Table 1 Patient characteristics according to *BRAF* or *KRAS* status *n* (%)

Characteristics	KRAS status			BRAF status		
	Wild-type *n* = 500	Mutant *n* = 312	*P* value	Wild-type *n* = 771	Mutant *n* = 40	*P* value
Age (yr)			0.11			0.40
mean ± SD	63.5 ± 10.3	64.7 ± 10.3		63.9 ± 10.3	65.4 ± 11.6	
Gender			0.02			0.006
Male	308 (62)	166 (53)		459 (60)	15 (38)	
Female	192 (38)	146 (47)		312 (40)	25 (63)	
Tumor location			0.37			< 0.001
Proximal	134 (27)	98 (31)		201 (26)	31 (78)	
Distal	213 (43)	125 (40)		332 (43)	5 (13)	
Rectum	153 (31)	89 (29)		238 (31)	4 (10)	
Histological grade			0.24			< 0.001
Well/moderate	472 (94)	288 (92)		728 (94)	31 (78)	
Poor/mucinous	28 (6)	24 (8)		43 (6)	9 (23)	
T stage			0.12			0.89
1	52 (10)	31 (10)		79 (10)	4 (10)	
2	106 (21)	46 (15)		145 (19)	7 (18)	
3	286 (57)	200 (64)		462 (60)	23 (58)	
4	56 (11)	35 (11)		85 (11)	6 (15)	
LN metastasis			0.18			0.96
Yes	180 (36)	127 (41)		292 (38)	15 (38)	
No	320 (64)	185 (59)		479 (62)	25 (63)	
TNM stage			0.09			0.92
I	125 (25)	58 (19)		173 (22)	10 (25)	
II	195 (39)	127 (41)		306 (40)	15 (38)	
III	180 (36)	127 (41)		292 (38)	15 (38)	
Adjuvant chemotherapy			0.44			0.57
Yes	217 (43)	144 (46)		344 (45)	16 (40)	
No	283 (57)	168 (54)		427 (55)	24 (60)	
MSI status			0.33			< 0.001
MSS/MSI-L	455 (91)	290 (93)		728 (94)	16 (40)	
MSI-H	45 (9)	22 (7)		43 (6)	24 (60)	

SD: Standard deviation; LN: Lymph node; TNM: Tumor-Node-Metastasis; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

Table 2 Univariate prognostic analysis of disease-free survival and overall survival

Characteristics	Disease-free survival		Overall survival	
	HR	95%CI	HR	95%CI
Age (yr)				
< 65	1	Reference	1	Reference
≥ 65	1.73	1.35-2.28	2.21	1.64-2.98
Gender				
Female	1	Reference	1	Reference
Male	1.57	1.20-2.06	1.57	1.16-2.13
Tumor location				
Proximal	1	Reference	1	Reference
Distal	0.92	0.67-1.25	0.9	0.64-1.26
Rectum	1.17	0.85-1.62	0.97	0.67-1.40
Histological grade				
Well/moderate	1	Reference	1	Reference
Poor/mucinous	1.53	0.97-2.42	1.43	0.84-2.42
AJCC stage				
I	1	Reference	1	Reference
II	2.6	1.61-4.19	2.26	1.36-3.75
III	4.68	2.95-7.42	3.49	2.14-5.70
Adjuvant chemotherapy				
No	1	Reference	1	Reference
Yes	1.24	0.96-1.60	1.29	1.10-1.51
MSI				
MSS/MSI-L	1	Reference	1	Reference
MSI-H	0.71	0.42-1.20	0.92	0.54-1.59
KRAS				
Wild-type	1	Reference	1	Reference
Mutant	1.35	1.04-1.74	1.44	1.08-1.92
BRAF				
Wild-type	1	Reference	1	Reference
Mutant	1.38	0.82-2.32	1.57	0.90-2.76

HR: Hazard ratio; CI: Confidence interval; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

However, the *BRAF* by MSI interaction test did not reach statistical significance ($P = 0.44$).

Survival analysis stratified by other potential variables

We also analyzed the prognostic value of *KRAS* and *BRAF* mutations for OS across strata of other potential prognostic factors (Figure 2). The prognostic effect of *KRAS* mutations appeared to be consistent across potential variables, and interactions between *KRAS* status and these factors were not significant. In contrast, *BRAF* mutations were significantly associated with poor OS in stage III, but not stage I-II, disease. Interactions between *BRAF* status and TNM stage showed suggestive statistical significance

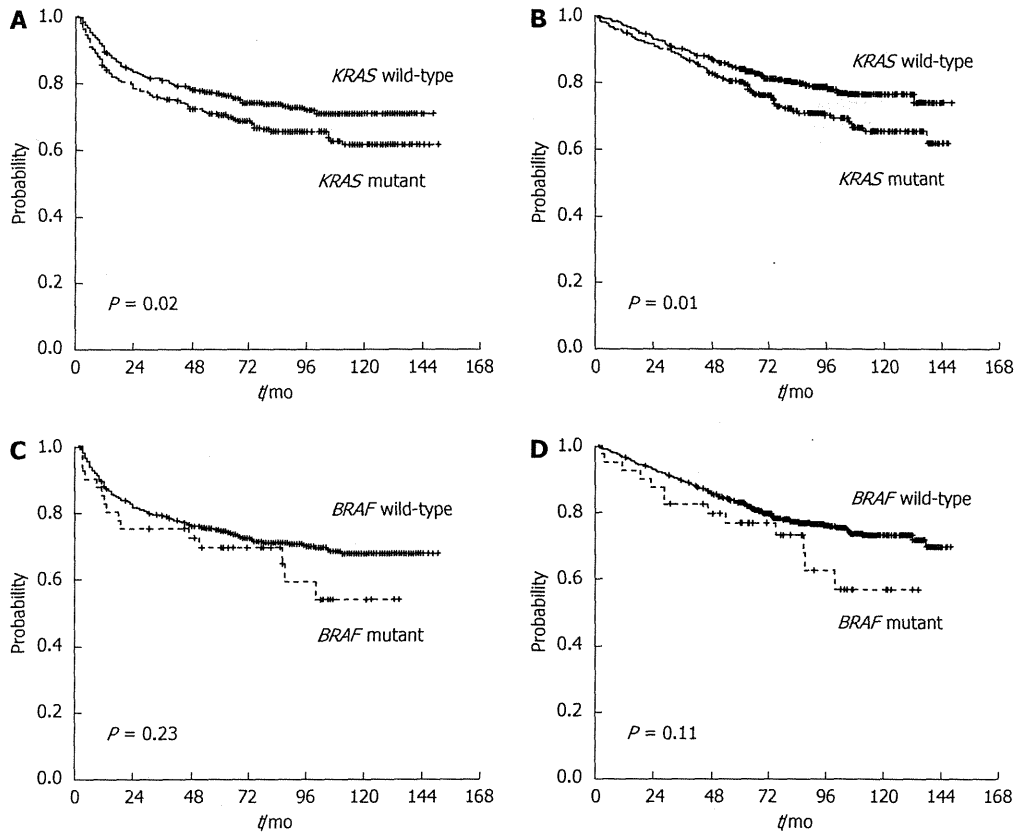


Figure 1 Kaplan-Meier curves for disease-free survival and overall survival according to *KRAS* or *BRAF* status. A: Disease-free survival (DFS) according to *KRAS* status; B: Overall survival (OS) according to *KRAS* status; C: DFS according to *BRAF* status; D: OS according to *BRAF* status.

Table 3 Prognostic effects of microsatellite instability, *KRAS*, and *BRAF* status in Cox proportional models

	Disease-free survival[1]		Overall survival[1]	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
MSI				
MSS/MSI-L	1 (reference)	0.14	1 (reference)	0.53
MSI-H	0.64 (0.35-1.16)		0.81 (0.42-1.56)	
KRAS				
Wild-type	1 (reference)	0.03	1 (reference)	0.01
Mutant	1.35 (1.03-1.75)		1.46 (1.09-1.97)	
BRAF				
Wild-type	1 (reference)	0.01	1 (reference)	0.02
Mutant	2.20 (1.19-4.06)		2.30 (1.15-4.71)	

[1]Covariates include age (< 65 or ≥ 65), gender, AJCC stage (Ⅰ/Ⅱ/Ⅲ), adjuvant chemotherapy (Yes/No), and MSI, *KRAS*, and *BRAF* status. CI: Confidence interval; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

Table 4 Prognostic Effects of *KRAS* and *BRAF* mutations according to microsatellite instability status

	KRAS		*BRAF*	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
DFS[1]				
MSS/MSI-L	1.37 (1.05-1.80)	0.95	2.06 (0.96-4.43)	0.91
MSI-H	1.34 (0.34-5.24)		2.46 (0.49-12.4)	
OS[1]				
MSS/MSI-L	1.49 (1.10-2.02)	0.70	2.74 (1.19-6.30)	0.44
MSI-H	1.39 (0.33-5.78)		1.18 (0.23-6.02)	

[1]Covariates include age, gender, AJCC stage (Ⅰ-Ⅱ/Ⅲ), adjuvant chemotherapy, and *KRAS* and *BRAF* status. HR: Hazard ratio; CI: Confidence interval; DFS: Disease-free survival; OS: Overall survival; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

(*P* = 0.10).

DISCUSSION

To our knowledge, this is the largest study to assess the prognostic value of *KRAS* and *BRAF* mutations for survival outcomes in CRC patients in Asian populations. Tumor specimens were prospectively collected from patients with curatively resected CRC (stage Ⅰ-Ⅲ); *KRAS* and *BRAF* mutations and MSI status were analyzed using a consistent methodology at a single institution. *KRAS* and *BRAF* mutations were associated with poor prognosis, independent of MSI status.

Many studies have examined associations of *KRAS* mutations with various clinical features, with no consistent results[5-8]. *KRAS* mutations were more frequent in females; however, these mutations were not associated with any other clinical variable.

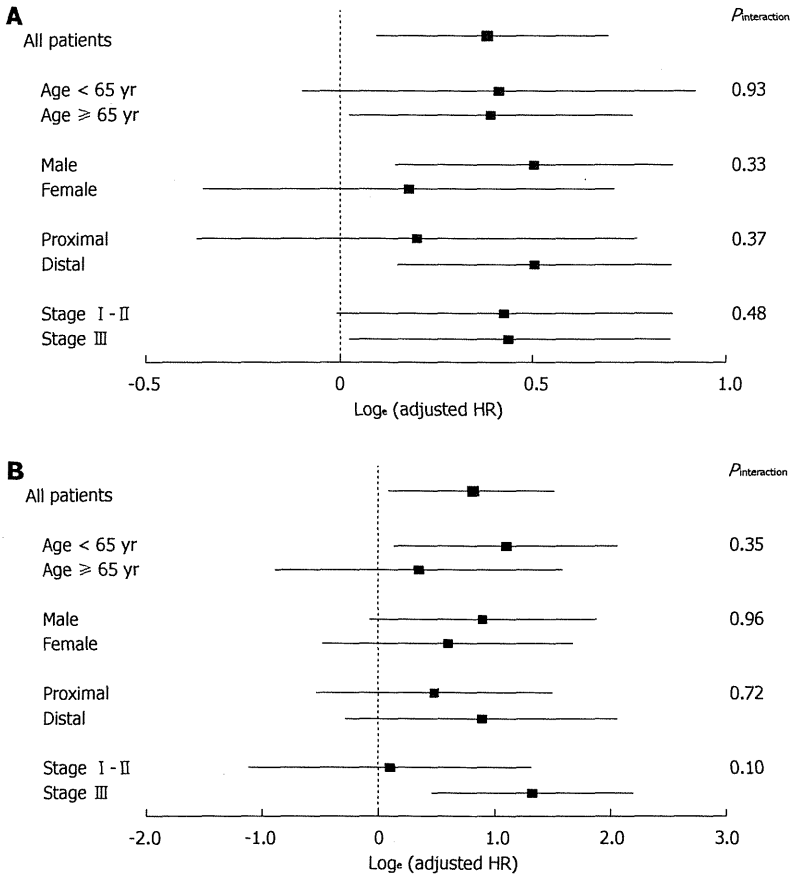


Figure 2 Stratified analysis of *KRAS* or *BRAF* status and overall survival. Loge [adjusted hazard ratio (HR)] and 95%CI for *BRAF* and *KRAS* mutant tumors (*vs* wild-type tumors) in various strata are shown. A: *KRAS* mutant tumors; B: *BRAF* mutant tumors.

Similarly, Watanabe *et al*[5] found relationships of *KRAS* mutations with the female gender and older age. In contrast, the Kirsten Ras Colorectal Cancer Collaborative Group study (RASCAL) demonstrated that *KRAS* mutations were associated with histological grade but no other variables[8]. In analysis of the PETACC-3 trial, Roth *et al*[6] reported associations of *KRAS* mutations with histological grade and tumor location but not gender. Such inconsistencies may be attributed to differences in the distribution of age, race, stage, or other factors among subject groups.

Currently, no convincing evidence exists that *KRAS* mutations are independent prognostic factors in CRC. In a Taiwanese study by Liou *et al*[14], KRAS mutations were not associated with inferior OS; however, the magnitude of multivariate HR (HR = 1.61; 95%CI: 0.91-2.84) was of the same order as that in the present study. A study from Japan revealed that the prognostic impact of *KRAS* mutations on recurrence-free survival was limited in patients with stage II CRC, and the association of *KRAS* mutations with OS was not observed[18]. Both studies had a small sample size and heterogeneous cohorts, including stage IV disease. In the large homogeneous cohort in this study, we found significant association of *KRAS* mutations with inferior DFS and OS. Because we previously found no difference in survival outcomes among different *KRAS* mutations, including those in exons 2, 3, and 4[23], prognostic analyses of specific codons for these mutations were not performed in the present study. Similarly, the RASCAL study indicated that *KRAS* mutations resulted in overall poorer prognosis[8], whereas subsequent analysis (RASCAL II) showed that only the glycine to valine substitution in codon 12 (G12V) was associated with poor prognosis in patients with Dukes' C disease[24]. Furthermore, recent randomized phase III trial results supported *KRAS* mutations as prognostic factors; 3-year DFS ranged from 72% to 75% across treatments for *KRAS* wild-type tumors, with 65% to 67% for *KRAS* mutant tumors[25]. In contrast, in the PETACC-3 trial, no association was found between *KRAS* mutations and poorer relapse-free survival or OS[6]. Although further research of the prognostic effect of *KRAS* mutations is needed, the influence of these mutations seems to be mild across previously reported studies.

The frequency of *BRAF* mutations (5%) and

MSI-H (8%) in our cohort was lower than that in Western populations (*BRAF*: 8%-20%, MSI-H: 11%-17%)[6,9,11-13,15,16] and comparable with that in Asian populations (*BRAF*: 4%-7%, MSI-H: 6%-12%)[14,18,26]. Generally, *BRAF* mutations and MSI-H are frequently observed in females, proximal tumors, and poorly differentiated tumors. In a systematic review including 9885 CRC patients, a *BRAF* mutation was associated with a proximal tumor location, poor differentiation, and female sex[27]. Consistent with this observation, *BRAF* mutations were more frequent in proximal tumors, poorly differentiated tumors, and females. Previous Western cohorts showed more patients with proximal and poorly differentiated tumors compared with Asian cohorts, including the current cohort. Thus, the discrepancy in *BRAF* mutations and MSI-H status between Western and Asian populations may be attributed to the different distribution of patients' characteristics such as gender, tumor location, histological grade, or racial and/or environmental differences.

Most previous studies found associations of *BRAF* mutations with poorer survival[6,10-12]. In meta-analysis of 26 independent studies (11773 patients), *BRAF* mutations increased the risk of mortality in CRC patients (HR = 2.25; 95%CI: 1.82-2.83)[28]. However, this evidence is mainly based on studies in Western populations; little is known regarding the prognostic role of *BRAF* mutations in Asian populations. In a Taiwanese study[14], *BRAF* mutations were associated with reduced OS, but MSI status was not estimated. In a Japanese study, Nakanishi *et al*[18] found no such association because of the insufficient number of patients with *BRAF* mutations. In the present study with larger sample size and homogeneous cohorts, we found associations of *BRAF* mutations with poorer DFS and OS in CRC patients with stage I-III disease, with the same order of magnitude of HR for OS as in the above meta-analysis. The prognostic effect of *BRAF* mutations on survival seems to be even stronger than that of *KRAS* mutations.

In contrast to previous reports[6,9,15-17], our analysis did not show that patients with MSI-H tumors exhibited better survival than those with MSS/MSI-L tumors. However, the number of patients with MSI-H tumors was too small to draw meaningful conclusions regarding the prognostic effect of MSI status. Therefore, additional larger studies are needed to clarify the prognostic impact of MSI status. Inconsistent results were reported regarding the prognostic effect of *BRAF* mutations according to MSI status[6,10,13]. Samowitz *et al*[10] found associations of BRAF mutations with poor survival in MSS/MSI-L, but not MSI-H tumors. Meanwhile, French *et al*[13] reported associations of *BRAF* mutations with poor survival in MSI-H tumors. In our analysis, associations of *BRAF* mutations with reduced OS were limited in MSS/MSI-L tumors. However, the *BRAF* by MSI interaction test was not significant; statistical power was considerably limited due to the small number of patients with MSI-H and *BRAF* mutant tumors. Larger studies are needed to clarify the modifying effect on the relation between *BRAF* mutations and survival outcome according to MSI status. Advantages of this study include comprehensive analysis of molecular markers using consistent methodology at a single institution, large sample size, and homogeneous cohort of Japanese patients. These results suggest that constitutive activation of the RAS/RAF/MAPK signaling pathway may be closely associated with clinical prognosis in CRC. Prognostic effects of *KRAS* and *BRAF* mutations seem to be consistent across most strata of clinical variables, while the adverse effect of *BRAF* mutations on OS may be attenuated in stage I-II CRC patients, with marginal statistical significance. The interaction of *BRAF* mutations with tumor stage warrants further research.

In conclusion, we found that Japanese CRC patients with *KRAS* or *BRAF* mutations have poorer survival, independent of MSI status. Additional investigations are warranted to clarify the interaction between these mutations and potential relevant factors, such as MSI status and tumor stage.

COMMENTS

Background

KRAS and *BRAF* mutations occur in 30%-40% and 4%-20% of colorectal cancers (CRCs), respectively. Microsatellite instability (MSI) is characterized by inactivation of the DNA mismatch repair system and is observed in 5%-15% of CRCs. MSI-high tumors are less likely to metastasize compared with the other phenotypes and have favorable survival outcomes. *KRAS* mutations are well known as predictive markers of resistance to epidermal growth factor receptor-targeted antibodies, and *BRAF* mutations are of current interest as a therapeutic target in metastatic CRCs. However, their prognostic value remains controversial for patients with curatively resected CRCs.

Research frontiers

Most previous studies investigating the prognostic role of *KRAS* and *BRAF* mutations in CRCs are from Western countries. Genetic background and geographical factors may influence mutation frequency and prognosis; however, few data are available regarding the prognostic role of these genetic alterations in Asian populations. Thus, clinical implications will be obtained by assessing the prognostic value of these mutations in a large cohort of CRCs in Japan, after adjustment for MSI status.

Innovations and breakthroughs

This study is the first large-scale study to demonstrate the prognostic impact of *KRAS* and *BRAF* mutations in Asian populations. After adjustment for relevant factors, including MSI, *KRAS* and *BRAF* mutations were independently associated with inferior disease-free survival and overall survival in patients with curatively resected CRCs. These findings will offer new insight into prognostic role of *KRAS* and *BRAF* mutations in CRCs.

Applications

BRAF and *KRAS* mutations may be useful as molecular markers for stratification of the clinical prognosis of curatively resected CRCs. Further investigation on whether the prognostic impact of *KRAS* and *BRAF* mutations could be modified by MSI status may provide more precise stratification of clinical outcomes in CRC.

Terminology

The protein product of the *KRAS* gene is a guanosine triphosphate/guanosine diphosphate-binding protein, and *KRAS* mutations play a key role in the development of various malignancies, including lung cancer, pancreatic cancer, and CRC. The protein product of the *BRAF* gene, a protein called B-Raf, is a serine/threonine protein kinase serving as downstream effector of the KRAS protein. *BRAF* mutations are involved in the development of many malignancies, *e.g.*, malignant melanoma, papillary thyroid cancer, and CRC.

Peer review

This is well written and illustrated paper. The authors investigate the prognostic role of *KRAS* and *BRAF* mutations after adjustment for MSI status. And they demonstrated that *KRAS* and *BRAF* mutations are associated with inferior survival, independent of MSI status in Asian colorectal cancer population. As the authors mentioned, in contrast to previous reports, their analysis did not show that patients with MSI-high tumors exhibited better survival than those with microsatellite-stable/MSI-low tumors.

REFERENCES

1 **Markowitz SD**, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. *N Engl J Med* 2009; **361**: 2449-2460 [PMID: 20018966 DOI: 10.1056/NEJMra0804588]

2 **Wang L**, Cunningham JM, Winters JL, Guenther JC, French AJ, Boardman LA, Burgart LJ, McDonnell SK, Schaid DJ, Thibodeau SN. BRAF mutations in colon cancer are not likely attributable to defective DNA mismatch repair. *Cancer Res* 2003; **63**: 5209-5212 [PMID: 14500346]

3 **Weisenberger DJ**, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, Kang GH, Widschwendter M, Weener D, Buchanan D, Koh H, Simms L, Barker M, Leggett B, Levine J, Kim M, French AJ, Thibodeau SN, Jass J, Haile R, Laird PW. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006; **38**: 787-793 [PMID: 16804544 DOI: 10.1038/ng1834]

4 **Yagi K**, Akagi K, Hayashi H, Nagae G, Tsuji S, Isagawa T, Midorikawa Y, Nishimura Y, Sakamoto H, Seto Y, Aburatani H, Kaneda A. Three DNA methylation epigenotypes in human colorectal cancer. *Clin Cancer Res* 2010; **16**: 21-33 [PMID: 20028768 DOI: 10.1158/1078-0432.CCR-09-2006]

5 **Watanabe T**, Yoshino T, Uetake H, Yamazaki K, Ishiguro M, Kurokawa T, Saijo N, Ohashi Y, Sugihara K. KRAS mutational status in Japanese patients with colorectal cancer: results from a nationwide, multicenter, cross-sectional study. *Jpn J Clin Oncol* 2013; **43**: 706-712 [PMID: 23657052 DOI: 10.1093/jjco/hyt062]

6 **Roth AD**, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010; **28**: 466-474 [PMID: 20008640 DOI: 10.1200/JCO.2009.23.3452]

7 **Samowitz WS**, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 1193-1197 [PMID: 11097226]

8 **Andreyev HJ**, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst* 1998; **90**: 675-684 [PMID: 9586664]

9 **Hutchins G**, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, Richman S, Chambers P, Seymour M, Kerr D, Gray R, Quirke P. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011; **29**: 1261-1270 [PMID: 21383284 DOI: 10.1200/JCO.2010.30.1366]

10 **Samowitz WS**, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, Wolff RK, Slattery ML. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res* 2005; **65**: 6063-6069 [PMID: 16024606 DOI: 10.1158/0008-5472.CAN-05-0404]

11 **Fariña-Sarasqueta A**, van Lijnschoten G, Moerland E, Creemers GJ, Lemmens VE, Rutten HJ, van den Brule AJ. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. *Ann Oncol* 2010; **21**: 2396-2402 [PMID: 20501503 DOI: 10.1093/annonc/mdq258]

12 **Ogino S**, Shima K, Meyerhardt JA, McCleary NJ, Ng K, Hollis D, Saltz LB, Mayer RJ, Schaefer P, Whittom R, Hantel A, Benson AB, Spiegelman D, Goldberg RM, Bertagnolli MM, Fuchs CS. Predictive and prognostic roles of BRAF mutation in stage III colon cancer: results from intergroup trial CALGB 89803. *Clin Cancer Res* 2012; **18**: 890-900 [PMID: 22147942 DOI: 10.1158/1078-0432.CCR-11-2246]

13 **French AJ**, Sargent DJ, Burgart LJ, Foster NR, Kabat BF, Goldberg R, Shepherd L, Windschitl HE, Thibodeau SN. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. *Clin Cancer Res* 2008; **14**: 3408-3415 [PMID: 18519771 DOI: 10.1158/1078-0432.CCR-07-1489]

14 **Liou JM**, Wu MS, Shun CT, Chiu HM, Chen MJ, Chen CC, Wang HP, Lin JT, Liang JT. Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese population. *Int J Colorectal Dis* 2011; **26**: 1387-1395 [PMID: 21553007 DOI: 10.1007/s00384-011-1229-1]

15 **Sinicrope FA**, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, Kim GP, Yothers G, Allegra C, Moore MJ, Gallinger S, Sargent DJ. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 2011; **103**: 863-875 [PMID: 21597022 DOI: 10.1093/jnci/djr153]

16 **Ribic CM**, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, Gallinger S. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; **349**: 247-257 [PMID: 12867608 DOI: 10.1056/NEJMoa022289]

17 **Popat S**, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609-618 [PMID: 15659508 DOI: 10.1200/JCO.2005.01.086]

18 **Nakanishi R**, Harada J, Tuul M, Zhao Y, Ando K, Saeki H, Oki E, Ohga T, Kitao H, Kakeji Y, Maehara Y. Prognostic relevance of KRAS and BRAF mutations in Japanese patients with colorectal cancer. *Int J Clin Oncol* 2013; **18**: 1042-1048 [PMID: 23188063 DOI: 10.1007/s10147-012-0501-x]

19 **Sobin LH**, Wittekind C. TNM Classification of Malignant Tumors, 6th edition. New York: Wiley-Liss, 2002

20 **Akagi K**, Uchibori R, Yamaguchi K, Kurosawa K, Tanaka Y, Kozu T. Characterization of a novel oncogenic K-ras mutation in colon cancer. *Biochem Biophys Res Commun* 2007; **352**: 728-732 [PMID: 17150185 DOI: 10.1016/j.bbrc.2006.11.091]

21 **Asaka S**, Arai Y, Nishimura Y, Yamaguchi K, Ishikubo T, Yatsuoka T, Tanaka Y, Akagi K. Microsatellite instability-low colorectal cancer acquires a KRAS mutation during the progression from Dukes' A to Dukes' B. *Carcinogenesis* 2009; **30**: 494-499 [PMID: 19147861 DOI: 10.1093/carcin/bgp017]

22 **Ishikubo T**, Nishimura Y, Yamaguchi K, Khansuwan U, Arai Y, Kobayashi T, Ohkura Y, Hashiguchi Y, Tanaka Y, Akagi K. The clinical features of rectal cancers with high-frequency microsatellite instability (MSI-H) in Japanese males. *Cancer Lett* 2004; **216**: 55-62 [PMID: 15500949 DOI: 10.1016/j.canlet.2004.07.017]

23 **Ogura T**, Kakuta M, Yatsuoka T, Nishimura Y, Sakamoto H, Yamaguchi K, Tanabe M, Tanaka Y, Akagi K. Clinicopathological characteristics and prognostic impact of colorectal cancers with NRAS mutations. *Oncol Rep* 2014; **32**: 50-56 [PMID: 24806883 DOI: 10.3892/or.2014.3165]

24 **Andreyev HJ**, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, Young J, Walsh T, Ward R, Hawkins N, Beranek M,

Jandik P, Benamouzig R, Jullian E, Laurent-Puig P, Olschwang S, Muller O, Hoffmann I, Rabes HM, Zietz C, Troungos C, Valavanis C, Yuen ST, Ho JW, Croke CT, O'Donoghue DP, Giaretti W, Rapallo A, Russo A, Bazan V, Tanaka M, Omura K, Azuma T, Ohkusa T, Fujimori T, Ono Y, Pauly M, Faber C, Glaesener R, de Goeij AF, Arends JW, Andersen SN, Lövig T, Breivik J, Gaudernack G, Clausen OP, De Angelis PD, Meling GI, Rognum TO, Smith R, Goh HS, Font A, Rosell R, Sun XF, Zhang H, Benhattar J, Losi L, Lee JQ, Wang ST, Clarke PA, Bell S, Quirke P, Bubb VJ, Piris J, Cruickshank NR, Morton D, Fox JC, Al-Mulla F, Lees N, Hall CN, Snary D, Wilkinson K, Dillon D, Costa J, Pricolo VE, Finkelstein SD, Thebo JS, Senagore AJ, Halter SA, Wadler S, Malik S, Krtolica K, Urosevic N. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer* 2001; **85**: 692-696 [PMID: 11531254 DOI: 10.1054/bjoc.2001.1964]

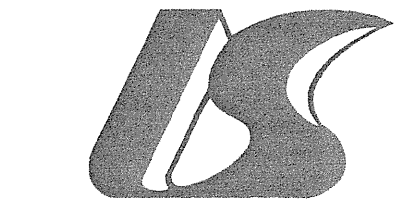
25 **Alberts SR**, Sargent DJ, Nair S, Mahoney MR, Mooney M, Thibodeau SN, Smyrk TC, Sinicrope FA, Chan E, Gill S, Kahlenberg MS, Shields AF, Quesenberry JT, Webb TA, Farr GH, Pockaj BA, Grothey A, Goldberg RM. Effect of oxaliplatin, fluorouracil, and leucovorin with or without cetuximab on survival among patients with resected stage III colon cancer: a randomized trial. *JAMA* 2012; **307**: 1383-1393 [PMID: 22474202 DOI: 10.1001/jama.2012.385]

26 **Lin CH**, Lin JK, Chang SC, Chang YH, Chang HM, Liu JH, Li LH, Chen YT, Tsai SF, Chen WS. Molecular profile and copy number analysis of sporadic colorectal cancer in Taiwan. *J Biomed Sci* 2011; **18**: 36 [PMID: 21645411 DOI: 10.1186/1423-0127-18-36]

27 **Clancy C**, Burke JP, Kalady MF, Coffey JC. BRAF mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis* 2013; **15**: e711-e718 [PMID: 24112392 DOI: 10.1111/codi.12427]

28 **Safaee Ardekani G**, Jafarnejad SM, Tan L, Saeedi A, Li G. The prognostic value of BRAF mutation in colorectal cancer and melanoma: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e47054 [PMID: 23056577 DOI: 10.1371/journal.pone.0047054]

P- Reviewer: Paoluzi OA, Sakakura C, Tajika M, Wang JY
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Wang CH



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ISSN 1007-9327

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2014;44(11)1032–1039
doi:10.1093/jjco/hyu128
Advance Access Publication 10 September 2014

Incidence and Clinical Features of Drug-induced Lung Injury in Patients with Advanced Colorectal Cancer Receiving Cetuximab: Results of a Prospective Multicenter Registry

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Received February 18, 2014; accepted August 5, 2014

Objective: We investigated the incidence and clinical features of drug-induced lung injury during cetuximab therapy in Japanese patients with colorectal cancer in a prospective multicenter registry based on a central registration system.

Methods: We investigated and followed up patients with or suspected of having drug-induced lung injury among 2006 patients with cetuximab-treated colorectal cancer. A subcommittee of medical oncologists, pulmonologists and a radiologist evaluated and discussed each case of drug-induced lung injury that occurred during cetuximab therapy.

Results: Sixty-six patients were identified and further examinations of drug-induced lung injury were conducted during the registration period. We analyzed time to onset, patient characteristics and factors associated with mortality. Cetuximab-related drug-induced lung injury occurred in 24 (1.2%) patients, and was rated as Grade 3 or worse in 15 (0.7%) patients. Fourteen patients received steroid pulse therapy. Ten patients with drug-induced lung injury died, of whom eight received steroid pulse therapy. The incidence of drug-induced lung injury was significantly higher in elderly patients, and in patients with prior interstitial lung disease. There was no particular trend in the time to onset. Patients with early onset of drug-induced lung injury (within 90 days) after starting cetuximab therapy had higher mortality than patients with later onset (over 90 days).

Conclusions: The incidence of drug-induced lung injury in cetuximab-treated patients was 1.2%. Because drug-induced lung injury is potentially serious, it is important to promptly initiate appropriate treatments. Considering that early onset drug-induced lung injury during cetuximab therapy is associated with a poor prognosis, close monitoring is mandatory for these patients.

Key words: biological therapy – cetuximab – chemotherapy – colorectal cancer – drug-induced lung injury

INTRODUCTION

Severe drug-induced lung injury (DLI) occurring during chemotherapy can result in respiratory failure, and may be fatal. Accurate diagnosis is complicated because it is difficult to distinguish DLI from cancer progression, infection or drug-induced heart failure (1). In addition, treatment with multiple drugs makes it difficult to identify the responsible drug.

The consequences of DLI are broadly classified into two categories: a direct cytotoxic effect in which the drug or an intermediate metabolite affects lung tissue directly, and an indirect cytotoxic effect in which the host's inflammatory response and immunological factors affect lung tissue (2,3). However, the etiology of DLI is unknown in many cases because its pathology varies between different drugs.

The incidence of DLI, including interstitial lung disease (ILD), during treatment with gefitinib, leflunomide and bleomycin, is higher in Japan than in the USA and Europe (4,5), suggesting there are racial differences in the risk of these lung disorders. Because ILD during gefitinib treatment occurred more frequently after its approval and marketing in Japan (6), physicians and the Pharmaceuticals Medical Devices Agency (PMDA) of Japan were interested in clarifying whether epidermal growth factor receptor (EGFR) inhibitors are associated with DLI in Japanese patients. The West Japan Thoracic Oncology Group retrospectively analyzed 1976 patients treated with gefitinib (7) and the incidence of gefitinib-induced ILD was 3.5%. Another prospective multicenter study reported that ILD occurred in 122 of 1872 (6.5%) of gefitinib-treated patients (6).

Cetuximab (Erbitux®) is a monoclonal antibody that specifically binds to EGFR and inhibits its downstream signaling. Clinical data for cetuximab as first-, second- and third-line therapy have been reported (8–12). In July 2008, cetuximab was approved in Japan for the treatment of EGFR-positive, curatively unresectable, advanced or recurrent colorectal cancer (CRC). This approval was granted on the condition that all patients scheduled for cetuximab treatment from September 2008 were to be included in a prospective registry study to evaluate the safety profile of this drug in clinical practice (13). Therefore, the safety data accumulated in the central registration system were collated and analyzed in a timely manner to determine the characteristics of DLI occurring during cetuximab therapy.

PATIENTS AND METHODS

Patients

Following the launch of cetuximab on 19 September 2008, all patients to be treated with cetuximab were enrolled in a central registration system in advance. Overall, 2126 patients across 637 institutions were registered between September 2008 and January 2009. Of these, 120 patients who did not receive cetuximab treatment were excluded from this study. Therefore, 2006 patients were included in the safety population. The median age of these patients was 64 years (range, 18–87 years), the male:female ratio was 1:0.6, and the median duration of treatment was 15.3 weeks (range, 1–73.9 weeks). CRC was EGFR positive in 98.5% of patients; 61.6% of patients had primary tumor sites in the colon and 38.6% in the rectum (including overlapping patients). In total, 93.2% of the patients received cetuximab as third-line or later therapy, and 99.7% had an Eastern Cooperative Oncology Group Performance Status (PS) score of 0 or 1. Furthermore, 405 patients (20.2%) had a history of other relevant diseases, including prior ILD in four patients (0.2%) and allergy in 306 patients (15.3%) (13). Except for four patients with prior ILD, the other patients had no evidence of interstitial changes before starting treatment, as determined from computed tomography (CT) images and the medical history recorded on case-report forms. Twenty-three cases of lung injury occurred during treatment with cetuximab and were reported by the primary physicians. Four-hundred and forty patients developed respiratory disorders, categorized by system organ class, and were further evaluated to identify possible cases of DLI.

Evaluation of ILD

For patients who developed respiratory disorders during treatment, the physicians completed a case report form designed to evaluate the clinical characteristics of DLI. To exclude non-respiratory responses and respiratory events associated with infusion reactions as causative events, we assessed the following factors in these patients: causal relationship with cetuximab, patient characteristics and disease course. Based on these assessments, 43 of 440 patients were suspected of having DLI.

The 43 suspected and 23 reported cases were then reviewed by a DLI subcommittee. The DLI subcommittee, which consisted of two pulmonologists, one radiologist and two medical oncologists, convened seven times between April 2009 and September 2010 to review the patient data recorded in the registry and case report forms completed by the primary physicians. The committee members reviewed chest X-ray and CT images, occurrence of pulmonary metastasis, smoking history, results of consultation with a pulmonologist, measurement of interstitial pneumonitis markers (KL-6 and SP-D), blood gas measurements, tests for bacterial and fungal infection, bronchoalveolar lavage, lung biopsy and autopsy. DLI was diagnosed based on a combination of clinical symptoms (e.g. coughing, dry cough, shortness of breath/exertional dyspnea, and fever), clinical laboratory test results and chest X-ray and CT findings (revealing ground-glass opacities or infiltrates) during cetuximab therapy.

Statistical Analysis

Calculations and analyses of collected data were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA). Fisher's exact test or Wilcoxon's two-sample test was used to analyze the relationship between patient characteristics and the

onset of DLI during cetuximab therapy. Factors associated with DLI mortality were also examined. Multivariate analysis using Cox's proportional hazard model was also performed in which the occurrence of DLI was used as the dependent variable and patient characteristics were used as independent variables. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Patients

Figure 1 summarizes the disposition of patients and how they were diagnosed with DLI. Of 2006 patients included in the safety population, 23 were reported by their physician to have lung disease and were further assessed by the DLI subcommittee. Of these patients, one was thought to have pneumonia not related to DLI. Of the 43 patients suspected of having DLI, two patients were diagnosed with cetuximab-related DLI, although they were originally reported by their primary physicians to have lymphangitis carcinomatosa and radiation pneumonitis. Therefore, 24 patients were ultimately diagnosed with cetuximab-related DLI, and data for these patients were further analyzed (Fig. 1).

Incidence of Cetuximab-related DLI and Patient Characteristics

The incidence of DLI during treatment with cetuximab was 1.2% ($n = 24/2006$ patients). Grade 3 or worse DLI occurred in 0.7% of patients ($n = 15$). The characteristics of patients with DLI are shown in Table 1. DLI occurred in 18 males and six females, and the median age was 70 years (range, 45–80 years). PS score was 0 in 19 patients and 1 in five patients.

Two patients received cetuximab as second-line therapy, while 22 received it as third-line therapy. One patient had prior ILD, and 13 had other medical histories. Twenty-two patients received cetuximab in combination with chemotherapy, including 17 who received cetuximab in combination with CPT-11 alone.

Of the 24 patients, 10 had a history of smoking and 10 were never smokers; smoking status was unknown in four patients. Image patterns of DLI were categorized according to the image findings evaluated by the DLI subcommittee. Images were classified as diffuse alveolar damage in eight patients and as ground-glass opacities in 14 patients; the images could not be determined in the other two patients.

Subgroup analyses based on patient characteristics revealed that the incidence of DLI was significantly higher in elderly patients (≥65 years) and in patients with prior ILD (Table 1). Therefore, we performed a multivariate analysis using Cox's proportional hazard model to investigate the relationship between DLI and patient characteristics, including sex, age (<65 vs ≥65 years old), treatment line (second-line vs third-line or later), PS (0 vs 1), prior ILD and combination chemotherapy (with vs without). The analysis showed that the incidence of DLI was significantly higher in patients with prior ILD (HR, 19.49; 95% CI, 1.22–311.73; $P = 0.036$).

Time to Onset

The median time to the onset of DLI from the start of cetuximab therapy was 101 days (range, 17–431 days; Fig. 2). DLI

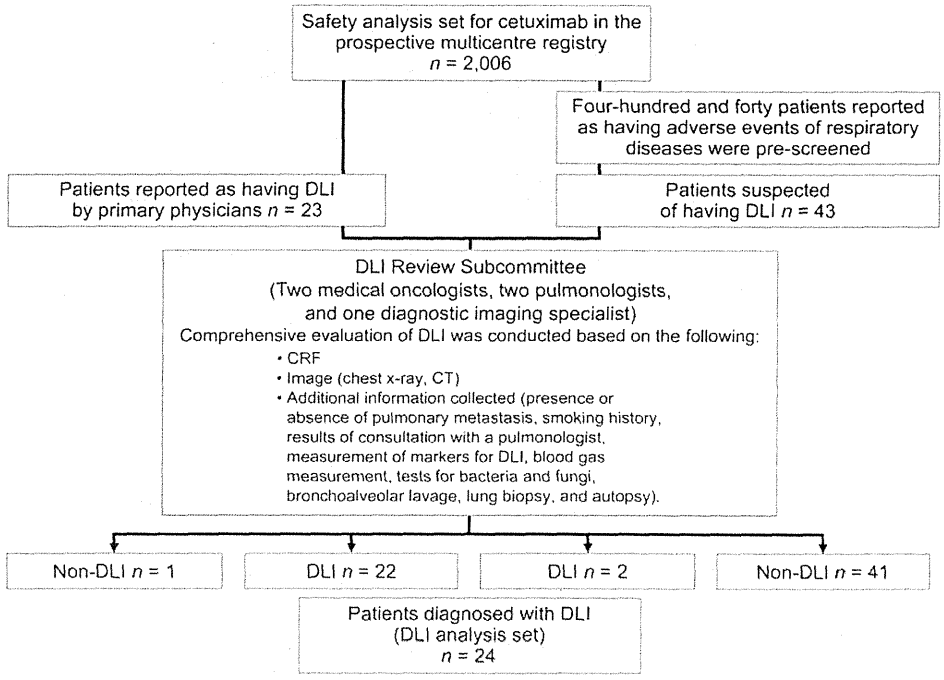

Figure 1. Registry profile and identification of patients with drug-induced lung injury (DLI).

Table 1. Incidence of drug-induced lung injury (DLI) during cetuximab therapy according to patient characteristics

Patient characteristic		Safety population	Number of patients with DLI	Incidence of DLI	*P* value
n		2006	24	1.2%	
Sex	Male	1234	18	1.46%	0.2083
	Female	772	6	0.78%	
Age	<65 years	1032	6	0.58%	0.0122
	≥65 years	971	18	1.85%	
	Unknown	3	0	0.0%	
PS	0	1370	19	1.39%	0.3762
	1	630	5	0.79%	
	2	2	0	0%	
	Other	4	0	0%	
Treatment line	Second line	133	2	1.50%	0.6711
	Third line or later	1869	22	1.18%	
	Other	4	0	0%	
Prior interstitial lung disease (ILD)	–	1955	21	1.07%	0.0442
	+	4	1	25%	
	Unknown	47	2	4.26%	
Complications	–	1019	11	1.08%	0.6833
	+	974	13	1.33%	
	Unknown	13	0	0%	
Combination chemotherapy	–	460	2	0.43%	0.2083
	+	1546	22	1.42%	
	CPT-11 alone	1255	17	1.35%	
	FOLFIRI	256	4	1.56%	
	Other	35	1	2.86%	

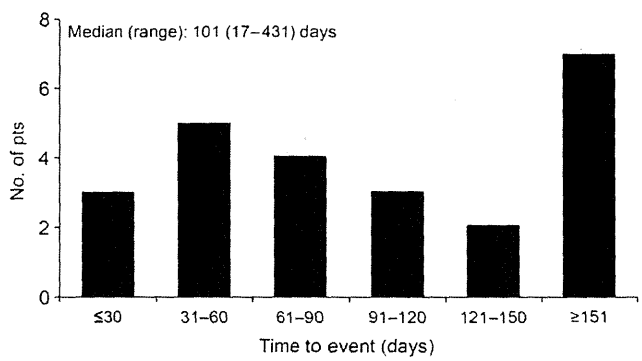

Figure 2. Time to the onset of DLI from the start of cetuximab administration.

occurred within 30 days of starting cetuximab therapy in three patients, from 31 to 60 days in five patients, from 61 to 90 days in four patients, and on Day 91 or later in 12 patients.

Treatment of DLI

Steroid pulse therapy was administered to 14 of 24 patients. The time from the onset of DLI (initial symptoms) to the start of steroid pulse therapy was 3 days in six patients, 4–7 days in six patients and ≥8 days in two patients.

Outcomes

In terms of the outcomes of DLI, 10 patients (41.7%) died, two patients showed full recovery, six patients had partial recovery, five patients showed no recovery, and the outcome was unknown in one patient. Eight of the patients who died had received steroid pulse therapy.

Factors Associated with Mortality

Univariate analyses were performed to investigate potential associations between mortality and patient characteristics, including sex, treatment line, PS, combination chemotherapy, pulmonary metastasis, time to onset, steroid pulse therapy including the timing and history of smoking. As a result, patients with early onset of DLI (within 90 days of starting cetuximab) had significantly higher mortality than those with later onset (over 90 days).

There were no significant associations between mortality and other characteristics, including smoking history and the time to the start of steroid pulse therapy. However, it is worth noting that two of the six patients who started steroid pulse therapy within 3 days died, compared with six of the eight patients who started steroid pulse therapy after 4 days (Table 2).

Case Report

A female patient in her 60s had primary colon cancer with liver and lymph node metastases (Fig. 3A) that was previously treated with FOLFOX (folinic acid, fluorouracil and oxaliplatin) and FOLFIRI (folinic acid, fluorouracil and irinotecan). Cetuximab (initial dose of 400 mg/m^2 with subsequent weekly doses of 250 mg/m^2) in combination with CPT-11 (140 mg/m^2, biweekly) was then started (Day 1). The patient experienced breathing difficulties 3 days after the sixth dose of cetuximab (~Day 57). Three days later (Day 60), chest X-ray and CT images showed ground-glass opacity (Fig. 3B and C), and she was diagnosed with DLI. The patient was hospitalized and treated with supplemental oxygen, antibacterial drugs and antifungal drugs (micafungin sodium, sulfamethoxazole-trimethoprim). On Day 61, treatment with methylprednisolone (1 g) was started. Piperacillin was added at a dose of 4 g/day on Day 62, and cyclophosphamide was added at a dose of 250 mg/day on Day 67. Despite these treatments, her symptoms did not improve (Fig. 3D), and signs of diffuse alveolar damage were found on Day 68 (Fig. 3E). The patient died 22 days after the onset of symptoms (Day 79).

Table 2. Univariate analysis of mortality among 24 patients with DLI during cetuximab therapy

		Number of patients (of 24)	Number of deaths (of 10)	*P* value
Sex	Male	18	7	0.6653
	Female	6	3	
Treatment line	Second line	2	1	1.000
	Third line or later	22	9	
PS	0	19	8	1.000
	1	5	2	
Combination chemotherapy	Cetuximab alone	2	0	0.4928
	In combination with CPT-11 or FOLFIRI	22	10	
Lung metastasis	−	10	5	0.6785
	+	14	5	
Classification of images	Diffuse alveolar damage	8	5	n/a
	Ground-glass opacity	14	5	
	Unable to be classified	2	0	
Time to onset of DLI from the start of cetuximab treatment	<90 days	12	8	0.0361
	≥91 days	12	2	
Steroid pulse therapy	−	10	2	0.1041
	+	14	8	
Time to the start of steroid pulse therapy from the onset of DLI	Within 3 days	6	2	0.2744
	4 days or later	8	6	
Smoking history	−	10	5	1.000
	+	10	4	
	Unknown	4	1	

DISCUSSION

This was the first registry to analyze and investigate the incidence and risk of DLI during cetuximab-based therapy in patients with CRC. In recent years, lung disease associated with EGFR inhibitors (gefitinib and erlotinib) used to treat non-small cell lung cancer (NSCLC) has become a social concern in Japan (4,6). As a condition for the approval of cetuximab in Japan, the PMDA requested to continuously monitor and analyze cases of DLI occurring during cetuximab therapy. Therefore, we examined all Japanese patients who had received cetuximab-based therapies between September 2008 and January 2009.

DLI occurred in 24 of 2006 patients (1.2%) included in the present registry, while 10 of these 24 patients died. In contrast, the incidence of cetuximab-related ILD ranged from 0.2 to 0.6% in clinical studies performed in other countries (Table 3). Based on these clinical trials, it seems that the incidence of DLI, including ILD, is greater in Japan than in other countries, suggesting that there is a racial difference in this disease. A good example of this difference is that the incidence of ILD during gefitinib treatment in the US was ~0.3% among 23 000 patients (FDA Approval Letter) while the incidence of ILD during gefitinib treatment was as high as 3.5–5.8% in Japan (7,14,15). As another example, the incidence of ILD in patients with leflunomide, a treatment for rheumatoid arthritis, was 0.02% (of 400 000 patients) and 1.2% (of 5054 patients) worldwide and in Japan, respectively (16,17). These results also highlight the differences in the incidence of ILD in Japan and other countries (5).

It is also important to compare the incidence in Japan and other Asian countries. In Taiwan, for example, the incidence of ILD was reported to be 5.8% among patients with NSCLC treated with gefitinib (18,19). Meanwhile, in a Chinese Phase II study of gefitinib in patients with NSCLC, the incidence of ILD was 7.5% (20). These reports suggest that Asian patients are more susceptible to DLI, including ILD, than Caucasian patients. In fact, a survey conducted in the USA examining the presence of idiopathic pulmonary fibrosis (IPF) at death between 1989 and 2007, showed that White and Black populations were less likely to die of/with IPF than Hispanic and other ethnic/racial populations (21). Although the reason for this racial difference has not been clarified, some studies have suggested that genetic differences, including MUC5B promoter polymorphism and telomere shortening, may play some roles in the onset of pulmonary fibrosis (22,23).

There have been several studies of DLI, including ILD, in patients with CRC. In a retrospective analysis of 734 Japanese patients with CRC given standard chemotherapy consisting of FOLFOX or FOLFIRI, ILD occurred in 11 patients (1.5%), four of whom died (24). In a large prospective post-marketing registry of bevacizumab, ILD occurred in six of 3727 patients (0.16%), and one of these patients died (24). In a post-marketing surveillance study of oxaliplatin, ILD occurred in 13 of 5008 patients (0.26%) (24). In our registry, DLI occurred in two patients (0.43%) treated with cetuximab alone, in 17 patients (1.35%) receiving cetuximab in combination with CPT-11, and in four patients (1.56%) receiving cetuximab in combination with FOLFIRI.

The variations in the occurrence rates of ILD in these earlier studies can be explained by differences in study settings. First, the retrospective analysis of FOLFOX- or FOLFIRI-related ILD was based on data from a single central hospital. Second, patients included in the post-marketing surveillance study of bevacizumab received bevacizumab as first- or second-line therapy, and the patients included in our registry received cetuximab as third-line or later therapy. Finally, the oxaliplatin-related ILD study suggests that the duration of oxaliplatin treatment may have effect on onset of ILD (24). For these reasons, the incidence rates of DLI, including ILD, should not be directly compared among studies.

Other EGFR inhibitors, such as erlotinib and gefitinib, are often used to treat NSCLC. Among gefitinib-treated patients, risk factors for gefitinib-related ILD included older age (≥55

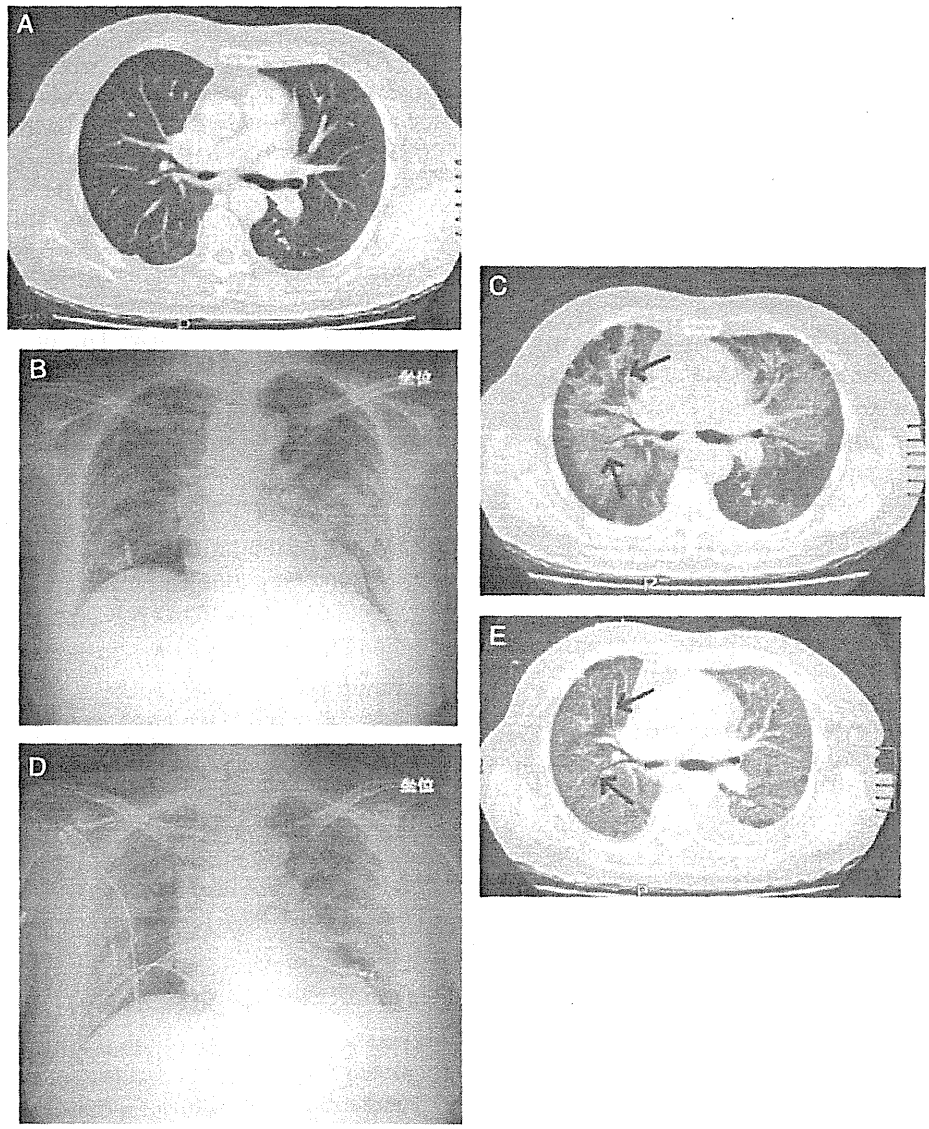

Figure 3. Case report of a female patient with recurrent colorectal cancer in her 60s. (A) Image before the onset of DLI taken at 32 days after starting cetuximab therapy. Metastatic nodes were observed in the inferior lobe of both lungs. An infiltrate and ground-glass opacity were not observed. (B) X-ray image taken 60 days after starting cetuximab therapy, 3 days after the onset of symptoms. Ground-glass opacity was predominantly observed in the bilateral upper lung field. (C) Computed tomography (CT) scan on Day 60. Ground-glass opacity was observed from the hilus to the middle section of both lungs. The lesion in the periphery of the lung field was lightly spared. Hypertransradiancy of bronchi (arrow) was observed, but there was no obvious traction bronchiectasis, and it could not be diagnosed as diffuse alveolar damage from the images. (D) X-ray image taken on Day 67. The abnormal shadows in both lung fields had spread and were exacerbated. (E) CT image taken on Day 68. The shadows in both lungs were exacerbated and extended to the periphery of each lung. Hypertransradiancy of bronchi with bellows-like dilation (arrow) was observed in the shadows, and was considered to be traction bronchiectasis. The organizing stage of diffuse alveolar damage was observed, which was considered to be a sign of lesion progression. Left pleural effusion was also observed.

years), smoking habit, poor PS ($\geq$2), and prior ILD (6). In our registry, older age and prior ILD were the primary factors associated with the onset of DLI. Although the sample size was limited, the results of multivariate analysis showed that prior ILD was the main risk factor for DLI during cetuximab therapy. It is noteworthy that the incidence of DLI in patients with CRC tends to be lower than that in patients with lung cancer, but mortality was similar. The incidence and risk factors for DLI in other cancers remain to be investigated.

In our registry, the median time to the onset of DLI was 101 days (range, 17–431 days) and there was no trend in the time to onset. In contrast, it was reported that ILD associated with gefitinib and erlotinib tends to occur within 4 weeks. Even though all of these drugs ultimately inhibit EGFR activity, the time to the onset of DLI, including ILD, varies by the type of cancer and the specific target site of each drug.

In our registry, patients with onset of DLI within 90 days had a worse prognosis than patients with later onset. For

Table 3. Incident rate of DLI, including ILD, in cetuximab studies

Trial		Regimen	*n*	All grades	≥Grade 3
Large prospective registry in Japanese patients[a]		Cetuximab alone	2006	24 (1.20%)	15 (0.7%)
		Cetuximab + CPT-11			
		Cetuximab + FOLFIRI			
Phase II in Japanese patients		Cetuximab + CPT-11	39	1 (2.60%)	–
Phase II	BOND	Cetuximab + CPT-11	327	–	
		Cetuximab alone			
	MABEL	Cetuximab + CPT-11	1147	3[b] (0.30%)	1 (0.10%)
	OPUS	Cetuximab + FOLFOX4	170	1[c] (0.60%)	–
Phase III	EPIC	Cetuximab + CPT-11	638	–	
	CO.17	Cetuximab alone	288	–	
	CRYSTAL	Cetuximab + FOLFIRI	600	1[c] (0.20%)	–

Evaluated by NCI-CTC Ver. 2.0.
[a]Evaluated by NCI-CTC Ver. 3.0.
[b]Not related to cetuximab.
[c]Counted adverse events related to both cetuximab and chemotherapy.
(Data source: Merck Serono internal documents).

example, in a case presented in this report, the patient in her 60s developed DLI within 90 days after cetuximab administration. Although steroid pulse therapy was initiated on the third day of her symptom, she did not recover and later died. The prognosis of gefitinib-treated patients with early onset of ILD was also poor (25). Therefore, it is necessary to closely monitor patients for the first 3 months of treatment for signs of DLI. It is also notable that, in our registry, two of six patients who started steroid pulse therapy early died compared with six of eight patients who started the treatment later after the onset of DLI, although this was not significantly different. The response of patients with DLI to steroid therapy varies depending on the etiology and pathogenesis of DLI. However, it is strongly recommended that in cases of suspected or confirmed DLI, the cetuximab-based chemotherapy should be discontinued immediately, and adequate approaches including consultation with a pulmonologist and steroid therapy (steroid pulse therapy with methylprednisolone 500–1000 mg/day for 3 days), should be implemented as soon as possible.

Our registry has several limitations. First, although this registry was planned before approval of cetuximab, chest X-rays or CT scans were not mandatory during treatment, except for patients with respiratory symptoms. In addition, although history of smoking increases the risk of DLI, smoking status was not recorded for all patients. Second, because this registry was conducted in 637 hospitals under the insurance reimbursement program, the characteristics of patients varied considerably compared with those in clinical trials. Nevertheless, we think that the incidence, mortality and risk factors identified in this registry will provide useful information to guide future treatment strategies.

Conclusion

Although DLI is rare, it can result in respiratory failure and may be fatal. Accurate diagnosis is complicated, and identifying the drug responsible for DLI is difficult. This registry examined 2006 patients treated with cetuximab following its approval in Japan. We reviewed all patients reported as having lung disease by their primary physicians, as well as patients suspected of having DLI during cetuximab therapy. The incidence of ILD in this registry was 1.2%. Older age and prior ILD were risk factors for the onset of DLI. Although no particular trend was noted in the time to onset, DLI occurring within 90 days of starting cetuximab had a poor prognosis. Therefore, we strongly recommend patients should be closely monitored, especially for the first 90 days of treatment with cetuximab.

Acknowledgements

We are grateful to all the physicians and patients for their cooperation in this study, as well as Merck Serono Co. Ltd, Japan and Bristol-Myers K.K., Japan.

Funding

This work was supported by Merck Serono Co. Ltd, Japan and Bristol-Myers K.K., Japan. The sponsors were involved in protocol development and data analysis through the contributions of S.I. and M.I., who are employees of the sponsors. Funding to pay the Open Access publication charges for this article was provided by Merck Serono Co., Ltd., Japan and Bristol-Myers K.K., Japan.

Conflict of interest statement

Taroh Satoh has received consulting fees and honoraria from Merck Serono Co. Ltd and Bristol-Myers K.K., and departmental research grants from Chugai Pharmaceutical Co. Ltd and Yakult Honsha Co. Ltd.

Akihiko Gemma has received lecture fees from Merck Serono Co. Ltd.

Shoji Kudoh has no potential conflicts of interest to declare.

Fumikazu Sakai has provided consultancy services to the Japanese Ministry of Health, Labour and Welfare, the Japanese Agency of the Environment and Restoration. Japanese Organization of Research for Thoracic Radiology, Takeda Pharmaceuticals Co. Ltd, Chugai Pharmaceutical Co. Ltd, MSD Co. Ltd, AstraZeneca Co. Ltd, Pfizer Co. Ltd, Bayer Co. Ltd, Jansen Pharmaceuticals Co. Ltd, Kyowa Kirin Co. Ltd, Bristol-Meyers Co. Ltd and Shionogi Pharmaceuticals Co. Ltd. Fumikazu Sakai has also received lecture fees from Daiichi-Sankyo Co. Ltd, Kyorin Pharmaceuticals Cl. Ltd and Dainihon-Sumitomo Pharmaceuticals Co. Ltd; research grants from the Japanese Ministry of Health, Labour and Welfare, the Japanese Agency of the Environment and Restoration, the Japanese Ministry of Environment, Eizai Co. Ltd, Bayer Co. Ltd, AstraZeneca Co. Ltd and LTT Bio Co. Ltd. Fumikazu

Sakai has also provided expert testimony on behalf of Maruyama Memorial Hospital and Osaka District Labour Agency of the Japanese Ministry of Health, Labour and Welfare.

Kensei Yamaguchi has received lecture fees from Merck Serono Co. Ltd, Bristol-Myers K.K. and Chugai Pharmaceutical Co. Ltd.

Toshiaki Watanabe has received consultancy fees, honoraria and research funding from Merck Serono Co. Ltd and Bristol-Myers K.K., and lecture fees and research funding from Taiho Pharmaceutical Co. Ltd, Chugai Pharmaceutical Co. Ltd, Yakult Honsha Co. Ltd and Takeda Pharmaceutical Company.

Megumi Ishiguro has received lecture fees from Merck Serono Co. Ltd, Bristol-Myers K.K., Taiho Pharmaceutical Co. Ltd, Chugai Pharmaceutical Co. Ltd and Yakult Honsha Co. Ltd.

Shogo Inoshiri is an employee of Merck Serono Co. Ltd.

Makiko Izawa is an employee of Bristol-Myers K.K.

Kenichi Sugihara has received lecture fees and research funding from Taiho Pharmaceutical Co. Ltd, Chugai Pharmaceutical Co. Ltd, Merck Serono Co. Ltd, Takeda Pharmaceutical Co. Ltd, Bristol-Myers K.K., Daiichi-Sankyo Co. Ltd and Kyowa Kirin Co. Ltd.

Yuh Sakata has received financial support to travel to meetings from Merck Serono Co. Ltd and Bristol-Myers K.K., and has received lecture fees from Taiho Pharmaceutical Co. Ltd, Yakult Honsha Co. Ltd and International Inc Synergy.

References

1. Inoue A, Saijo Y, Maemondo M, et al. Severe acute interstitial pneumonia and gefitinib. *Lancet* 2003;361:137–9.
2. Cooper JA, Jr, White DA, Matthay RA. Drug-induced pulmonary disease. Part 1: cytotoxic drugs. *Am Rev Respir Dis* 1986;133:321–40.
3. Cooper JA, Jr, White DA, Matthay RA. Drug-induced pulmonary disease. Part 2: noncytotoxic drugs. *Am Rev Respir Dis* 1986;133:488–505.
4. Tsuboi M, Le Chevalier T. Interstitial lung disease in patients with non-small-cell lung cancer treated with epidermal growth factor receptor inhibitors. *Med Oncol* 2006;23:161–70.
5. Azuma A, Kudoh S. High prevalence of drug-induced pneumonia in Japan. *Jap Med Assoc J* 2007;50:405–11.
6. Kudoh S, Kato H, Nishiwaki Y, et al. Interstitial lung disease in Japanese patients with lung cancer: a cohort and nested case–control study. *Am J Respir Crit Care Med* 2008;177:1348–57.
7. Ando M, Okamoto I, Yamamoto N, et al. Predictive factors for interstitial lung disease, antitumor response, and survival in non-small cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2006;24:2549–56.
8. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;351:337–45.
9. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007;357:2040–8.
10. Sobrero AF, Maurel J, Fehrenbacher L, et al. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;26:2311–9.
11. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408–17.
12. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008;359:1757–65.
13. Ishiguro M, Watanabe T, Yamaguchi K, et al. A Japanese post-marketing surveillance of cetuximab (Erbitux®) in patients with metastatic colorectal cancer. *Jpn J Clin Oncol* 2012;42:287–94.
14. Takano T, Ohe Y, Kusumoto M, et al. Risk factors for interstitial lung disease and predictive factors for tumor response in patients with advanced non-small cell lung cancer treated with gefitinib. *Lung Cancer* 2004;45:93–104.
15. Hotta K, Kiura K, Tabata M, et al. Interstitial lung disease in patients with non-small cell lung cancer receiving gefitinib: an analysis of risk factors and treatment outcomes in Okayama Lung Cancer Study Group. *Cancer J* 2005;11:417–24.
16. Sawada T, Inokuma S, Sato T, et al. Leflunomide-induced interstitial lung disease: prevalence and risk factors in Japanese patients with rheumatoid arthritis. *Rheumatology (Oxford)* 2009;48:1069–72.
17. McCurry J. Japan deaths spark concerns over arthritis drug. *Lancet* 2004;363:461.
18. Chou CL, Ko HW, Wang CW, Yu CT, Kuo HP, Huang CD. Erlotinib-associated near-fatal interstitial pneumonitis in a patient with relapsed lung adenocarcinoma. *Chang Gung Med J* 2010;33:100–5.
19. Shih YN, Chiu CH, Tsai CM, Perng RP. Interstitial pneumonia during gefitinib treatment of non-small-cell lung cancer. *J Chin Med Assoc* 2005;68:183–6.
20. Lin WC, Chiu CH, Liou JL, Chen YM, Perng RP, Tsai CM. Gefitinib as front-line treatment in Chinese patients with advanced non-small-cell lung cancer. *Lung Cancer* 2006;54:193–9.
21. Swigris JJ, Olson AL, Huie TJ, et al. Ethnic and racial differences in the presence of idiopathic pulmonary fibrosis at death. *Respir Med* 2012;106:588–93.
22. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med* 2011;364:1503–12.
23. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178:729–37.
24. Shimura T, Fuse N, Yoshino T, et al. Clinical features of interstitial lung disease induced by standard chemotherapy (FOLFOX or FOLFIRI) for colorectal cancer. *Ann Oncol* 2010;21:2005–10.
25. Hotta K, Kimura K, Tabata M, et al. Interstitial lung disease in Japanese patients with non-small cell lung cancer receiving gefitinib: an analysis of risk factors and treatment outcomes in Okayama Lung Cancer Study Group. *Cancer J* 2005;11:417–24.

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2014;44(6)541–546
doi:10.1093/jjco/hyu049
Advance Access Publication 24 April 2014

Severe Infusion Reactions to Cetuximab Occur within 1 h in Patients with Metastatic Colorectal Cancer: Results of a Nationwide, Multicenter, Prospective Registry Study of 2126 Patients in Japan

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Received November 8, 2013; accepted March 24, 2014

Objective: Infusion reactions are common adverse reactions associated with antibody preparations. However, no studies have examined the time to onset of serious infusion reactions after administering cetuximab. We aimed to investigate the timing and severity of IRs affecting Japanese patients after administration of cetuximab.

Methods: Study subjects were identified from a nationwide prospective registry of 2126 metastatic colorectal cancer patients scheduled to receive cetuximab. Infusion reactions were examined in 2006 patients with adequate safety data.

Results: Infusion reactions of any grade occurred in 114 patients (5.7%), including Grade 3–4 infusion reactions in 22 patients (1.1%). Premedications were antihistamine plus corticosteroid (88.9% of patients with infusion reactions), antihistamine alone (9.2%) or corticosteroid alone (1.1%). In 95 patients (83.3%), infusion reactions occurred after the first dose. Twenty of the 22 Grade 3–4 infusion reactions occurred within 1 h of the first dose (the timing of the infusion reaction was unknown in one patient while another infusion reaction occurred after the fourth dose). Infusion reactions resolved in 111/114 patients (97.4%) while one patient recovered with sequelae, one patient died and one patient failed to recover within the follow-up period. Thirteen patients (15.7% of patients with infusion reactions) with Grade 1–2 infusion reactions showed recurrence after readministration of cetuximab; the recurrent infusion reactions were less severe than the initial reactions.

Conclusions: Grade 3–4 infusion reactions occurred in 1.1% of colorectal cancer patients, and most occurred within 1 h of receiving the first dose of cetuximab. Therefore, patients should be carefully observed following cetuximab infusion, especially during the first hour after the first infusion.

Key words: cetuximab – chemotherapy – colorectal cancer – infusion reaction

INTRODUCTION

Infusion reactions (IRs) are occasionally associated with antibody preparations. In the field of anticancer therapy, IRs caused by several molecular-targeting drugs have been reported, including rituximab (1,2), trastuzumab (3,4), bevacizumab (5) and panitumumab (6). Mild-to-moderate IRs include chills, pyrexia and dizziness, which are often associated with hypersensitivity and allergic symptoms. Severe IRs include anaphylactoid symptoms, such as dyspnea, bronchospasm, urticaria, hypotension, loss of consciousness and shock, or even myocardial infarction or cardiac arrest in some patients. Therefore, IRs should be treated promptly to avoid exacerbation. It has been suggested that patients should be carefully observed after receiving the first dose of any antibody preparation (7). However, to our knowledge, no one has examined the time to onset of IRs after administering antibody preparations.

Cetuximab is an epidermal growth factor receptor (EGFR) inhibitor that is used to treat unresectable advanced or recurrent colorectal cancer. It is a human/mouse chimeric monoclonal immunoglobulin G1 antibody that inhibits the EGFR (8,9). Serious adverse reactions to cetuximab include IRs, cutaneous reactions and interstitial lung disease (11–13). An earlier clinical study identified Grade 3 and 4 IRs in 2% and 5% of 1373 patients, respectively, along with one death (10). The authors also reported that ~90% of the IRs of any grade occurred after the first dose (10). To ameliorate and/or prevent IRs, premedication with an antihistamine before administering cetuximab is recommended. Indeed, the Monoclonal Antibody Cetuximab in a European Pre-License (MABEL) study showed that administration of a corticosteroid combined with an antihistamine reduced the incidence of IRs (14,15).

In Japan, the pharmaceutical regulatory authorities require patient registration studies to be conducted for all anticancer agents after approval is granted. The objective of such studies is to confirm, in the real-world clinical setting, the accuracy of the safety profile determined during the clinical studies that were conducted to support approval. These studies, also known as postmarketing surveillance studies, examine the safety and efficacy of anticancer agents in consecutively and prospectively registered patients. Registration commences immediately after the launch of the drug and is continued until the required number of patients has been enrolled. For cetuximab, a multicenter, prospective study was planned with a target sample size of 1800 patients. The following parameters were designated as priority items for assessment: IRs, cutaneous toxicity, interstitial pneumonia, hypomagnesemia and cardiotoxicity. A summary of the results of this study has already been reported (16). The present analysis focused on the incidence, timing of onset and severity of IRs affecting Japanese patients in the real-world clinical setting. We also attempted to identify risk factors for IRs occurring after the initial dose or after readministration of cetuximab.

PATIENTS AND METHODS

Patients

A more detailed description of this registry study is provided elsewhere (16). In brief, 2126 patients scheduled to receive cetuximab at 637 institutions in Japan were prospectively enrolled in a central registry between 19 September 2008 and 5 January 2009 (16). Only patients receiving cetuximab in accordance with the approved indications were enrolled. All of the patients had EGFR-positive colorectal cancer, no history of hypersensitivity to any ingredients of the drug, a performance status (PS) of 0–1, no interstitial lung disease and a resistant/refractory tumor or intolerance of prior chemotherapy. Cetuximab was administered by intravenous infusion once weekly, with no limit on the number of doses or duration of treatment. The first dose (400 mg/m^2) was infused over 2 h, and subsequent doses (250 mg/m^2) were given over 1 h (10). When the study was planned, there were no Japanese data on the efficacy or safety of cetuximab combined with oxaliplatin-based regimens, so irinotecan or FOLFIRI regimens (folinic acid, fluorouracil and irinotecan) could be used instead. The product information for cetuximab states that an antihistamine should be given as premedication before administration and concomitant use of a corticosteroid may reduce the risk of IRs. Therefore, premedication with an antihistamine and/or corticosteroid was recommended.

Evaluation and Analysis

The observation period was defined as the time between the first and last doses of cetuximab. The attending physicians were instructed to submit case report forms documenting safety and efficacy in Weeks 4 and 16 after the first dose, and after the final dose. The safety and efficacy results were described in an earlier report (16). Adverse event data were compiled by the Central Data Centre, and the severity of adverse events was assessed according to Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. Adverse events are described using CTCAE terminology. Priority items included the presence or absence and severity of IRs, cutaneous disorders, interstitial lung disease, electrolyte abnormalities (e.g. hypomagnesemia), cardiotoxicity, gastrointestinal disorders, thrombosis/embolism, delayed wound healing and ocular disorders (e.g. keratitis). Detailed information on IRs was obtained from the case report forms, and included seriousness, date of onset, time from starting treatment to onset, outcome and premedication(s), and whether or not readministration was attempted.

Statistical Analysis

Data were compiled and analyzed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The incidence of IRs was evaluated in relation to patient characteristics (sex, age, stage, PS, complications, previous illnesses, history of allergy, premedication and concomitant drugs) in univariate analyses

using the χ^2 test, Fisher's exact test, or Wilcoxon two-sample test as was appropriate. In all analyses, $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Patient Characteristics

A total of 154 patients who were enrolled did not receive cetuximab and were excluded from analysis. The remaining 2006 patients were included in the safety analysis set. Their median age was 64 years (range: 18–87 years), and the male/female ratio was 1 : 0.6. The PS was 0 or 1 in 2000 patients (99.7%) and 3 or 4 in six patients (0.3%). Concurrent diseases were present in 974 patients (48.6%), including hypertension in 447 patients (22.3%), diabetes mellitus in 284 patients (14.2%), liver dysfunction in 93 patients (4.6%), hyperlipidemia in 86 patients (4.3%) and heart diseases in 79 patients (3.9%). Overall, 405 patients (20.2%) had a history of clinically relevant diseases, including allergy in 306 patients (15.4%), heart disease in 27 patients (1.3%) and interstitial lung disease in four patients (0.2%).

The median duration of cetuximab treatment was 15.3 weeks (1–73.9 weeks), and 1869 patients (93.2%) received cetuximab as third-line or later treatment. The number of doses of cetuximab was ≤3 in 12.6%, 4–15 in 44.9%, 32–47 in 12.7% and ≥48 in 3.7% of patients. Four hundred and sixty patients (22.9%) received cetuximab alone while 1546 patients (77.1%) received cetuximab in combination with chemotherapy. The chemotherapy regimens included irinotecan in 1255 patients (62.6%) and FOLFIRI in 256 patients (12.8%). Premedication was given to 1991 patients (99.3%).

Incidence and Severity of IRs

IRs were reported in 114/2006 patients (5.7%) and were classified as Grade 3 in 13 patients and Grade 4 in nine patients (Grades 3–4: 1.1%). The most common IRs were classified (using CTCAE terminology) as general disorders and administration site conditions (e.g. infusion-related reactions and pyrexia), which occurred in 114 (5.7%, any grade) of patients, including 1.1% classified as Grades 3–4. Other IRs (which were observed in individuals who were also classified as having general disorders and administration site reactions) included respiratory, thoracic and mediastinal disorders (e.g. dyspnea) in 26 patients (1.3%; Grades 3–4: 0.4%), skin and subcutaneous tissue disorders in 23 patients (1.1%; Grades 3–4: 0.1%), vascular disorders (e.g. flushing) in 15 patients (0.7%), immune system disorders (including anaphylactic shock and hypersensitivity) in 14 patients (0.7%; Grades 3–4: 0.4%), nervous system disorders (≤0.1%), cardiac disorders (≤0.1%), gastrointestinal disorders (0.1%) and investigations (0.3%).

Timing of IRs

Of the 114 patients, 95 (83.3%), 6 (5.3%), 5 (4.4%), 3 (2.6%) and 4 (3.5%) experienced IRs after the first, second, third, fourth and fifth or subsequent doses of cetuximab, respectively (Fig. 1). Grade 3–4 IRs occurred in 22 patients (Grade 3 in 13 patients and Grade 4 in nine patients). Among them, 20 patients (90.9%) experienced IRs after the first dose and one patient (4.5%) did so after the fourth dose, while the timing was unknown for one patient (4.5%).

The timing of each symptom after the start of cetuximab infusion in the 22 patients with Grade 3 or 4 IRs is shown in Fig. 2. The median time to the onset of reactions in these 22 patients was 10 min (range: 2 min to 8 h). In 18 patients, IRs occurred within 15 min after the start of infusion. Except for fever at 8 h after the start of cetuximab infusion in one patient (which was considered by the investigator to be at least partly related to a biliary tract infection in this patient), all of the other IRs occurred within 1 h of starting infusion. Interestingly, all of the Grade 4 IRs occurred within 15 min of starting infusion.

Outcome

The IR resolved/improved in 111/114 patients (97.4%). One patient experienced flushing (a vascular symptom and IR) and other symptoms associated with the infusion, although the nature of these symptoms was not reported. This patient did not recover from these symptoms during the follow-up period of this survey. One patient who had pruritus recovered with sequelae. One patient with Grade 4 IRs died. In this patient, the Grade 4 IRs were dyspnea and low blood pressure, which were detected 10 min after the start of infusion. The median time to recovery was 1 day (range: 0–366 days) in patients with Grade 1–2 IRs and 1 day (range: 1–15 days) in patients with Grade 3–4 IRs.

Incidence of IRs According to Premedication

Premedication consisted of an antihistamine plus a corticosteroid in 1783 patients (88.9% of the patients in the safety analysis set), an antihistamine alone in 185 patients (9.2%) and a corticosteroid alone in 23 patients (1.1%). The incidence of IRs was similar between patients pretreated with an

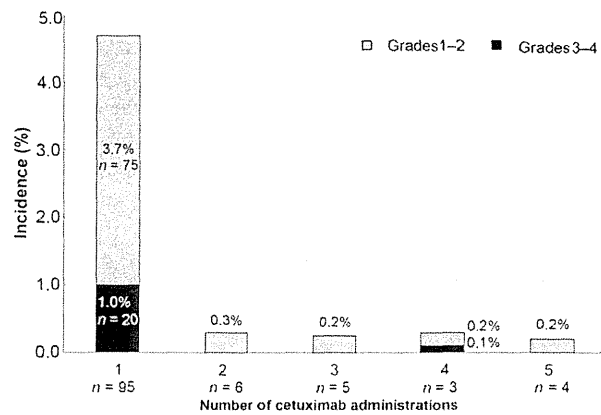

Figure 1. Timing of infusion reactions (IRs) in relation to the number of cetuximab doses. One patient who had a Grade 3 event with unknown timing was excluded from the analysis.

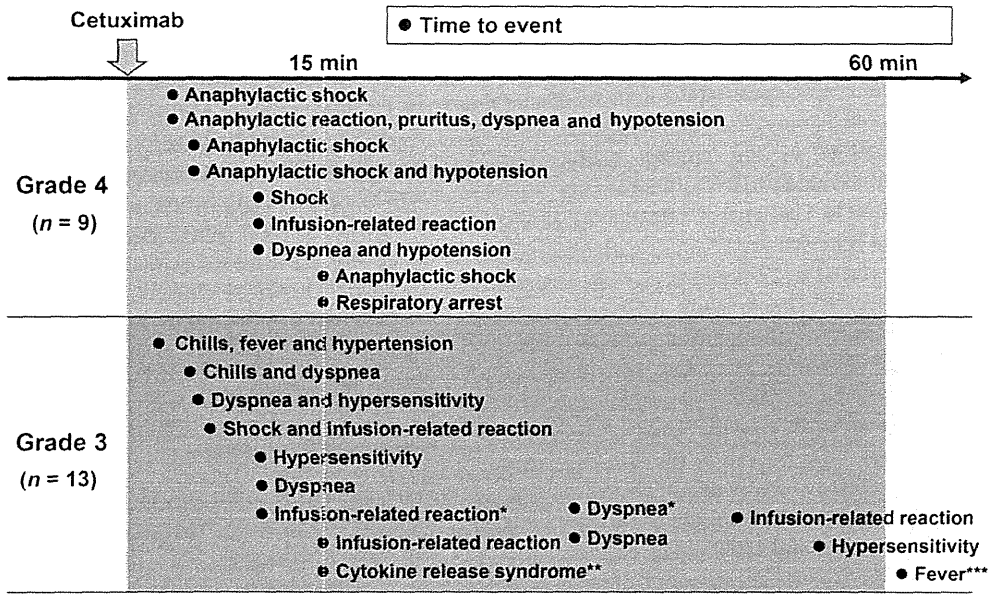

Figure 2. Time to onset of Grade 3–4 IRs. The median time was 10 min (range: 2 min to 8 h). Dots indicate individual patients. Asterisks indicate multiple events in the same patient. *Identical patients. **This patient had dyspnea, bronchospasm, tachycardia and nausea. ***Considered related to biliary tract infection and liver metastasis.

antihistamine plus corticosteroid and those pretreated with an antihistamine alone [5.9% (105/1783) versus 4.3% (8/185)]. The incidence of Grade 3–4 IRs was 1.1% (20/1783 patients) in patients pretreated with an antihistamine plus corticosteroid and 0.5% in those pretreated with an antihistamine alone (1/185 patients).

Recurrence of IRs after Cetuximab Readministration

Overall, 85 of 114 patients who experienced IRs after the first infusion underwent cetuximab readministration (83 patients with Grade 1–2 IRs and two patients with Grade 3–4 IRs). Of these 85 patients, 14 (16.3%) developed an IR following readministration. The product information for cetuximab in Japan and other countries states that it should be immediately discontinued in patients with Grade 3–4 IRs and that such patients should not receive readministration. However, it was readministered in two patients with Grade 3–4 IRs, but no adverse events were observed in either patient.

Risk Factors for IRs

Univariate analysis was conducted to identify possible risk factors for IRs. It was found that the incidence of IRs was significantly higher in patients with a history of heart disease or interstitial lung disease than in patients without a history of such diseases (8.6 versus 5.0%, $P = 0.0077$). The incidence of IRs was also significantly higher in patients treated with cetuximab alone than in those treated with cetuximab plus chemotherapy (8.0 versus 5.0%, $P = 0.0158$). Furthermore, the incidence of IRs was slightly, but not significantly, higher in patients with a history of allergy than in patients without a history of allergy (8.4 versus 5.3%, $P = 0.0822$). No association was found with other characteristics, such as sex, age, chemotherapy stage and PS.

DISCUSSION

The present survey showed that IRs occurred in 114/2006 patients (5.7%) included in the safety analysis set while Grade 3–4 IRs occurred in 22 patients (1.1%). The majority of Grade 3–4 IRs were detected within 1 h of starting infusion, which suggests that careful observation is necessary for ≥ 1 h after first administering cetuximab in routine clinical practice.

Most of the IRs occurred after the first dose of cetuximab, including 90% of Grade 3–4 IRs, although one patient developed a severe IR after the fourth dose. Therefore, careful observation is particularly important after the first dose but physicians should continue to be vigilant for possible IRs after subsequent doses of cetuximab.

With regard to the timing of IRs, all of the Grade 3–4 reactions occurred within 1 h of starting infusion, except in one patient who developed an IR at 8 h. However, that patient's symptom was fever, which is not a typical IR symptom and was considered to be at least partly related to biliary tract infection and liver metastasis.

The IRs resolved/improved in 97.4% of the patients, with a median recovery time of 1 day. IRs caused by cetuximab appear to resolve promptly by appropriate treatment. However, persistence of the IR, recovery with sequelae and death were reported in one patient each. Clinicians should be fully aware of these risks of IRs and inform patients of them before starting cetuximab therapy.

IRs associated with cetuximab were extensively described in the MABEL study (14,15), a multinational, Phase 2 study that examined the efficacy and safety of cetuximab combined with irinotecan in 1147 patients with metastatic colorectal cancer refractory to irinotecan monotherapy. A similar number of patients received this combination in the present study (1546). Premedication was administered to 1991/2006 patients (99.3%) in our study and to 1122/1147 patients (97.8%) in the MABEL study. In that study, IRs occurred in 175/1122 patients (15.6%) and Grade 3–4 IRs occurred in 27 patients (2.4%), compared with 5.7 and 1.1%, respectively, in our study. Although we found no difference in the incidence or IRs between patients receiving premedication with an antihistamine alone and those treated with an antihistamine plus a corticosteroid, it is notable that 88.9% of our patients received antihistamine plus corticosteroid premedication compared with 61.0% in the MABEL study. Therefore, it seems likely that concomitant corticosteroid use was protective against IRs. Indeed, the lower rate of Grade 3–4 IRs in our study (1.1%) compared with that in the MABEL study (2.4%) may be attributable to a higher proportion of patients premedicated with antihistamines combined with corticosteroids. The lack of a difference in the incidence of IRs between patients receiving premedication with an antihistamine alone and those treated with an antihistamine plus a corticosteroid in the present study is probably due to the small number of events and the small number of patients treated with an antihistamine alone.

The incidence of IRs varies greatly among antibody preparations. For example, the incidence of IRs occurring within 24 h after the start of administration is ~90% for rituximab (chimeric), ~40% for trastuzumab (humanized) and<3% for bevacizumab (humanized) (1,3,5). The incidence of IRs caused by cetuximab in the present survey (5.7%) was higher than that caused by panitumumab (3%: 43/1336 patients; Grade 3–4 IRs: 1%, six patients) (6), which is a fully human anti-EGFR antibody.

Regarding readministration of cetuximab after the occurrence of mild-to-moderate IRs (Grades 1–2), cetuximab was readministered to 83 of 92 patients with Grade 1–2 IRs, and 13 (15.7%) developed recurrent IRs. However, none of those IRs was more severe than the initial reactions. Based on these results, it may be necessary to change the premedication or reduce the infusion rate in patients with Grade 1–2 IRs who undergo readministration of cetuximab.

Some limitations of this study should be discussed. First, this was a nonrandomized and uncontrolled study. Therefore, it is possible that there is a bias in the data obtained; however, given the sample size of 2006 patients, we believe such a bias is unlikely. Second, the case report forms did not allow the clinicians to record the cause of death or sequelae in patients who did not recover from IRs; therefore, it is possible that there were serious events related to cetuximab infusion that we were unable to identify. Likewise, because the symptoms were reported by the investigators, those symptoms that did not recover by the end of the follow-up period were classified as unrecovered. Third, although the quality of the data is different from that of controlled prospective clinical trials, this prospective registry provided us with valuable information on the safety and effectiveness of cetuximab in real-world usage.

In summary, Grade 3–4 IRs caused by cetuximab occurred in 1.1% of patients in this prospective registry. Most Grade 3–4 IRs occurred within 60 min after the initial administration. Therefore, we think that patients should be very carefully observed following infusion of cetuximab, especially during the first hour after initial administration. Notably, there were no severe IRs after readministration of cetuximab after Grade 1–2 IRs. Therefore, clinicians should consider the potential risks/benefits when readministering cetuximab after IRs and ensure the patients are fully informed of the possible risks of cetuximab therapy.

Acknowledgements

We are grateful to the physicians, patients and their families for their cooperation. We also thank Nicholas D. Smith, PhD and Daniel McGowan, PhD, for their editorial support.

Funding

This work was supported by Bristol-Myers K.K and Merck Serono Co., Ltd., Japan. Funding to pay the Open Access publication charges for this article was provided by Bristol-Myers K.K and Merck and Serono Co., Ltd., Japan.

Conflict of interest statement

Kensei Yamaguchi has provided consultancy services to, and received honoraria from Merck Serono Co. Ltd. and Bristol-Myers K.K.; Toshiaki Watanabe has received honoraria and research funding from Merck Serono Co. Ltd. and Bristol-Myers K.K.; Taroh Satoh has received honoraria from Merck Serono Co. Ltd. and Bristol-Myers K.K.; Makiko Izawa is an employee of Bristol-Myers K.K.; Shogo Inoshiri is an employee of Merck Serono Co. Ltd.; Kenichi Sugihara has received honoraria and research funding from Merck Serono Co. Ltd. and Bristol-Myers K.K.; Yuh Sakata has received honoraria from Merck Serono Co. Ltd. and Bristol-Myers K.K. Megumi Ishiguro has no conflict of interest to declare.

References

1. Rituxan® (rituximab). *Package Insert*. South San Francisco, CA: Genentech, Inc. (Revised: 04/2011).
2. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large B-cell lymphoma. *N Engl J Med* 2002;346:235–42.
3. Herceptin® (trastuzumab). *Package Insert*. South San Francisco, CA: Genentech, Inc. (Revised: 10/2010).
4. Cook-Bruns N. Retrospective analysis of the safety of Herceptin immunotherapy in metastatic breast cancer. *Oncology* 2001;61(Suppl 2): 58–66.
5. Avastin® (bevacizumab). *Package Insert*. South San Francisco, CA: Genentech, Inc. (Revised: 12/2011).
6. Vectibix® (panitumumab). *Package Insert*. Thousand Oaks, CA: Amgen Inc. (Revised: 12/2011).
7. Lenz HJ. Management and preparedness for infusion and hypersensitivity reactions. *Oncologist* 2007;12:601–9.