Statistical Product and Service Solutions, SPSS 10.0 with estimation of both the time to progression and overall survival time by the Kaplan-Meier method (Kaplan and Meier, 1958) and comparing between the two groups with use of the log-rank test (Peto and Peto, 1972).

RESULTS

Thirty five patients from October, 2007 to June, 2009 with metastatic colorectal carcinoma with tumor progression after previous first-line therapy were entered onto the study. The patients had progression on previous oxaliplatin or irinotecan-based chemotherapy. All patients were evaluable for response and toxicity. Patients' characteristics in Table 1 showed well-balanced data at baseline with no statistically significant difference between treatment groups. Median age was 57 and 58 years in patients of cetuximab with and without sorafenib respectively. Median chemotherapy cycles were 3 in both groups. Male/female ratio of total cases was 22/13. ECOG performance status (2) constituted 70.6 and 66.7% of cetuximab (E group) and cetuximab-sorafenib (EN group), respectively. Wild K-ras cases constituted 64.7 and 61.1% of (E) and (EN) groups respectively as shown in Table 1.

Table 2 showed that partial response was higher in combined cetuximab-sorafenib group that constituted (33.3%; 95% CI; 18.1-41.3%) compared to (17.6%; 95% CI; 12.5-23.2%) in cetuximab group (p = 0.44). Disease control (partial response and stationary disease) was higher in (EN) group that constituted 44.4% compared to 35.2% in (E) group. Table 3 showed the response in both treatment groups in relation to K-ras status. Cases with mutant K-ras had disease progression in 35.3% and 38.9% in (E) and (EN) groups respectively (p = 1). Response of treatment was stratified according to K-ras status in Table 4. All cases of partial response had wild K-ras that constituted 40.9% compared to 0% in mutant type (p = 0.013), while all cases of mutant K-ras (13 patients) had disease progression compared to 36.4% of wild type (p = 0.0001).

Univariate and multivariate analysis of different factors (age, gender, performance status, K-ras type and treatment regimen) revealed that K-ras status had statistically significant p-values (0.03 and 0.04, respectively) in progression-free survival as presented in Table 5.

Table 1: Baseline characteristics of 35 patients with metastatic colon cancer

Characteristics		E (17 cases)		EN (18 cases)		p-value
Medien age		58 years		57 years		NS
Median chemotherapy cycles		3		3		NS
Gender	Male	11	64.7%	11	61.1%	0.83
	Female	6	35.3%	7	38.9%	0.83
Performance	0-1	5	29.4%	6	33.3%	0.80
status	2	12	70.6%	12	66.7%	0.80
K-ras	Wild	11	64.7%	11	61.1%	0.83
	Mutant	6	35.3%	7	38.9%	0.83

E: Cetuximab, N: Sorafenib, NS: not significant

Table 2: Response in both treatment arms

Response	E (17 cases)	EN (18 cases)	p-value
PR	3 (17.6%)	6 (33.3%)	0.44
SD	3 (17.6%)	2 (11.1%)	0.66
DP	11 (64.7%)	10 (55.6%)	0.73

E: Cetuximab, N: Sorafenib, PR: Partial response, SD: Stationary disease, DP: Disease progression

Table 3: Response in both treatment arms in relation to K-ras status

Response	E (17 cases)	EN (18 cases)	p-value
PR			
Wild K-ras	3 (17.6%)	6 (33.3%)	0.44
Mutant K-ras	---	---	1.00
SD			
Wild K-ras	3 (17.60%)	2 (11.1%)	0.66
Mutant K-ras	---	---	1.00
DP			
Wild K-ras	5 (29.4%)	3 (16.7%)	0.44
Mutant K-ras	6 (35.3%)	7 (38.9%)	1.00

E: Cetuximab, N: Sorafenib, PR: Partial response, SD: Stationary disease, DP: Disease progression

Table 4: Response of treatment in relation to K-ras status

Response	Wild K-ras (22 cases)	Mutant K-ras (13 cases)	p-value
PR	9 (40.9%)	---	0.0130*
SD	5 (22.7%)	---	0.1340
DP	8 (36.4%)	13 (100%)	0.0001*

PR: Partial response, SD: Stationary disease, DP: Disease progression

Table 5: The p-values for univariate and multivariate analysis of progression-free and overall survivals in relation to different factors

	PFS		OS	
Factors	Univariate	Multivariate	Univariate	Multivariate
Age	0.31	0.19	0.18	0.53
Gender	0.19	0.75	0.46	0.5
PS	0.41	0.45	0.15	0.64
K-ras	0.03*	0.04*	0.05	0.19
Treatment regimen	0.22	0.13	0.12	0.37

PFS: Progression free survival, OS: Overall survival, PS: Performance status. *Statistically significant

Treatment outcome including mean and median progression-free and overall survival in relation to treatment regimens was presented in Table 6 and (Fig. 1a, b), while treatment outcome was stratified according to K-ras status in Table 7 and Fig. 2a, b and 3a, b.

With a median period of follow up of 8.5 months (1.1-13.7 months), current study results showed that addition of sorafenib to cetuximab in treatment of metastatic colon cancer had higher median OS (7 months; 95% CI; 4-10 months) in (EN) group compared to (5 months; 95% CI; 4- 6 months) in (E) group but without statistically significant difference (p = 0.49). The median PFS was similar (2 months; 95% CI; 2-2 months) and (2 months; 95% CI; 1- 3 months) in (E) and (EN) groups respectively as shown in Table 6 and Fig. 1.

Progression-free survival had higher statistically significant difference in patients with wild K-ras compared to mutant K-ras cases (p = 0.0001). Median PFS in wild K-ras patients was (3 months; 95% CI; 2- 4 months) and (4 months; 95% CI; 2-6 months) in (E) and (EN) groups compared to (2 months; 95% CI; 1-2 months) and(2 months; 95% CI; 0-3 months) in mutant K-ras patients of both groups, respectively as shown in Table 7 and Fig. 2a and b. Median OS was higher in wild K-ras patients (5 months; 95% CI; 3-7 months) and (8 months; 95% CI; 6-10 months) in (E) and (EN) groups compared to (3 months; 95% CI; 0-7 months) and (3 months; 95% CI; 2-4 months) in mutant K-ras patients of both groups respectively (p = 0.09) as shown in Table 7 and Fig. 3a and b.

Toxicity of cetuximab (E) and combined cetuximab-sorafenib (EN) was nearly similar without statistical significant difference except for hand and foot syndrome, it occurred in 33.3% in (EN) cases compared to 0% in (E) group with (p = 0.02) as presented in Table 8.

Leucopenia occurred in 0% and 11.1% in (E) and (EN) groups respectively, while gastro-intestinal toxicities (vomiting, diarrhea and constipation) occurred in 23.5% in (E) group compared to 22.2- 38.9% in (EN) group. Headache was higher in combined cetuximab- sorafenib group that constituted 50% of cases compared to 23.5% in cetuximab group (p = 0.16).

Skin reactions occurred in most of cases (88.2 and 88.9%) in (E) and (EN) groups, respectively. There were no treatment-related deaths.

Table 6: Progression free and overall survival in both treatment groups

Treatment outcome	E (17 cases)	EN (18 cases)	P (Log rank)
PFS			
Mean	3 ms (95% CI; 2- 4)	3 ms (95% CI; 2- 4)	0.80
Median	2 ms (95% CI; 2- 2)	2 ms (95% CI; 1- 3)	
OS			
Mean	6 ms (95% CI; 5- 8)	7 ms (95% CI; 6- 9)	0.49
Median	5 ms (95% CI; 4- 6)	7 ms (95% CI; 4- 10)	

PFS: Progression free survival, OS: Overall survival, E: Cetuximab, N: Sorafenib, ms: Months, P: p-value by log rank.

Table 7: Progression free and overall survival in relation to K-ras status in both treatment groups

	Wild K-ras (22 cases)		Mutant K-ras (13 cases)		
Treatment outcome	E (11 cases)	EN (11 cases)	E (6 cases)	EN (7 cases)	p-value
PFS					
Mean	4 ms (95% CI; 3- 5)	4 ms (95% CI; 3- 6)	2 ms (95% CI; 1- 2)	1 ms (95% CI; 1- 2)	0.0001*
Median	3 ms (95% CI; 2- 4)	4 ms (95% CI; 2- 6)	2 ms (95% CI; 1- 2)	2 ms (95% CI; 0- 3)	
OS					
Mean	7 ms (95% CI; 5- 8)	9 ms (95% CI; 7-10)	6 ms (95% CI; 2- 9)	5 ms (95% CI; 2- 8)	0.0920
Median	5 ms (95% CI; 3- 7)	8 ms (95% CI; 6-10)	3 ms (95% CI; 0- 7)	3 ms (95% CI; 2- 4)	

PFS: Progression free survival, OS: Overall survival, E: Cetuximab, N: Sorafenib, ms: Months, *: Significant p-value by log rank

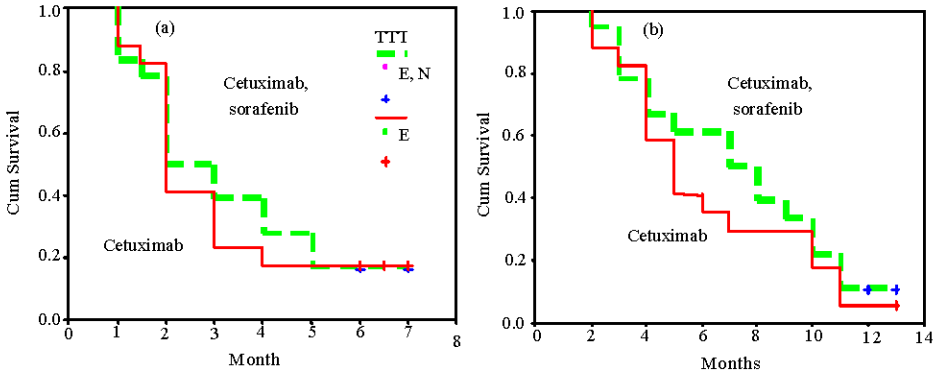

Fig. 1: (a) Progression-free survival (PFS) and (b) overall survival in both treatment groups

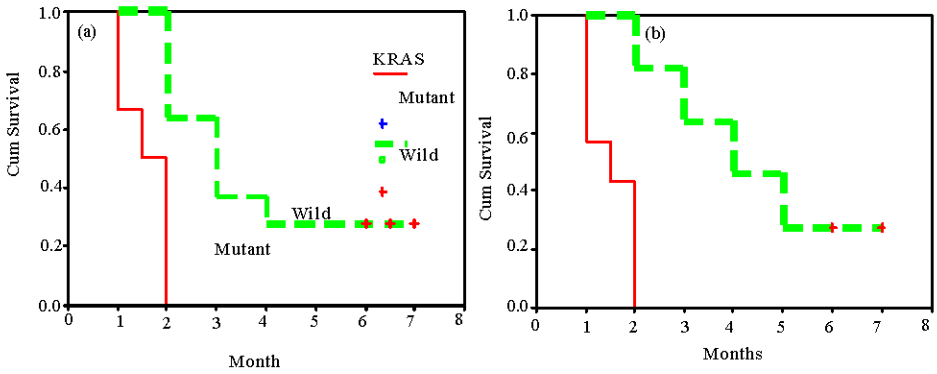

Fig. 2: Progression-free survival in relation to K-ras status in both treatment groups, (a) cetuximab and (b) cetuximab, sorafenib)

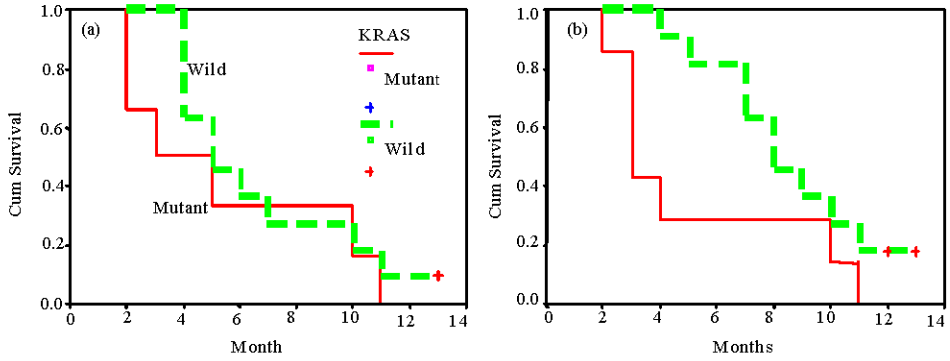

Fig. 3: Overall survival in relation to K-ras status in both treatment groups, (a) cetuximab and (b) cetuximab, sorafenib)

Table 8: Side effects in both treatment arms

	E		EN		
Sice effects	No.	%	No.	%	p-value
leucopenia	---		2	11.1	0.49
vomiting	4	23.5	6	33.3	0.71
diarrhea	4	23.5	7	38.9	0.47
constipation	4	23.5	4	22.2	1.00
headache	4	23.5	9	50.0	0.16
Asthenia, malaise	8	47.1	10	55.6	0.74
hypertension	--		2	11.1	0.49
Skin reaction	15	88.2	16	88.9	1.00
Alopecia	--		3	16.7	0.23
Hand & foot syndrome	--		6	33.3	0.02*
Nail inflammation	8	47.1	9	50.0	1.00
Sensory neuropathy	--		2	11.1	0.49
Infusion reaction	1	5.9	1	5.6	1.00
hypomagnesaemia	7	41.2	8	44.4	1.00

E: Cetuximab, N: Sorafenib, *P: Statistically significant

Table 9: Cost of chemotherapy treatment in (Egyptian pounds)

Cost of one cycle (1 month) of:	Egyptian pounds
Cetuximab	35,960±170
Cetuximab, sorafenib	71,850±130

1 Egyptian Pound = 0.18 American dollars)

Average cost of one cycle of 4 weeks of cetuximab was 35,960±170 compared to 71,850±130 Egyptian pounds for cetuximab-sorafenib regimen as shown in Table 9.

DISCUSSION

Colorectal cancer is one of the most common cancers worldwide and a leading cause of cancer death. The targeted drug cetuximab (Erbitux) - monoclonal antibodies that inhibit EGFR are used to treat patients with chemotherapy-resistant metastatic colorectal cancer. The frequency of EGFR tyrosine kinase mutations is very low or absent in metastatic colorectal cancer and it is not clear if these mutations really confer a greater susceptibility to EGFR-targeted antibodies (Lenz *et al*., 2006; Ji *et al*., 2006). Mutations in other genes in the EGFR pathway, such as PI3K, K-ras and B-raf, are more frequent in colorectal cancer but their relationship with response to EGFR-targeted antibodies is less well studied and also conflicting. In particular, although some studies suggest that patients with these mutations may be resistant to targeted therapy, other studies do not show such relationship (Moroni *et al*., 2005; Lievre *et al*., 2006).

Recent retrospective evidence from several randomized studies has established that advanced colorectal cancer patients with tumors harboring a mutation in the K-ras gene do not derive benefit

from the administration of epidermal growth factor receptor-directed monoclonal antibodies (Jimeno *et al*., 2009).

Present study showed that K-ras mutations occurred in 37.1% of all patients in both groups which was slightly higher, then results of Di Nicolantonia *et al.* (2008), who found it in 30% of cases while Lievre *et al.* (2006) and Amado *et al.* (2008) reported that K-ras mutations occurred in 43% of cases. These variable incidences of K-ras mutations could be attributed to different patient populations and sample size in different studies.

K-ras mutation in the current study was present in 50% of all non-responding cases of all patients and in 42.9 and 58.3% of (E) and (EN) groups, respectively. Partial response occurred only in nine out of 22 (40.9%) of wild K-ras patients which reflects that K-ras gene is not the only factor determining response to treatment. Partial response was higher in wild K-ras patients of (EN) group as it occurred in 6 out of 11 cases (54.5%) compared to 3 from 11 cases (27.3%) of (E) group which revealed that addition of sorafenib had beneficial effect in targeting other factors than K-ras which matched results of Benvenuti *et al.* (2007) and Di Nicolantonia *et al.* (2008), who reported that targeting B-raf -mutated cell lines with combination of cetuximab and sorafenib resulted in higher response rates than those observed with exposure to either agent alone.

Benvenuti *et al.* (2007) studied the B-raf mutations (B-raf V600E allele) and identified them in 11 patients (10% of the evaluated population; 14% of wild K-ras patients) and found that B-raf mutated tumors had statistically significant shorter PFS and overall survival irrespective of K-ras status.

Present study showed that partial response had higher statistically significant incidence in wild K-ras compared to mutant K-ras cases that coincides with results of Cappuzzo *et al.* (2008) and Amado *et al.* (2008) who found response rates to EGFR antibodies were (6.3% versus 26.5%) and (17% versus 0%), for wild versus mutant K-ras groups, in both studies, respectively.

Blocking K-ras/B-raf pathway with cetuximab, sorafenib had partial response in only 33.3% of cases, while disease progression occurred in 55.6% of cases that could be attributed to other signaling pathways.

Although, K-ras mutations may be a common genetic aberration involved in carcinogenesis, mutations of other genes, such as phosphatase and tensin homologue (PTEN), B-raf or PI3K, that can lead to unrestricted growth of cancer cells. Loss of PTEN activity has been associated with lack of efficacy of cetuximab in patients with colorectal cancer (Frattini *et al*., 2007; Karapetis *et al*., 2008).

Phosphatidylinositol 3-kinases (PI3Ks) are heterodimeric kinases composed of regulatory and catalytic subunits that are involved in the control of cell proliferation, survival and motility. The PI3K catalytic subunit, P110alpha (PIK3CA) has been reported to be somatically mutated and activated in several cancers. Activation of PIK3CA leads to plasma membrane recruitment and activation of Akt and downstream survival mechanisms. PIK3CA mutations have been reported to be associated with resistance to EGFR-targeted monoclonal antibodies in patients with metastatic colorectal cancers. In a study involving 110 patients with metastatic colorectal cancers, PIK3CA mutations were found to be significantly associated with reduced objective response rates following treatment with EGFR-targeted monoclonal antibodies and shorter progression-free survival (Sartore-Bianchi *et al*., 2009). PI3K signaling is inhibited by activity of phosphatidylinositol phosphatase, PTEN that acts as a tumor suppressor by negatively regulating the Akt signaling pathway. Additionally, loss of PTEN expression occurs by mechanisms including promoter methylation and silencing or loss of heterozygosity. Though, the association with response to EGFR-targeted therapy in metastatic colorectal cancer and loss of PTEN expression does not appear to be as strongly correlated as response and PIK3CA mutations, the consideration of both tumor PTEN expression status and PIK3CA mutation status may contribute to predicting response to EGFR-targeted therapies (Sartore-Bianchi *et al*., 2009; Egloff and Grandis, 2009).

Jhawer *et al.* (2008) reported that cell lines with both PIK3CA mutant/PTEN null and Ras/BRAF mutant had significantly maximal resistance to cetuximab than cell lines that did not harbor mutations in both of these pathways. Jhawer *et al.* (2008) had mutation screening analysis of colon cancer cell lines and found that 56% with Ras/BRAF mutations harbored a synchronous PIK3CA/PTEN mutation and 9 of the 10 cell lines (90%) harboring PIK3CA/PTEN mutations contained synchronous Ras/BRAF mutations. This finding was consistent with previous reports indicating significant overlap between the presence of PIK3CA and K-Ras/BRAF mutations within the same colorectal tumor (Parsons *et al*., 2005; Velho *et al*., 2005).

Two prior studies failed to observe a link between PIK3CA mutation status and cetuximab response in patients with colon cancer (Moroni *et al*., 2005; Lievre *et al*., 2006). However, in both of these

studies, PTEN mutation status of the tumors was not examined and very few patients with PIK3CA mutations, 3 of 31 (Moroni *et al*., 2005) and 2 of 30 (Lievre *et al*., 2006), were identified.

Perrone *et al*. (2009) reported that K-RAS mutations and PI3KCA/PTEN deregulation significantly correlate with resistance to cetuximab. In line with this, patients carrying K-ras mutations or with activated PI3K profiles can benefit from targeted treatments only by switching off molecules belonging to the downstream signaling of activated EGFR, such as mammalian target of rapamycin.

Analysis of different factors affecting outcome in current study revealed that K-ras status had statistical significant effect on progression-free survival but not on overall survival. Median progression-free and overall survival for cetuximab arm was 2 and 5 months respectively that matched the results of Saltz *et al.* (2004) as they constituted 1.4 and 6.4 months, respectively.

Roock *et al*. (2008) reported that progression-free and overall survival was statistically higher in wild K-ras patients than mutant K-ras cases and median overall survival was significantly higher in K-ras wild type versus mutants (43 versus 27.3 weeks) that coincides with our results even though median overall survival in current study was statistically insignificant. The higher incidence of survivals in (cetuximab-sorafenib) group than monotherapy cetuximab arm could be attributed to extended target therapy towards B-raf as beneficial effect with addition of sorafenib.

Loupakis *et al*. (2009) reported that 36% of patients with PTEN-positive tumors were responders compared with 5% of PTEN-negative tumors ($p = 0.007$), while the median progression-free survival was 4.7 months and 3.3 months, respectively ($p = 0.005$). Patients with PTEN-positive metastases and K-ras wild type had longer progression-free survival compared with other patients.

Reversible skin toxicity (acneiform rash and allergic reactions), asthenia and hypomagnesmia were the most common adverse events in both groups that coincides with Saltz *et al*. (2004). Hand-foot syndrome (palmar-plantar erythrodysesthesia) occurred in 1/3 cases of (EN) group that matched the results of Ratain *et al*. (2006). The tolerability of cetuximab makes it an attractive option for combination with other cancer treatments. Sorafenib, unlike other tyrosine kinase inhibitors, had no severe toxic effects on heart or thyroid gland that matched Escudier *et al*. (2007), who reported that serious adverse events including cardiac ischemia, infarction or hypertension occurred in 2% of cases.

Several studies show that efficacy of EGFR-targeted antibodies is confined to patients with wild type K-ras and genotyping of tumors should be considered before treatment. The absence of K-ras mutations does not guarantee an improved likelihood of response to EGFR-targeted antibodies. Investigation of other genetic and epigenetic biomarkers will be useful to further refine the responder population (Silvestris *et al*., 2009). Prospective studies to test the efficacy of combined therapies simultaneously targeting EGFR and the RAS/RAF/MAPK signaling pathways for metastatic colorectal cancer are needed.

Present study showed that addition of sorafenib to cetuximab had higher response rate and insignificant increase in overall survival compared to monotherapy cetuximab group. K-ras mutation had significant effect on response and progression-free survival. Ligand binding to EGFR results in signal transduction via the Ras/Raf/MEK/MAPK and the PI3K/AKT pathway. It was hypothesized that colon cancer cell lines with constitutively activated signaling downstream of EGFR would not be dependent on ligand binding to EGFR for their growth and, in turn, would be refractory to cetuximab. A prior screening of colon tumors for PTEN expression status and PIK3CA and Ras/B-raf mutation status could help stratify patients likely to benefit from this therapy.

CONCLUSION

The study reflects that mutation status of molecular markers such as K-ras and B-raf is a predictor of response, so genotyping of tumors is needed for defining the patient population that is likely to benefit from targeted therapy. Combination of therapy that simultaneously targets K-ras and B-raf and other signaling pathways could be a useful approach to increase number of patients who may benefit from anti- EGFR therapy, however, large scale prospective randomized trials are needed to properly determine which patients are best candidates for these targeted agents.

REFERENCES

Amado, R.G., M. Wolf, M. Peeters, E.V. Cutsem and S. Siena *et al*., 2008. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J. Clin. Oncol., 26: 1626-34.

Benvenuti, S., A.S. Bianchi, F.D. Nicolantonio, C. Zanon and M. Moroni *et al*., 2007. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res., 67: 2643-2648.

Cappuzzo, F., M.V. Garcia, G. Finocchiaro, M. Skokan and S. Gajapathy *et al*., 2008. Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. Br. J. Cancer, 99: 83-99.

Ciardiello, F. and G. Tortora, 2001. A novel approach in the treatment of cancer targeting the epidermal growth factor receptor. Clin. Cancer Res., 7: 2958-2970.

Cunningham, D., Y. Humblet, S. Siena, D. Khayat and H. Bleiberg *et al*., 2004. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N. Engl. J. Med., 351: 337-45.

Di Nicolantonio, F., M. Martini, F. Molinari, A. Sartore-Bianchi and F. Arena *et al*., 2008. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J. Clin. Oncol., 264: 5705-5712.

Egloff, A.M. and J.R. Grandis, 2009. Improving response rates to EGFR-targeted therapies for head and neck squamous cell carcinoma candidate predictive biomarkers and combination treatment with src inhibitors. J. Oncol., 2009: 12-12.

Escudier, B., T. Eisen, W.M. Stadler, C. Szczylik and S. Oudard *et al*., 2007. Sorafenib in advanced clear-cell renal-cell carcinomas. N. Engl. J. Med., 356: 125-34.

Fan, Z., H. Masui, I. Altas and J. Mendelsohn, 1993. Blockade of epidermal growth factor receptor function by bivalent and monovalent fragments of 225 anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res., 53: 4322-4328.

Frattini, M., P. Saletti, E. Romagnani, V. Martin and F. Molinari *et al*., 2007. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br. J. Cancer, 97: 1139-1145.

Gollob, J.A., S. Wilhelm, C. Carter and S.L. Kelley, 2006. Role of raf kinase in cancer therapeutic potential of targeting the Raf/MEK/ERK signal transduction pathway. Semin. Oncol., 33: 392-406.

Jhawer, M., S. Goel, A.J. Wilson, C. Montagna and Y.H. Ling *et al*., 2008. PIK3CA mutation/pten expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer Res., 68: 1953-1953.

Ji, H., D. Li, L. Chen, T. Shimamura and S. Kobayashi *et al*., 2006. The impact of human EGFR kinase domain mutations on lung tumorigenesis and *in vivo* sensitivity to EGFR-targeted therapies. Cancer Cell., 9: 485-95.

Jimeno, A., W.A. Messersmith, F.R. Hirsch, W.A. Franklin and S.G. Eckhardt, 2009. KRAS mutations and susceptibility to cetuximab and panitumumab in colorectal cancer. Cancer J., 15: 110-113.

Kaplan, E.L. and P. Meier, 1958. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc., 53: 457-481.

Karapetis, C.S., S.K. Ford, D.J. Jonker, C.J.O. Callaghan and D. Tu *et al*., 2008. K-ras mutations and benefit from cetuximab in Advanced colorectal cancer. NEJM, 359: 1757-1765.

Klapper, L.N., M.H. Kirschbaum, M. Sela and Y. Yarden, 2000. Biochemical and clinical implications of the ErbB HER signaling network of growth factor receptors. Adv. Cancer Res., 77: 25-79.

Kupsch, P., B.E. Henning, K. Passarge, H. Richly and K. Wiesemann *et al*., 2005. Results of a phase I trial of sorafenib (BAY 43-9006) in combination with oxaliplatin in patients with refractory solid tumors including colorectal cancer. Clin. Colorectal Cancer, 5: 188-196.

Lenz, H.J., E.V. Cutsem, S. K. Ford, J.R. Mayer and G. Ph *et al*., 2006. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan oxaliplatin and fluoropyrimidines. J. Clin. Oncol., 24: 4914-21.

Lievre, A., J.B. Bachet, D.L. Corre, V. Boige and B. Landi *et al*., 2006. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res., 66: 3992-95.

Lopez-Crapez, E., T. Livache, P. Caillat and D. Zsoldos, 2004. Detection of K-ras mutations by a microelectronic DNA chip. Meth. Mol. Med., 97: 337-354.

Loupakis, F., L. Pollina, I. Stasi, A. Ruzzo and M. Scartozzi *et al*., 2009. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J. Clin. Oncol., 27: 2622-9.

Messa, C., F. Russo, M.G. Caruso and A.D. Leo, 1998. EGF TGF-alpha and EGF-R in human colorectal adenocarcinoma. Acta Oncol., 37: 285-289.

Moroni, M., S. Veronese, S. Benvenuti, G. Marrapese and A.S. Bianchi *et al*., 2005. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to anti-EGFR treatment in colorectal cancer a cohort study. Lancet Oncol., 6: 279-86.

National Cancer Institute, Cancer Therapy Evaluation Program, 2003. Common terminology criteria for adverse events v3.0 (CTCAE). http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Parsons, D.W., T.L. Wang, Y. Samuels and A. Bardelli *et al*., 2005. Colorectal cancer mutations in a signaling pathway. Nature, 436: 792-792.

Perrone, F., A. Lampis, M. Orsenigo, M.D. Bartolomeo and A. Gevorgyan *et al*., 2009. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. Ann. Oncol., 20: 84-90.

Peto, R. and J. Peto, 1972. Asymptotically efficient invariant test procedures. J. R. Stat. Soc. A., 135: 185-185.

Porebska, I., A. Harlozinska and T. Bojarowski, 2000. Expression of the tyrosine kinase activity growth factor receptors (EGFR, ERB B2, ERB B3) in colorectal adenocarcinomas and adenomas. Tumour. Biol., 21: 105-115.

Ratain, M.J., T. Eisen, W.M. Stadler, K.T. Flahert and S.B. Kaye *et al*., 2006. Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. J. Clin. Oncol., 24: 2505-12.

Roock, W.D., H. Piessevaux, J.D. Schutter, M. Janssens and G.D. Hertogh *et al*., 2008. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann. Oncol., 19: 508-15.

Salomon, D.S., R. Brandt, F. Ciardiello and N. Normanno, 1995. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit. Rev. Oncol. Hematol., 19: 183-232.

Saltz, L.B., N.J. Meropol, P.J. Sr. Loehrer, M.N. Needle, J. Kopit and R.J. Mayer, 2004. Phase II trial of cetuximab in patients with refractory Colorectal cancer that expresses the epidermal growth factor receptor. J. Clin. Oncol., 22: 1201-1208.

Sartore-Bianchi, A., M. Martini, F. Molinari, S. Veronese and M. Nichelatti *et al*., 2009. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res., 69: 1851-1857.

Silvestris, N., T. Stefania, S. Daniele, R. Antonio and S. Gianni *et al*., 2009. KRAS mutations and sensitivity to anti-EGFR monoclonal antibodies in metastatic colorectal carcinoma an open issue. Expert Opin. Biol. Ther., 9: 565-577.

Sridhar, S.S., D. Hedley and L.L. Siu, 2005. Raf kinase as a target for anticancer therapeutics. Mol. Cancer Ther., 4: 677-685.

Therasse, P., S.G. Arbuck, E.A. Eisenhauer, J. Wanders and R.S. Kaplan *et al*., 2000. New guidelines to evaluate the response to treatment in solid tumors European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J. Natl. Cancer Inst., 92: 205-216.

Velho, S., C. Oliveira, A. Ferreira, A.C. Ferreira and G. Suriano *et al*., 2005. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur. J. Cancer, 41: 1649-1654.

Wilhelm, S.M., C. Carter, L. Tang, D. Wilkie and A. McNabola *et al*., 2004. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res., 64: 7099-109.

Wilhelm, S., C. Carter, M. Lynch, T. Lowinger and J. Dumas *et al*., 2006. Discovery and development of sorafenib a multikinase inhibitor for treating cancer. Nat. Rev. Drug Discov., 5: 835-844.