의학석사 학위논문

EGFR 증폭이 있는 직결장암 환자들의 임상적 특징 및 치료 패턴 연구

Clinical characteristics of and treatment pattern for EGFR-amplified colorectal cancer

울산대학교 대학원

의 학 과

김 성 은

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이 논문을 의학석사 학위 논문으로 제출함

2024 년 2 월

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국문요약

연구배경: EGFR 증폭은 전이성 대장암 환자 중 1-8%에서 보고되었지만 그 예후 및 예측에 대한 영향은 아직 충분히 다뤄지지 않았다. 최근 EGFR 증폭이 있는 환자의 임상 및 유전자 특성과 예후의 개선이 연관이 있다는 것을 보고한 연구가 있었다.

연구 방법: 2016 년 1 월부터 2021 년 12 월까지 서울아산병원에서 SureDesign 을 통해 설계된 244 개 유전자 패널(AMC Oncopanel)을 통해 차세대 염기서열 분석(NGS)을 받은 전이성 대장암 환자들을 의무기록에서 파악하였다. EGFR 복제수 변이를 CNVkit 로 처리하여 적어도 5 개 이상의 EGFR 복제수 변이가 있는 환자를 선별하여, 해당 케이스를 변이 염기 비율에서 추론한 종양 순도에 따라 보정하였다. 보정된 복제수 변이가 6 이상인 환자들을 EGFR 증폭군(EGFR amp+)으로 정의하였으며 그들의 임상적 특성을 EGFR 증폭이 없는 환자군(EGFR amp-)과 비교하였다.

연구결과: 2,421 명의 환자 중 35 명(1.4%)이 EGFR amp+이었으며 복제수 변이의 중간값은 7, 범위는 6 에서 363 이었다. 그 중 33 명(94%)은 RAS 와 BRAF V600E 변이가 없었으며 2 명은 KRAS 변이가 있었다. 모든 35 명의 환자는 현미부수체 안정(microsatellite stable, MSS)이었고, 2,386 명 중 78 명(3.3%)은 현미부수체 불안정성(microsatellite instability, MSI)을 보였다. EGFR 증폭의 존재 여부에 따라 임상적 특성은 유의하게 다르지 않았으나 EGFR amp+의 경우 복막 전이 빈도가 상대적으로 낮았다(8.6% vs. 21.8%, p<0.001). 전체 생존율(overall survival)은 EGFR amp+에서(전체 생존율 중앙값(mOS) 76 개월, [95% 신뢰 구간(confidence interval, CI) = 21-131]) EGFR amp-보다 양호했으나(mOS 37 개월, [95% CI = 35-39]) 통계적으로 유의하지 않았다(p = 0.15). 질병 경과 중 항 EGFR 항체 기반 항암화학요법을 받은 572 명 환자 중에서 EGFR amp+ 환자 16 명은 mOS 79 개월로(95% CI=38-120)로 556 명의 EGFR amp- 환자(mOS 39 개월, [95% CI = 36-42])보다 더 길었다(p = 0.05, 위험비 = 2.07, [95% CI = 0.98-4.61]). 초치료로 항 EGFR 항체를 포함한 항암화학요법을 받은 환자들의 무진행 생존(PFS)을 비교하였을 때 EGFR amp+과 EGFR amp-에서 통계적으로 유의한 차이를 보이지 않았다(20 개월 vs 14 개월, p = 0.416).

연구결론: 전이성 대장암에서 EGFR 증폭은 RAS 또는 BRAF 변이가 없고 현미부수체 안정 상태 종양에서 풍부하게 나타났으며 복막 전이의 빈도가 낮았다. 항 EGFR 항체 기반 항암치료를 받은 환자에서 EGFR 증폭이 있는 환자의 유리한 예후를 시사하나, 초치료에서의 이득은 분명하지 않았다. EGFR 증폭이 폐암에서와는 다르게 적어도 항 EGFR 기반 치료에 대한 저항성을 보이지는 않는 것으로 생각된다.

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Introduction

Anti-EGFR antibody is an important agent in the treatment of metastatic colorectal cancer (mCRC). Research on biomarkers for predicting therapeutic response of anti-EGFR antibody have been carried out. While RAS and BRAF mutations have been known to be associated with the resistance to anti-EGFR antibodies, the therapeutic implication of EGFR amplification has not been known well. Various studies were conducted to decipher the biologic significance of EGFR amplification in mCRC, but its prognostic and predictive impact has not been elucidated well [1-3]. According to a recent international cohort study, EGFR amplification was observed in approximately 4% of mCRC, predominantly in left sided colon and rectal cancer harboring wild-type RAS and BRAF [4]. Overall survival was anticipated favorable among patients without EGFR amplification than those with EGFR amplification, but use of anti-EGFR antibody was not related to overall survival.

Indeed, EGFR amplification is known to be associated with resistance to anti-EGFR tyrosine kinase inhibitors (TKI) in lung cancer [5, 6]. Relatively little is known about its other clinical significance. Studies showed that patients with EGFR amplification was enriched in other genetic mutations compared to those without amplification, suggesting higher chances of developing resistance to conventional treatments by activating various signaling pathways from co-occurring mutations [1, 7, 8]. Therefore, it is considered that anti-EGFR antibody treatment alone may have limitations in EGFR amplified patients, and anti-EGFR antibody in combinations with anti-EGFR TKI, MEK inhibitor, immune check point inhibitor is expected to yield better outcomes. Thus, understanding clinical and pathologic features of patients with EGFR amplification will provide important foundation for further treatment options.

This study is aimed to compare clinicopathologic features and clinical outcome of mCRC based on the EGFR amplification status in a single center.

Methods

Study design and subject

As shown in Figure 1, a total of 2,421 patients with mCRC who underwent next-generation sequencing (NGS) through a targeted 244-gene panel (AMC Oncopanel, designed through SureDesign in Asan Medical Center) from January 2016 to December 2021 were identified from the electronic medical records.

EGFR copy numbers processed with CNVkit [9] were screened and the slides of the cases with at least 5 EGFR copies from the pipeline were reviewed for the tumor purity inferred from variant allelic fraction pattern, to adjust the copy number [2]. Patients whose adjusted copy number was 6 or greater were defined to be EGFR-amplified (EGFR amp+) and copy number less than 6 was defined as EGFR-nonamplified (EGFR amp-) (Figure 2).

Their clinicopathologic features including mutation profile, microsatellite instability (MSI) status, primary tumor location, age, sex, stage at diagnosis, metastatic sites and histologic type were collected.

This study was approved from the Institutional Review Board of Asan Medical Center, Seoul, Republic of Korea (IRB number: 2022-1355).

Figure 1. Patient selection process

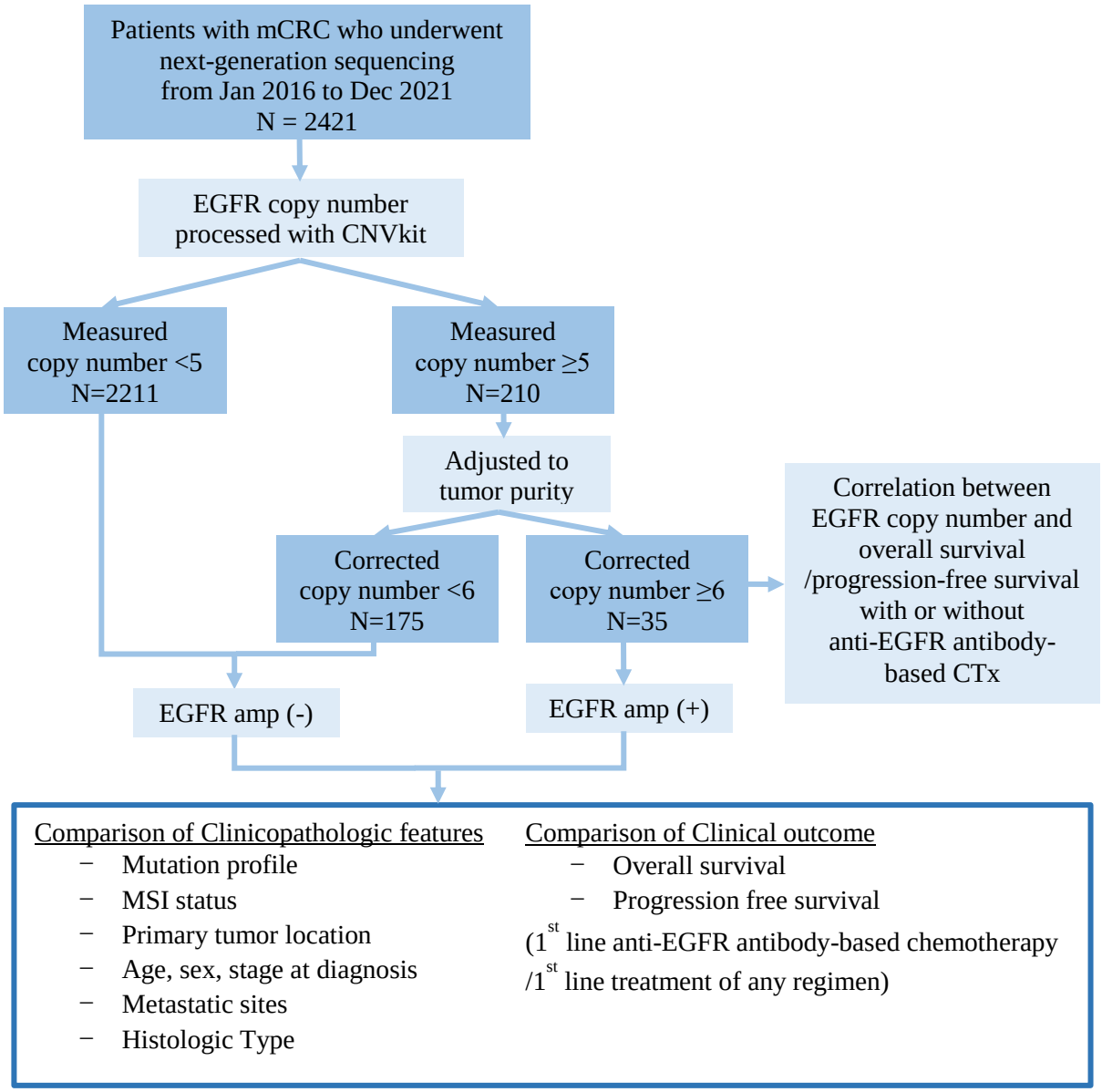
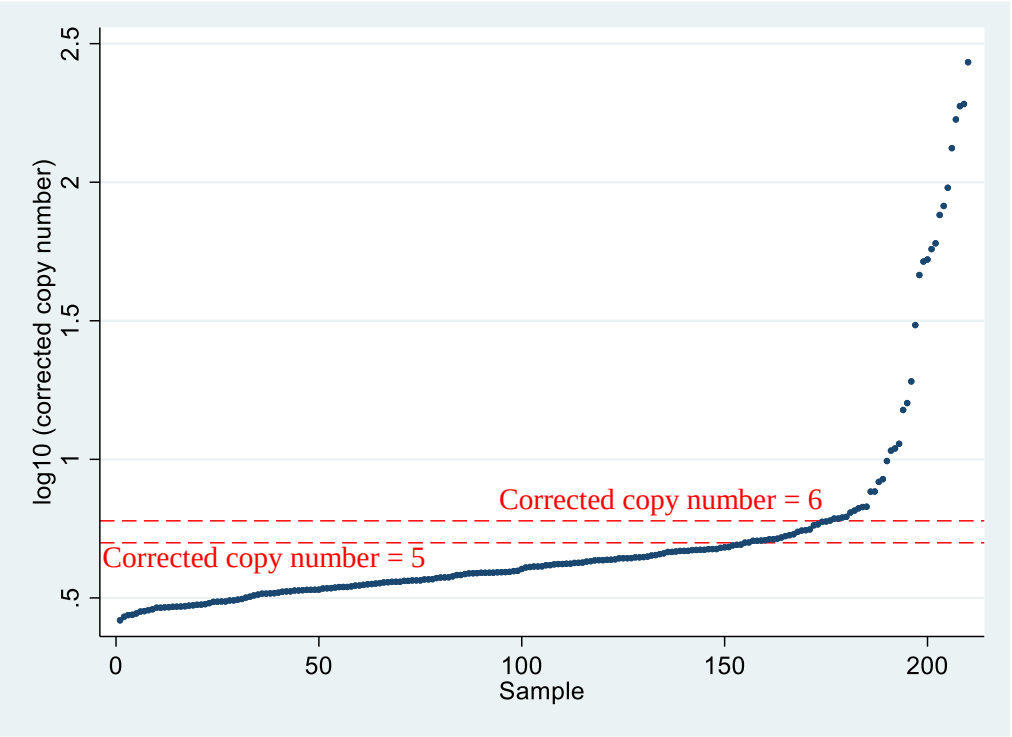


Figure 2. Ranked corrected EGFR copy number for cases with estimated copy number by CNV kit ≥5



Molecular analysis

Various genomic data other than EGFR amplification on NGS was also analyzed. KRAS/NRAS/BRAF mutations were identified in all subjects and patients were further divided into subgroups by the presence of the mutations. Microsatellite instability status was also identified in all patients according to the criteria which was previously published [10].

Statistical analysis

Clinicopathologic features among patients with or without EGFR amplification was estimated using chi-square test or Fisher's exact test in categorical variable and t-test or Mann Whitney test in continuous variable, as appropriate.

Overall survival (OS) was defined as the time from diagnosis of metastatic colorectal cancer until death or last follow-up of patient. Progression-free survival (PFS) was calculated as the time from the first day of treatment of a certain chemotherapy regimen until death, progression of disease evaluated by RECIST criteria or attending oncologist or last follow-up of patients. Both clinical outcome was assessed by means of the Kaplan-Meier method and Cox proportional hazards regression models were used to figure out covariates associated with OS or PFS.

All tests were 2-sided at alpha equals 5%. Statistical analyses were performed using SPSS 23.0.

Results

Clinicopathologic characteristics

As shown in Table 1, among 2,421 mCRC patients who underwent next-generation sequencing from January 2016 to December 2021, 210 had measured copy number ≥5 processed through CNVkit, and the adjustment to the exact cellularity was done for these patients. Their corrected copy number is shown in Figure 2, which shows most of them (n=175) had copy number less than 6, leaving 35 patients with EGFR amplification (copy number ≥6). Thus, the overall prevalence of EGFR amplification in this population was 1.4% (35/2,421). The correlation between measured and corrected EGFR copy number is shown in Figure 3, suggesting most patients had marginal values (less than 10) of copy number and corrected copy numbers were generally lower than measured ones. All EGFR amp+ cases were RAS/BRAF wild type, except for 2 patients with KRAS mutations. Among 2,386 EGFR amp- patients, 1,143 patients (48%) were RAS/BRAF wild type. EGFR amp+ was associated with a predilection for rectum compared to RAS, BRAF wild type, EGFR amp- and RAS or BRAF mutants, EGFR amp- (48.6% vs 42.3% vs 45.2%), respectively. All 35 EGFR amp+ patients were microsatellite-stable (MSS), while 78 out of 2,386 EGFR amp- patients (3.3%) showed microstatellite instability. EGFR amp+ patients tended to have fewer peritoneal seeding at presentation (8.6% vs 21.8%, $p<0.001$). The proportion of patients who received anti-EGFR agent throughout the course was 45.7% vs 23.3% respectively in EGFR amp+ and EGFR amp-. Among these cases, 7 EGFR amp+ patients received anti-EGFR agent as first line chemotherapy (20.0%), compared with 346 patients treated with anti-EGFR agent as first line chemotherapy among EGFR amp- (14.5%). 5-FU, folinic acid, and either oxaliplatin or irinotecan doublet were combined in almost all patients with first-line anti-EGFR agent (100% vs 99.7%).

Clinicogenomic characteristics

Co-alterations of EGFR amplified colorectal patients were analyzed and summarized into OncoPrint (Figure 4). APC mutation was the most common alteration found, followed by TP53 mutations. Sidedness of tumor was also described together, showing prominent left colon distribution.

Table 1. Patient characteristics

	EGFR amp+		EGFR amp-		P VALUE
	RAS, BRAF wt (n=33)	RAS or BRAF mt (n=2)	RAS, BRAF wt (n=1,143)	RAS or BRAF mt (n=1,243)	
Mean age, year (range)	57.61 (34-79)	51.50 (51-52)	56.81 (19-90)	58.10 (25-90)	0.027
Male sex, n (%)	22 (66.7%)	0	749 (65.5%)	685 (55.1%)	<0.001
Initial stage					0.171
I-III	13 (39.4%)	1 (50.0%)	317 (28.7%)	418 (32.6%)	
IV	20 (60.6%)	1 (50.0%)	775 (70.3%)	854 (66.6%)	
Histology					0.047
W/D or M/D	31 (93.9%)	2 (100%)	962 (84.2%)	1049 (84.4%)	
P/D, SRCC, mucinous	2 (6.1%)	0	218 (19.1%)	180 (14.5)	
Others	0	0	12 (1.0%)	14 (1.1%)	
Primary tumor site					<0.001
Right colon	6 (18.2%)	0	199 (17.4%)	348 (28.0%)	
Left colon	10 (30.3%)	2 (100%)	423 (37.0%)	317 (25.5%)	
Rectum	17 (51.5%)	0	484 (42.3%)	562 (45.2%)	
≥2		0	30 (2.6%)	11 (0.9%)	
Metastatic sites					<0.001
Liver	22 (62.9%)	0	716 (62.6%)	673 (54.1%)	
Lung	8 (22.9%)	0	257 (22.5%)	480 (38.6%)	
Peritoneum	3 (8.6%)	0	221 (19.3%)	299 (24.1%)	
No. of metastatic sites					0.569
1	15 (45.5%)	1 (50.0%)	605 (52.9%)	645 (51.9%)	
> 1	18 (54.5%)	1 (50.0%)	520 (45.5%)	582 (46.8%)	
MMR status					0.649
dMMR	0	0	40 (3.5%)	38 (3.1%)	

pMMR	33 (100%)	2 (100%)	1095 (95.8%)	1200(96.5%)	
Unknown	0	0	8 (0.7%)	5 (0.4%)	
Primary tumor resection					0.254
Yes	28 (84.8%)	2 (100%)	919 (80.4%)	972 (78.2%)	
No	5 (15.2%)	0	223 (19.5%)	271 (21.8%)	
Metastasectomy					<0.001
Yes	14 (42.4%)	1 (50.0%)	483 (42.3%)	424 (34.1%)	
No	19 (57.6%)	1 (50.0%)	660 (57.7%)	819 (65.9%)	
1st-line chemotherapy					<0.001
Bevacizumab+doublet	16 (48.5%)	2 (100%)	556 (48.4%)	942 (75.8%)	
Cetuximab+doublet	7 (21.2%)	0	331 (29.0%)	15 (1.3%)	
Doublet	4 (12.1%)	0	152 (13.3%)	152 (12.2%)	
Others	2 (6.1%)	0	23 (2.0%)	23 (1.6%)	
Anti-EGFR CTx throughout course					<0.001
Yes	16 (48.5%)	0	507 (44.4%)	49 (3.9%)	
No	13 (39.4%)	2 (100%)	556 (48.6%)	1089 (87.6%)	

* wt = Wild type, mt = Mutants / W/D = Well-differentiated, M/D =Moderately-differentiated, P/D = Poorly-differentiated, SRCC = Signet ring cell carcinoma / MMR = Mismatch repair, dMMR = deficient MMR, pMMR = proficient MMR / CTx = Chemotherapy

Figure 3. Measured vs. corrected copy number

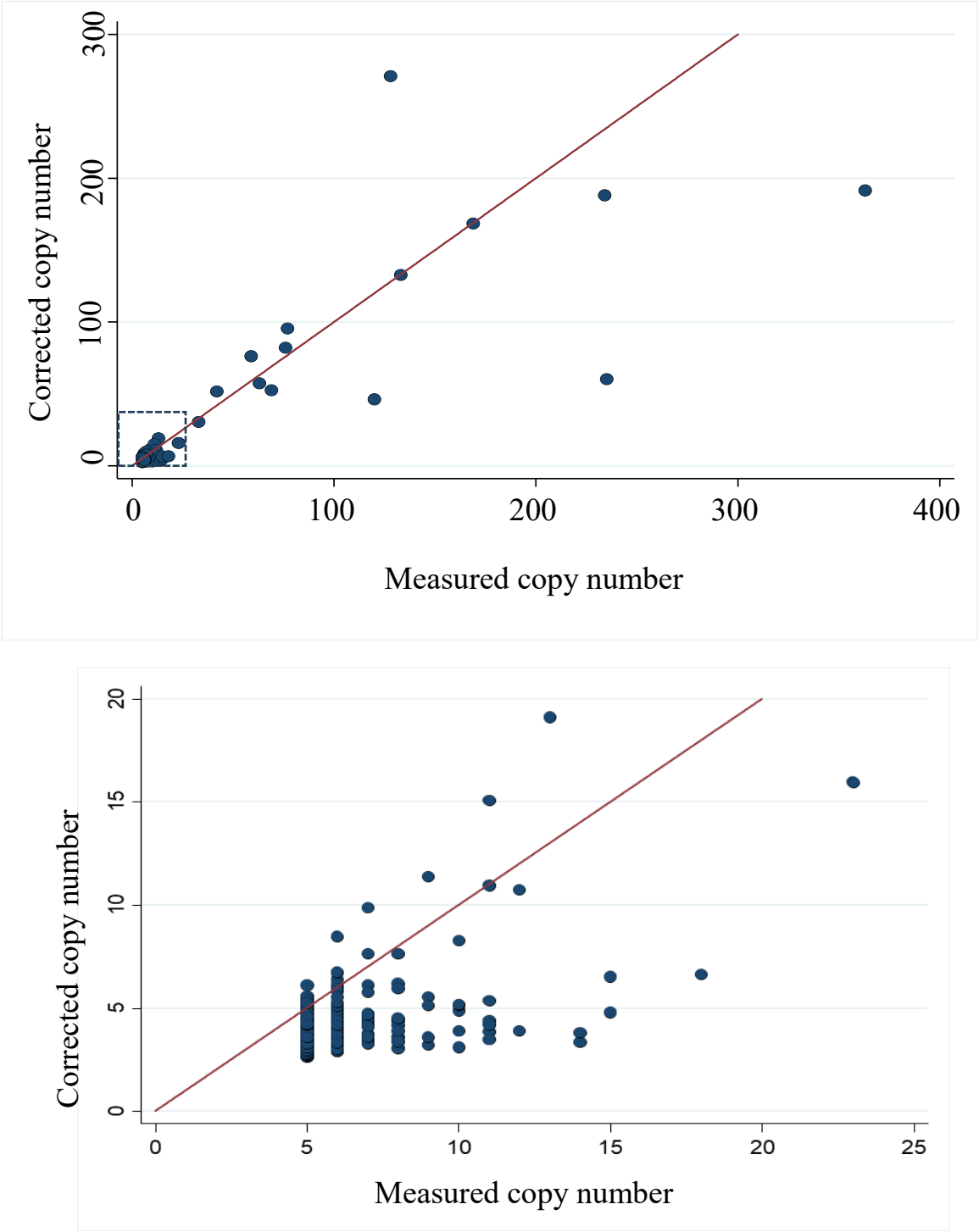
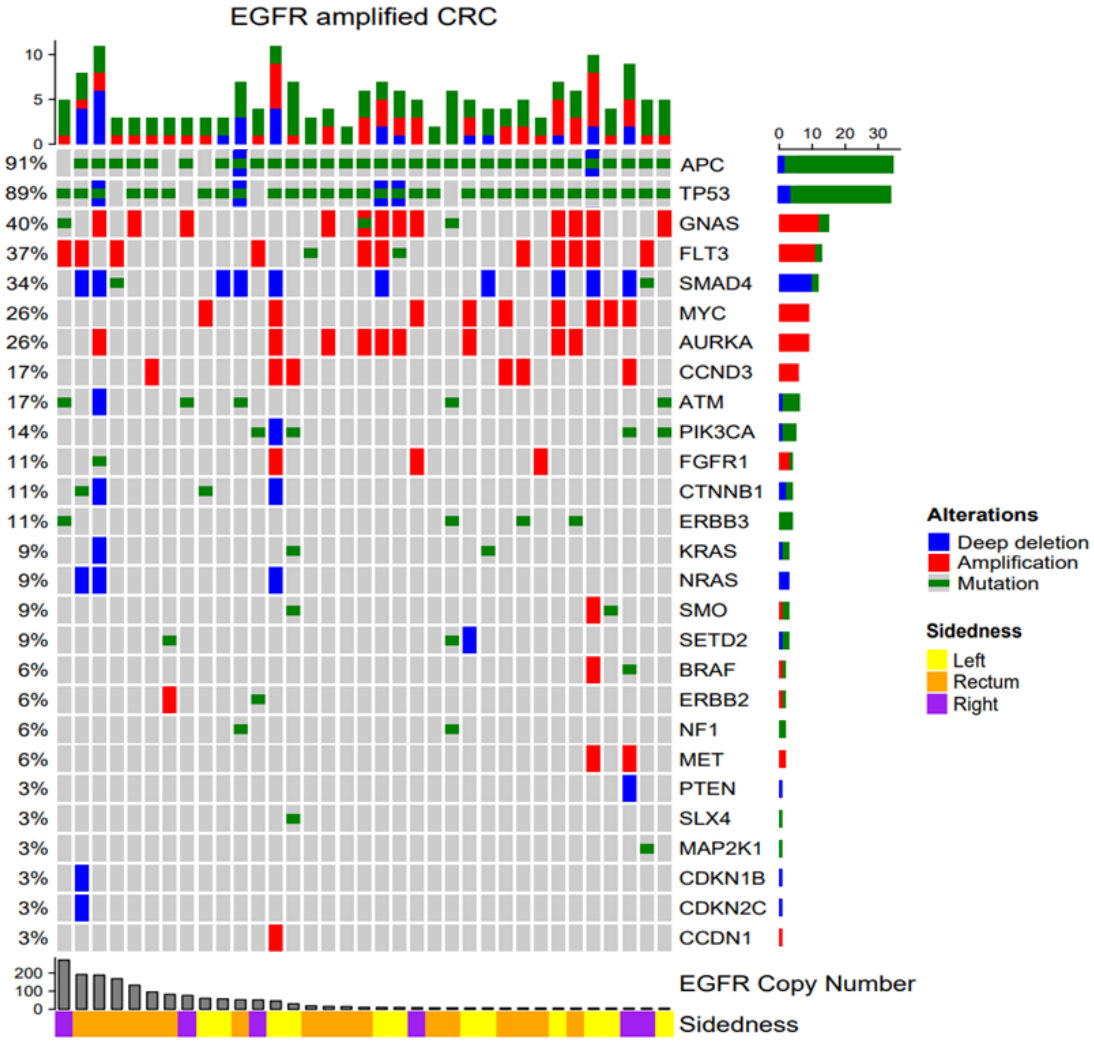


Figure 4. OncoPrint for EGFR amplified patients



Clinical outcome of EGFR amplification in metastatic colorectal cancer

Median follow-up was 22.0 months (range 1-207 months). Overall survival (OS) tended to be better with EGFR amp+ (median OS = 76 months, 95% confidence interval [CI] = 21-131) than EGFR amp- (median OS = 37 months, [95% CI = 35-39]) but the difference did not reach statistical significance (p = 0.15) (Figure 5). Among 572 patients with RAS/BRAF wild type tumors who received anti-EGFR antibody-based chemotherapy in their course of diseases, median OS was significantly better in 16 EGFR amp+ patients with 79 months (95% CI = 38-120) than 39 months (95% CI = 36-42) in 556 EGFR amp-patients (p=0.05, adjusted HR = 2.07, [95% CI = 0.98-4.61]) (Figure 6 , Table 2). When we adjusted the survival outcome according to EGFR amplification and other clinical factors including primary location of tumor, number of metastatic sites, stage at diagnosis, primary tumor resection and metastasectomy, EGFR amplification did not have an impact on survival outcomes in all patients and those who received anti-EGFR antibodies (Table 3). Only 7 out of 35 EGFR amp+ patients were given front-line anti-EGFR chemotherapy, and their progression-free survival (PFS) did not differ from the PFS of EGFR amp- patients treated with first-line anti-EGFR chemotherapy (median PFS 20 vs. 14 months, p = 0.416) (Figure 7).

Figure 5. Comparison of overall survival between groups with EGFR amplification and without EGFR amplification.

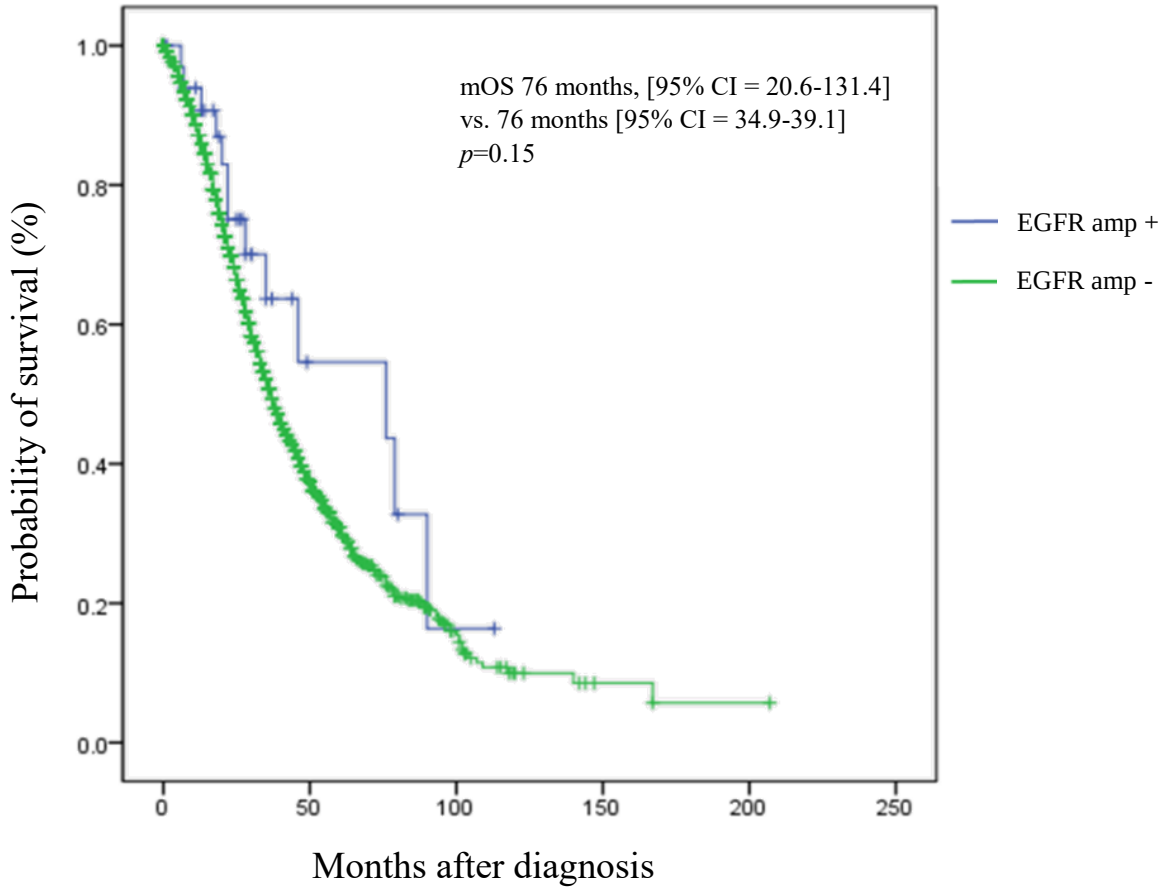


Figure 6. Comparison of overall survival between groups with EGFR amplification and without EGFR amplification among patients who received anti-EGFR antibody-based chemotherapy throughout disease course

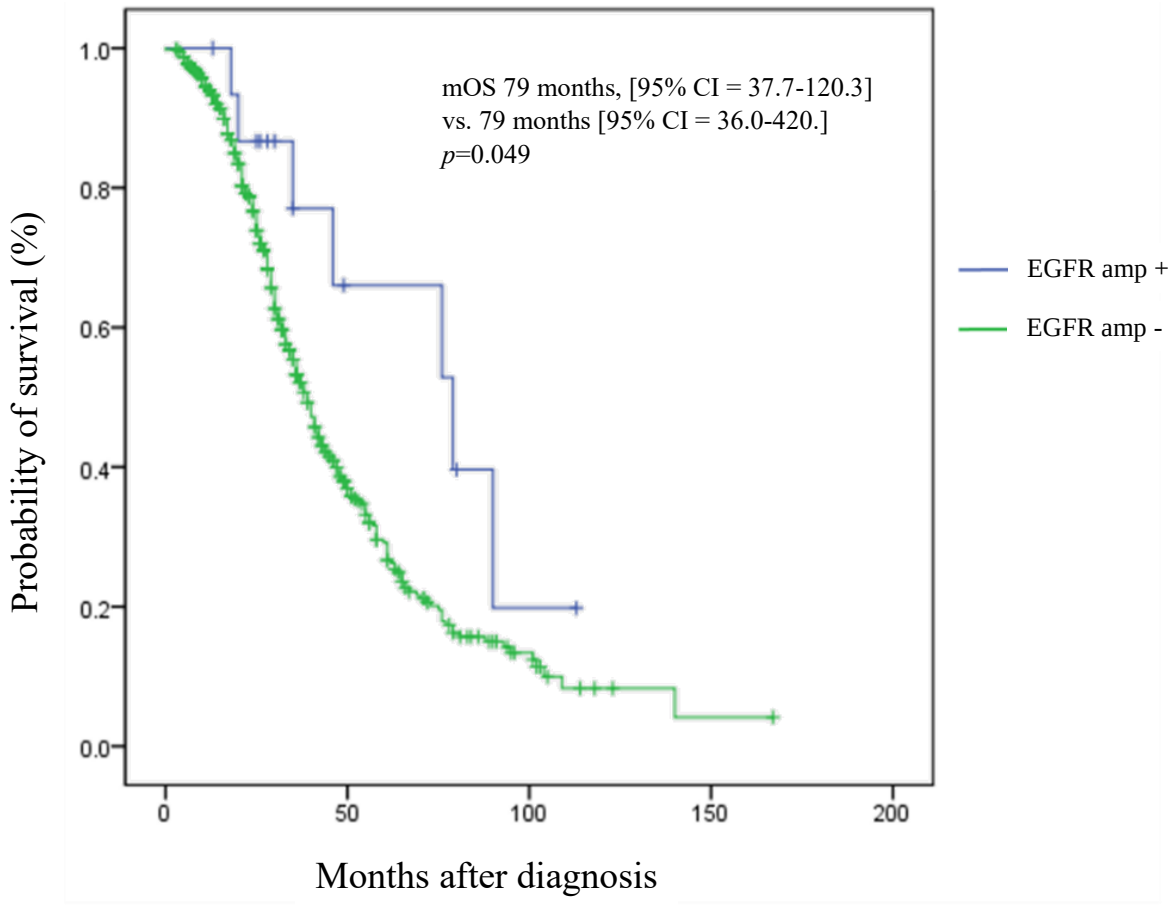


Figure 7. Comparison of progression-free survival between groups with EGFR amplification and without EGFR amplification among patients who received anti-EGFR antibody-based chemotherapy as 1st line chemotherapy

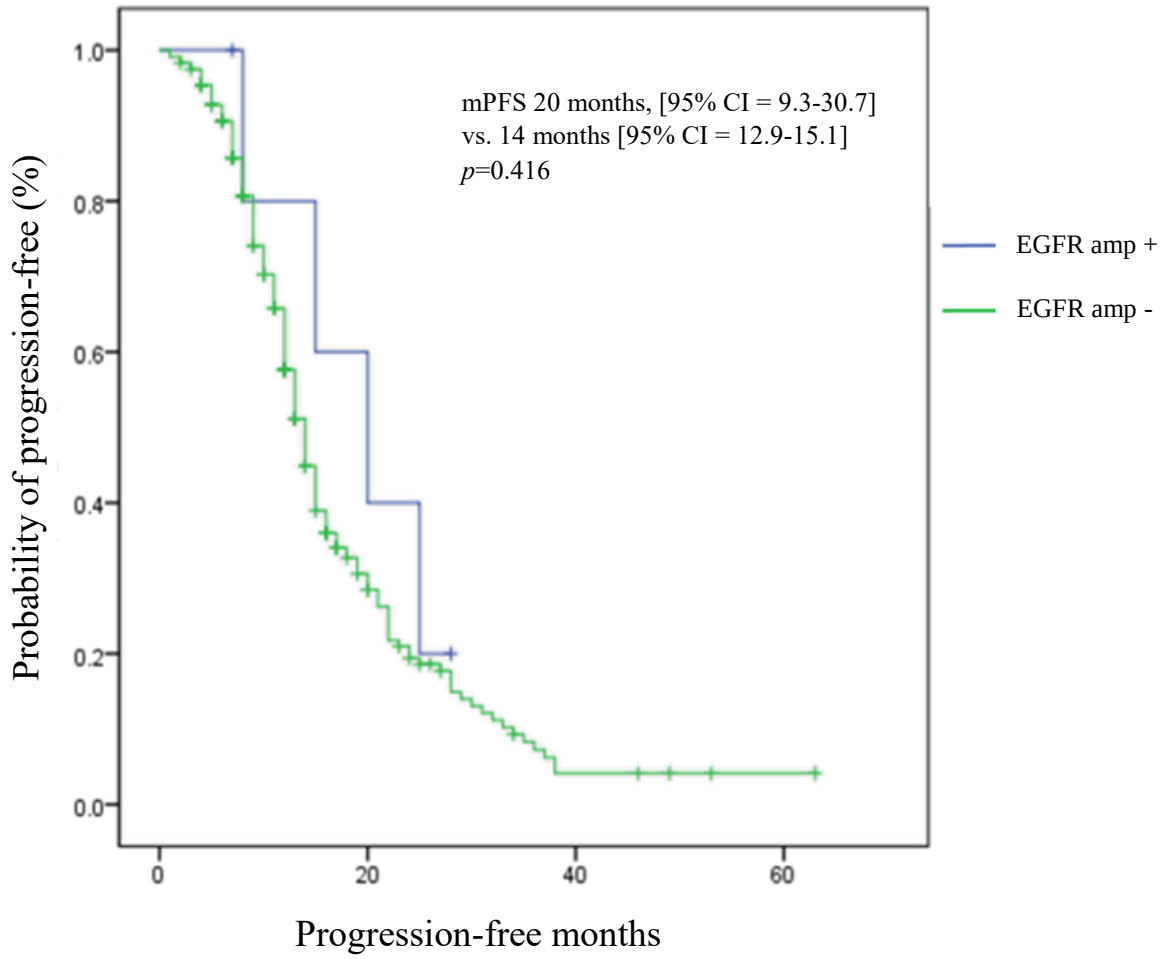


Table 2. Cox regression analysis for overall survival among patients who have received anti-EGFR antibody-based chemotherapy throughout disease course (Univariate analysis)

	HR	95% CI	P VALUE
EGFR amp			
+	ref		
-	2.074	0.979-4.614	0.05
Sidedness			
Right	ref		
Left & rectum	0.595	0.440-0.806	<0.001
No. of metastatic sites			
1	ref		
>1	1.743	1.397-2.174	<0.001
RAS/RAF mutation			
Wild-type	ref		
Mutant	2.046	1.477-2.836	<0.001
Stage at diagnosis			
I-III	ref		
IV	1.241	0.957-1.608	0.10
Primary tumor resection			
Yes	ref		
No	3.015	2.324-3.911	<0.001
Metastasectomy			
Yes	Ref		
No	3.193	2.501-4.076	<0.001

Table 3. Cox regression analysis for overall survival among patients who have received anti-EGFR antibody-based chemotherapy throughout disease course (Multivariate analysis)

	HR	95% CI	P VALUE
EGFR amp			
+	ref		
-	1.846	0.864-3.920	0.114
Sidedness			
Right	ref		
Left & rectum	0.733	0.535-1.005	0.054
No. of metastatic sites			
1	ref		
>1	1.433	1.136-1.808	<0.001
RAS/RAF mutation			
Wild-type	ref		
Mutant	2.004	1.441-2.786	<0.001
Stage at diagnosis			
I-III	Ref		
IV	1.179	0.890-1.561	0.250
Primary tumor resection			
Yes	ref		
No	1.951	1.458-2.824	<0.001
Metastasectomy			
Yes	Ref		
No	2.437	1.849-3.214	<0.001

Discussion

In this study, we showed the clinical features and significance of EGFR amplified mCRC. EGFR amplification was more common in microsatellite-stable tumors with RAS/BRAF wild type gene and was associated with infrequent peritoneal seeding. The prognostic impact of EGFR amplification was noticeable in anti-EGFR chemotherapy-treated patients, but the overall survival and progression-free survival was not statistically significant in this study.

There is no consensus on the appropriate EGFR amplification cut-off value. In another study conducted by Italian institutions, EGFR gene copy numbers, determined by FISH assay, was linked to the response to cetuximab therapy with the cut-off value of 2.92 [11]. In this analysis, we adopted the cut-off value of copy number at least 6 from a multinational cohort study published in 2021 [4]. EGFR copy number estimated from NGS results is generally considered inaccurate, because it is largely affected by tumor cellularity. To address this limitation, we screened EGFR copy numbers processed using a CNV kit and identified 210 cases with copy numbers of 5 or higher. After adjusting for tumor cellularity, only 27% (56/210) of cases had EGFR copy numbers with 5 or higher, as shown in Figure 2. This implies that copy number extracted from NGS pipeline was generally overestimated and correction by the exact tumor cellularity should be performed, particularly when copy number is at borderline around 5 to 6.

Previous studies on EGFR amplification have reported prevalence raging from approximately 1 to 8% [4, 12, 13]. In this study, the prevalence of EGFR amplification was 1.4%, using the aforementioned cut-off value. One study that examined genomic landscape of EGFR amplification using NGS data from Guardant Health reported a prevalence of 16.3% in colorectal cancer using CtDNA [1]. The higher prevalence in this study compared to others can be attributed to the use of a lower cut off-value, with a median amplification level of 2.55. As the frequency of co-occurring alterations vary with the level of EGFR amplification, it should be evaluated whether the proportions of co-alterations, particularly RAS mutations, increase when cut-off value is set at a lower level. This analysis showed improved overall survival with anti-EGFR agent chemotherapy and therefore, could justify the cut-off value of copy number 6. The multinational cohort, from which this study adopted the cut-off value, also showed longer overall survival among EGFR amplification although it did not influence the response to anti-EGFR agent therapy. Further investigation is needed to determine the optimal level of cut-off value of EGFR amplification, considering its correlation with diverse co-alterations, especially in hyper-amplified cases, which play a crucial role in developing resistance to anti-EGFR therapy.

The clinical features of EGFR amplified colorectal cancer in this study align with previous studies, demonstrating a left-side dominant distribution, rare peritoneal seeding, and mutually exclusive RAS/BRAF mutations [4, 14]. EGFR amplified patients from this data exhibit diverse co-alterations other than RAS/BRAF mutations, with APC mutations being the most common, followed by TP53 mutations. This may suggest that EGFR amplified colorectal cancer might be classified as an extreme type of consensus molecular subtype 2 (CMS2) of colorectal cancer, which is also prominent in left colon cancer [15]. The CMS 2 subtype is characterized by higher somatic copy number alteration, enriched copy number gains of oncogenes and losses in tumor suppressor genes, along with upregulated MYC downstream signaling and frequent APC mutations. The predictive and prognostic value of CMS subtypes was assessed for patients participated in a phase III trial comparing cetuximab with bevacizumab (CALGB/SWOG 80405). It showed CMS 2 subtype benefited the most from cetuximab, suggesting the need to evaluate EGFR amplified colorectal cancer in relation to CMS 2 subtype and their reliability as prognostic markers [16].

Previous studies on the benefits of anti-EGFR agents in EGFR amplified patients have produced varying results. A phase II trial did not find correlation between EGFR copy number and the efficacy of anti-EGFR agents [3], while a single center retrospective study revealed potential to responsiveness to cetuximab only in EGFR-negative tumors [17]. Although not reaching statistical significance, this study estimated improved overall survival among EGFR amplified patients. Anti-EGFR agent treated EGFR amplified patients were also associated with lower hazard ratio. Our results suggest that EGFR amplified colorectal cancer is a subtype that can benefit from anti-EGFR agents. This finding is consistent with a meta-analysis of 19 studies regarding predictive value of EGFR gene copy number for anti-EGFR monoclonal antibody treatments, where EGFR amplification was associated with improved outcomes [18]. One possible explanation for the result is that EGFR amplification is related to high reactive oxygen species level, leading to increased chemosensitivity [19]. It is suggested that EGFR signaling is abundant in distal colon cancer, which might lead to better expectations for EGFR targeted treatment [14]. However, little is specifically proved or found regarding the efficacy of therapy and further investigation is needed.

Recently, the possible activity of bevacizumab plus erlotinib combination has been proposed, as resistance to EGFR blockade might be attributed to VEGF, which shares a common downstream pathway with EGFR [20-22]. Trials in non-small cell lung cancer have displayed improved progression-free survival in combination therapy compared to erlotinib alone. Preliminary data from the GERCOR

DREAM trial in colorectal cancer suggested that combination of bevacizumab and erlotinib as maintenance therapy might be superior in terms of progression-free survival and overall survival [23, 24]. Notably, the anti-tumor activity of erlotinib did not depend on KRAS mutation in this trial, unlike other anti-EGFR agents like cetuximab or panitumumab. However, the same combination in lung cancer showed significantly better outcomes in cases with L858R mutation [25], suggesting that anticipations for maximal efficacy in EGFR signaling in colorectal cancer and EGFR amplified patients could be made.

The strength of this study is that despite its low prevalence, we gathered over two thousand cases of EGFR amplification. However, this study has several limitations. First, this study is a retrospective study based on medical record in a single center, leading to some inevitable information loss. Selection bias may also exist due to the characteristics of our center and the severity of cases it handles. Nevertheless, the inclusion of over two thousand patients with diverse features helps mitigate this bias. Second, long term data was often not available due to some patients being lost to follow-up when they were referred to local centers for later-line treatments and supportive care. This could have influenced overall survival and progression-free survival, had long term data been more available.

Conclusion

In conclusion, this single-center analysis elucidates the clinical features and significance of EGFR amplified metastatic colorectal cancer, implying that unlike lung cancer, at least EGFR amplification dose not confer resistance to anti-EGFR antibody in metastatic colorectal cancer, and further suggesting its favorable prognostic impact in patients treated with anti-EGFR chemotherapy.

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Abstract

Background: EGFR amplification has been reported in 1-8% of metastatic colorectal cancer (mCRC) patients, but its prognostic and predictive value has not been addressed well so far. Recently better prognosis with favorable clinicogenomic features was known to be associated with EGFR amplification.

Methods: Patients with mCRC who underwent next-generation sequencing through a targeted 244-gene panel (AMC OncoPanel, designed through SureDesign in Asan Medical Center) from January 2016 to December 2021 were identified from the electronic medical records. Their EGFR copy numbers processed with CNVkit were screened and the cases with at least 5 EGFR copies were reviewed to adjust to corrected tumor purity that was inferred from variant allelic fraction pattern. Patients whose adjusted copy number ≥6 were defined to be EGFR-amplified (EGFR amp+) and their clinical characteristics were compared with those without EGFR amplification (EGFR amp-).

Results: Among 2,421 patients, 35 patients (1.4%) were EGFR amp+ (the median of copy number = 7, range 6 - 363). 33 (94%) were RAS and BRAF V600E wild-type (wt), while 2 had KRAS mutations. All 35 patients were microsatellite-stable (MSS), while 78 out of 2,386 EGFR amp- (3.3%) showed microsatellite instability. Clinical characteristics were not significantly different according to the presence of EGFR amplification, but EGFR amp+ tended to have fewer peritoneal seeding at presentation (8.6% v. 21.8%, $p<0.001$). Overall survival (OS) tended to be better with EGFR amp+ (median OS 76 (mOS) months, 95% confidence interval (CI) = 21-131]) than EGFR amp- (mOS 37 months, [95% CI = 35-39]) but the difference did not reach statistical significance ($p = 0.15$). Among 572 patients who received anti-EGFR antibody-based chemotherapy (anti-EGFR CTx) in their course of diseases, mOS was better in 16 EGFR amp+ patients with 79 months (95% CI = 38-120) than 39 months (95% CI = 36-42) in 556 EGFR amp- patients ($p=0.05$, adjusted hazard ratio (HR) = 2.07, [95% CI = 0.98-4.61]). Only 7 out of 35 EGFR amp+ patients were given front-line anti-EGFR CTx, and their progression-free survival (PFS) did not differ from the PFS of EGFR amp- treated with front-line anti-EGFR CTx (20 vs 14 months, $p = 0.416$).

Conclusion: EGFR amp+ in mCRC was enriched in RAS/BRAFwt MSS tumors and was associated with infrequent peritoneal seeding. The favorable prognostic impact of EGFR amplification was suggested in anti-EGFR CTx-treated patients, but the benefit from front-line anti-EGFR antibody in this group was not notable in our study. At least EGFR amplification does not seem to confer resistance to anti-EGFR antibody in metastatic colorectal cancer.