original report

Impact of the *Bim* Deletion Polymorphism on Survival Among Patients With Completely Resected Non–Small-Cell Lung Carcinoma

abstract

Purpose A deletion polymorphism of the *Bim* gene has been reported to be a prognostic factor for patients with non–small-cell lung cancer (NSCLC) treated with epidermal growth factor receptor-tyrosine kinase inhibitors in the Asian population. We investigated the impact of the *Bim* deletion polymorphism on survival among patients with completely resected NSCLC.

Patients and Methods The *Bim* polymorphism was detected by polymerase chain reaction analysis. We measured overall survival (OS) and recurrence-free survival rates in 411 patients and postrecurrence survival (PRS) in 94 patients who experienced recurrence and received additional anticancer therapy.

Results The *Bim* deletion polymorphism was detected in 61 patients (14.8%). OS rates were significantly lower for patients with the *Bim* deletion polymorphism than for those with the wild-type sequence. On multivariable analysis, the *Bim* deletion polymorphism was identified as an independent prognostic factor for OS (hazard ratio, 1.98; 95% CI, 1.17 to 3.36; $P = .011$). Among the 94 patients who experienced recurrence and were treated with anticancer therapy, patients with the *Bim* deletion polymorphism showed significantly poorer PRS than those with the wild-type sequence (median, 9.8 months *v* 26.9 months, respectively; $P < .001$). Multivariable analysis revealed that the *Bim* deletion polymorphism was an independent predictor of PRS (hazard ratio, 3.36; 95% CI, 1.75 to 6.47; $P < .001$). This trend remained apparent in subgroup analyses stratified by *EGFR* status, histology, and therapeutic modality.

Conclusion The *Bim* deletion polymorphism is a novel indicator of shortened PRS among patients with recurrent NSCLC treated with anticancer therapy in the Asian population.

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INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide.[1] Even after radical surgery in patients with early-stage non–small-cell lung cancer (NSCLC), 30% to 40% of patients experience recurrence within 5 years.[2,3] Postoperative recurrent disease is usually treated as metastatic NSCLC. Although molecule-targeted drug therapies such as epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have produced considerable survival benefits in patients with both advanced disease and postoperative recurrence of *EGFR*-mutated NSCLC,[4-8] the majority of patients eventually become refractory to these therapies.

B-cell chronic lymphatic leukemia lymphoma 2-like 11 (BCL2L11), or BIM, is a proapoptotic member of the Bcl-2 protein family and is a key modulator of EGFR-TKI–induced apoptosis in NSCLC cell lines.[9] Ng et al[10] reported a common intronic deletion with a 2,903-base pair (bp) polymorphism in the gene encoding BIM. This deletion polymorphism leads to impaired expression of BH3-containing BIM isoforms, resulting in resistance to EGFR-TKIs in patients with NSCLC who have *EGFR* mutations. Interestingly, this deletion polymorphism was observed only in East Asian populations.[10] Several clinical studies of East Asian populations have indicated that the *Bim* deletion polymorphism is an independent prognostic factor for progression-free survival in advanced *EGFR*-mutated NSCLC treated with EGFR-TKIs[11,12] and cytotoxic chemotherapy.[13] The *Bim* deletion polymorphism is expected to

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be a novel biomarker in anticancer therapy against inoperable NSCLC, especially adenocarcinoma. Patients with NSCLC who have recurrence after curative surgery have a more favorable prognosis than those with advanced-stage disease at initial presentation, because patients with NSCLC who have postoperative recurrence have different characteristics from those with stage IV disease.[14,15] However, there have been no studies regarding the prognostic power of the *Bim* deletion polymorphism in postoperative patients with lung cancer, including those with non-adenocarcinoma histology, or the influence of the polymorphism on postrecurrence treatment.

We hypothesized that the *Bim* deletion polymorphism affects survival among patients with postoperative recurrent NSCLC who have received anticancer therapy. In this study, we investigated the impact of the *Bim* deletion polymorphism on the outcomes of patients with completely resected NSCLC.

PATIENTS AND METHODS

Patients and data collection

A total of 565 patients with NSCLC who underwent pulmonary resection at Gunma University Hospital between June 2003 and December 2013 were identified in our database. Among these patients, 481 underwent complete resection (lobectomy or greater with systematic lymph node dissection) without induction chemotherapy or radiotherapy. We excluded patients with residual lesions (macroscopically or microscopically apparent), as well as those with pathologic stage IV disease and those without adequate documentation. Consequently, 411 patients were eligible for inclusion in this study. Histologic diagnoses were made on the basis of WHO criteria,[16] and disease stage was determined according to the TNM Classification of Malignant Tumors, 7th edition. This study was approved by the ethics committee of Gunma University Hospital. Informed consent for a global genome analysis of samples was obtained from each patient before inclusion in the study. Institutional review board approval for the study was obtained for the analysis of Bim and other genes in those samples.

Diagnosis of Recurrence and Survival Analysis

Patients were followed at 3-month intervals for the first 2 years and at 6-month intervals thereafter on an outpatient basis. Follow-up evaluation included a physical examination, chest radiography, and blood analysis, including analysis of pertinent tumor markers. Computed tomography of the chest and abdomen or positron emission tomography-computed tomography was performed annually. When symptoms or signs of recurrence were detected, further evaluations were performed. Recurrence was diagnosed based on compatible physical examination and diagnostic imaging findings, and the diagnosis was confirmed histologically when clinically feasible. The date of recurrence was defined as the date of histologic confirmation, or in patients whose diagnosis was based on clinical evidence, the date of recognition of recurrent disease by the attending physician. Local recurrence was defined as disease recurrence at the surgical margin, ipsilateral hemithorax, or mediastinum. Distant metastasis was defined as disease recurrence in the contralateral lung or outside the hemithorax and mediastinum.

The overall survival (OS) period was defined as the time between the date of surgery and the date of death as a result of any cause. Patients who were lost to follow-up were censored from analysis at the time of the last negative follow-up. For the patients who developed recurrent disease during follow-up, postrecurrence survival (PRS) was measured from the date of initial recurrence to the date of death as a result of any cause or the date on which the patient was last known to be alive. Recurrence-free survival (RFS) was measured from the date of surgery to the date of initial recurrence.

DNA Extraction and Gene Analysis

After surgical removal of the tumor, a portion of each sample was immediately frozen and stored at −80°C before DNA extraction. Genomic DNA was extracted from a 3- to 5-mm cube of tumor tissue by using DNA Mini Kits (QIAGEN, Hilden, Germany) and was subsequently diluted to a concentration of 20 ng/μL. *EGFR* mutations in lung cancer tissue were analyzed by peptide nucleic acid–enriched sequencing, as described previously.[17] Presence of the *Bim* deletion polymorphism was analyzed by first extracting DNA from peripheral blood mononuclear cells by using a QIAamp DNA Blood Mini Kit (QIAGEN, Venlo, the Netherlands) followed by polymerase chain reaction assay as described previously.[11]

Statistical Analysis

Statistical analyses were conducted by using SPSS software for Windows, version 12.0 (SPSS, Chicago, IL) and Power and Sample Size Calculation software, version 3.1.2 (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). All categorical variables were analyzed by using the χ^2 test. Continuous variables were compared by

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using the independent samples *t* test. Survival was analyzed by using the Kaplan-Meier method, and statistical analysis was performed by using the log-rank test. Prognostic groups were assessed by using Cox proportional hazards regression analysis. Variables significantly associated with OS and PRS on univariable analysis were tested by multivariable analysis using a Cox proportional hazards regression model. A two-tailed *P* value of less than .05 was taken to indicate statistical significance. On the basis of previous reports,[12,13,18] we assumed that 13.7% of Japanese patients had the Bim deletion polymorphism and an OS of 24.8 and 16.8 months, respectively, for patients with advanced NSCLC who received anticancer therapy in the *Bim* wild-type and *Bim* deletion groups. Under these assumptions, with a two-tailed α of .05 and power at 0.8, 64 patients with the *Bim* deletion polymorphism and 403 patients with the wild-type sequence were required to evaluate the effect of the *Bim* deletion polymorphism on PRS for anticancer therapy.

RESULTS

Clinicopathologic Characteristics

Patient characteristics are presented in Table 1. All patients were Japanese. The median age at the

Table 1 – Baseline Patient Characteristics and *Bim* Deletion Polymorphism Distribution

Variable	All (n = 411)	*Bim* Polymorphism Status		*P*
		Wild Type (n = 349) No. (%)	Deletion Polymorphism (n = 61) No. (%)	
Median age, years	67.6	67.9	66.5	.294
Sex				.483
Female	175	152 (86.9)	23 (13.1)	
Male	236	198 (83.9)	38 (16.1)	
Smoking status				.086
Never smoker	157	140 (89.2)	17 (10.8)	
Ever smoker	254	210 (82.7)	44 (17.3)	
Histology				.064*
ADC	297	259 (87.2)	38 (12.8)	
SQC	93	74 (79.6)	19 (20.4)	
Other	21	17 (81.0)	4 (19.0)	
Tumor size, cm				.124
≤ 3	248	215 (86.7)	33 (13.3)	
> 3	163	135 (82.8)	28 (17.2)	
Node metastases				.007
N0	303	267 (88.1)	36 (11.9)	
N1-2	108	83 (76.9)	25 (23.1)	
Vascular invasion				.197
Negative	256	223 (87.1)	33 (12.9)	
Positive	155	127 (81.9)	28 (18.1)	
Lymphatic invasion				.024
Negative	238	211 (88.7)	27 (11.3)	
Positive	173	139 (80.3)	34 (19.7)	
Pathologic stage				.001
I	275	246 (89.5)	29 (10.5)	
II or III	136	104 (76.5)	32 (23.5)	
EGFR gene				1.000
Wild type	276	235 (85.1)	41 (14.9)	
Mutation	135	115 (85.2)	20 (14.8)	

Abbreviations: ADC, adenocarcinoma; EGFR, epidermal growth factor receptor; SQC, squamous cell carcinoma.
*ADC *v* SQC and other.

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time of surgery was 67.6 years (range, 36 to 90 years), and the study population consisted of 175 females and 236 males. On the basis of the histology of the lesions, the study population included 297 adenocarcinomas, 93 squamous cell carcinomas, 12 large-cell neuroendocrine carcinomas, seven large-cell carcinomas, and two adenosquamous cell carcinomas. With regard to diagnosis, 275 patients were classified as pathologic stage I, and 136 patients were classified as stage II or III. *EGFR* mutation was detected in 135 tumors (32.8%) consisting of 133 adenocarcinomas and two squamous cell carcinomas. The *Bim* deletion polymorphism was detected in 61 patients (14.8%). The percentage of patients according to sex, smoking history, histology, and *EGFR* mutational status did not differ significantly between the *Bim* wild-type and *Bim* deletion polymorphism groups, although the percentage of lymph node metastases, positive lymphatic invasion, and advanced stage in the patients with *Bim* deletion polymorphism was significantly higher than in those with the wild-type *Bim* sequence (Table 1).

Prognostic Impact of *Bim* Polymorphism on OS and RFS

Factors associated with OS and RFS, as revealed through univariable analysis, included sex, smoking status, histology, vascular invasion, lymphatic invasion, pathologic stage, *EGFR* gene status, and *Bim* polymorphism. In the multivariable analysis, pathologic stage, *EGFR* gene mutation, and the *Bim* deletion polymorphism were independent factors associated with OS, and pathologic stage and lymphatic invasion were independent factors associated only with poor RFS (Table 2). The *Bim* deletion polymorphism was independently associated with OS but not RFS in 411 patients. The 5-year OS rate was significantly lower for patients with the *Bim* deletion polymorphism compared with those with wild-type *Bim* (58.8% *v* 78.9%, respectively; $P < .001$; Fig 1A). To eliminate bias, we analyzed survival by using propensity score matching (Data Supplement). The 5-year OS in the propensity score–matched analysis was also significantly poorer in patients with *Bim* deletion than in those with wild-type *Bim* (58.8% *v* 80.3%, respectively; $P = .036$; Fig 1B).

In addition, we investigated RFS among patients who developed recurrence. As of October 2014, 109 patients had experienced recurrence. Patient characteristics are shown in the Data Supplement. In the univariable analysis, the variables associated with RFS in patients with recurrence were vascular invasion and *Bim* deletion polymorphism, and these remained as independent factors in the multivariable analysis (Data Supplement). Furthermore, patients with the *Bim* deletion polymorphism showed significantly shortened RFS compared with those with wild-type *Bim* (median, 9.8 *v* 13.9 months, respectively; $P = .003$; Fig 2A).

Prognostic Impact of *Bim* Polymorphism on PRS

To determine the impact of the *Bim* deletion polymorphism on outcome after recurrence, we investigated 94 (86%) of 109 patients with recurrent disease who received additional anticancer therapy, including cytotoxic chemotherapy, EGFR-TKIs, or radiotherapy with curative intent. The characteristics of the 94 patients who received anticancer therapies are summarized in Table 3. The median time to follow-up was 16.4 months (range, 2.0 to 91.8 months), median age at recurrence was 68.6 years (range, 37 to 80 years), and the patients consisted of 38 females and 56 males. There were 65 patients with adenocarcinoma and 29 with non-adenocarcinomas (23 squamous cell carcinomas, four large-cell neuroendocrine carcinomas, and two large-cell carcinomas). Sixteen patients (17%) harbored the *Bim* deletion polymorphism, and 29 patients (31%) harbored

Table 2 – Multivariable Analysis of Predictors of OS and RFS

Variable	OS		RFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Female sex	1.008 (0.456 to 2.229)	.985	1.056 (0.562 to 1.985)	.866
Ever smoker	0.817 (0.330 to 2.020)	.662	0.974 (0.487 to 1.945)	.940
ADC histology	0.656 (0.375 to 1.147)	.139	0.776 (0.495 to 1.218)	.271
Positive vascular invasion	1.211 (0.704 to 2.084)	.489	1.039 (0.675 to 1.601)	.861
Positive lymphatic invasion	1.210 (0.674 to 2.171)	.524	1.673 (1.032 to 2.711)	.037
Pathologic stage II or III	5.213 (2.913 to 9.327)	< .001	4.738 (2.994 to 7.498)	< .001
EGFR mutated	0.358 (0.172 to 0.743)	.006	0.762 (0.466 to 1.245)	.277
Bim deletion polymorphism	1.979 (1.166 to 3.357)	.011	1.231 (0.781 to 1.939)	.370

Abbreviations: ADC, adenocarcinoma; *EGFR*, epidermal growth factor receptor; HR, hazard ratio; OS, overall survival; RFS, recurrence-free survival.

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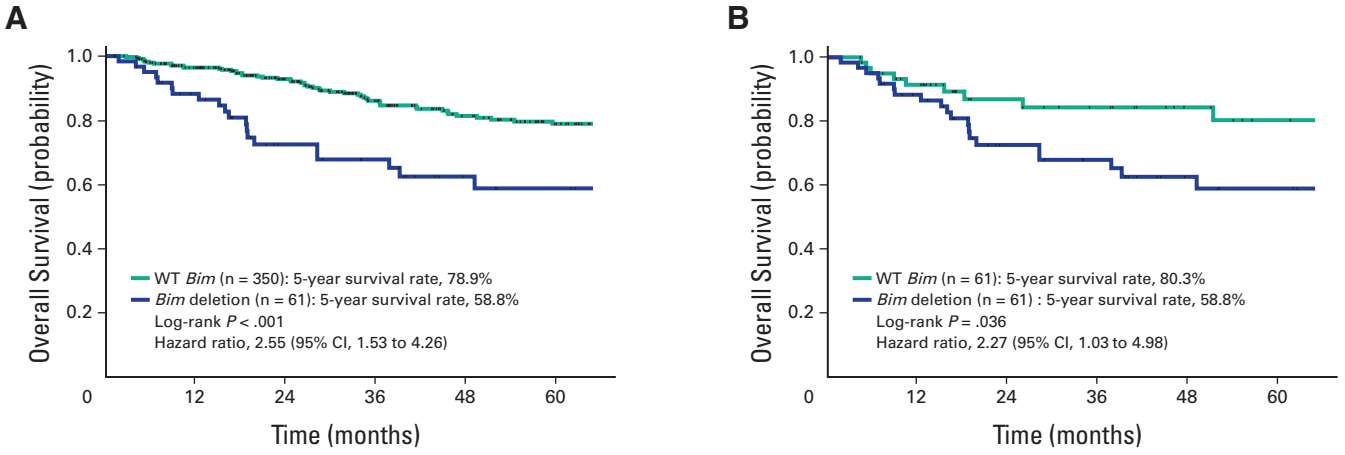

Figure 1 –
Kaplan-Meier curves for overall survival according to the *Bim* polymorphism. Overall survival in (A) 411 patients with non–small-cell lung cancer and (B) propensity score–matched patients (n = 122). WT, wild type.

EGFR-mutated tumors. Thirty-seven patients (39%) showed local recurrence only, and 59 patients (61%) showed distant recurrence. Recurrence in multiple foci was detected in 65 patients (69%). Treatment for recurrence consisted of platinum-based chemotherapy in 43 patients (46%), radiotherapy in 43 patients (46%), and EGFR-TKIs in 33 patients (35%). No significant differences in age, sex, tumor histology, smoking status, pathologic stage, site of recurrence, number of recurrent foci, *EGFR* gene status, or therapeutic modality were observed between patients with the *Bim* deletion polymorphism and those with wild-type *Bim* (Table 3).

Univariable analysis indicated that RFS shorter than 12 months, *EGFR* gene status, and *Bim* polymorphism influenced PRS, all of which remained as independent prognostic factors for PRS in the multivariable analysis (Table 4). Median PRS was 26.9 months among those with wild-type *Bim* and 11.4 months among those with the *Bim* deletion polymorphism ($P < .001$; Fig 2). Subset analysis of PRS showed that patients with wild-type *Bim* consistently showed prolonged survival compared with those with the deletion polymorphism when stratified by *EGFR* gene status (mutated: median, 61.0 *v* 23.2 months; $P < .001$; Fig 3A; wild-type: median, 19.7 *v* 9.8 months; $P = .001$; Fig 3B) or tumor histology (adenocarcinoma: median, 33.9 *v* 11.4 months; $P = .009$; Fig 3C; non-adenocarcinoma: median, 19.7 *v* 9.8 months; $P = .013$; Fig 3D). When analyzed according to therapeutic modality, the median PRS was significantly shorter in patients with the *Bim* deletion polymorphism compared with those with the wild-type *Bim* or *EGFR*-mutated NSCLC treated with EGFR-TKIs (median, 38.1 *v* 23.2 months, respectively; $P = .007$; Fig 4A), those treated with cytotoxic chemotherapy alone (median, 18.5 *v* 6.2 months, respectively; $P = .003$; Fig 4B), and those treated with radiotherapy alone (median, 26.9 *v* 11.4 months, respectively; $P = .046$; Fig 4C). No bias was observed in the distribution of the *Bim* deletion polymorphism in terms of platinum or taxane use among the 23 patients who received cytotoxic chemotherapy. Similarly, there was no significant difference in the distribution of the *Bim* deletion polymorphism according to the radiotherapy method (conventional or cyberknife) or total

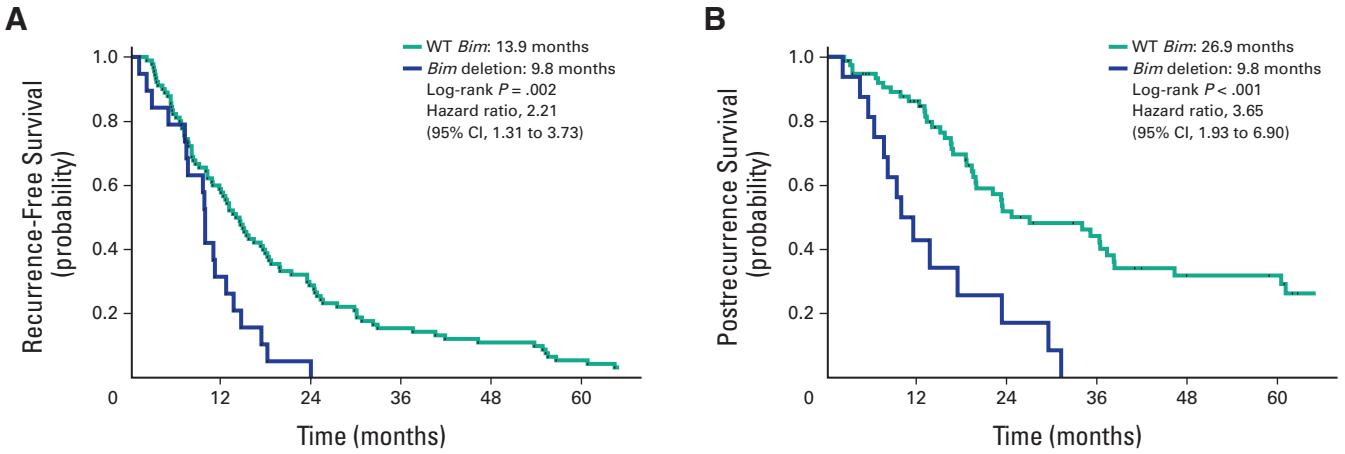

Figure 2 –
Kaplan-Meier survival curves for patients who developed recurrent disease classified according to the *Bim* deletion polymorphism. (A) Recurrence-free survival in 109 patients who developed recurrence. (B) Postrecurrence survival in 94 patients who received anticancer therapy after recurrence. WT, wild type.

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Table 3 – Characteristics of Patients Who Received Anticancer Therapy After Recurrence and *Bim* Deletion Polymorphism Distribution (n = 94)

Variable	All (n = 94)	*Bim* Polymorphism Status		*P*
		Wild Type (n = 78) No. (%)	Deletion Polymorphism (n = 16) No. (%)	
Median age (years)	68.6	69.2	66.1	.284
Sex				1.000
Female	38	32 (84.2)	6 (15.8)	
Male	56	46 (82.1)	10 (17.9)	
Smoking status				.405
Never smoker	33	29 (87.9)	4 (12.1)	
Ever smoker	61	49 (80.3)	12 (19.7)	
Histology				.244*
ADC	65	56 (86.2)	9 (13.8)	
SQC	23	18 (78.3)	5 (21.7)	
Other	6	4 (66.7)	2 (33.3)	
Pathologic stage				.135
I	29	27 (93.1)	2 (6.9)	
II or III	65	51 (78.5)	14 (21.5)	
Recurrence-free survival, months				.093
< 12	39	29 (74.4)	10 (25.6)	
≥ 12	55	49 (89.1)	6 (10.9)	
Site of recurrence				.579
Local only	37	32 (86.5)	5 (13.5)	
Distant	57	46 (80.7)	11 (19.3)	
Recurrent foci				.573
Single	30	24 (80.0)	6 (20.0)	
Multiple	64	54 (84.4)	10 (15.6)	
EGFR gene				1.000
Wild type	65	54 (83.1)	11 (16.9)	
Mutation	29	24 (82.8)	5 (17.2)	
Treatment				
Platinum-based chemotherapy	43	35 (81.4)	8 (18.6)	.786
Radiotherapy	43	34 (79.1)	9 (20.9)	.415
EGFR-TKI	33	30 (90.1)	3 (9.1)	.160

Abbreviations: ADC, adenocarcinoma; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; SQC, squamous cell carcinoma.
*ADC *v* SQC and other.

radiation dose among the 22 patients who received radiotherapy alone (Table 5).

DISCUSSION

The *Bim* deletion polymorphism has been investigated in inoperable advanced NSCLC and identified as a heritable factor conferring resistance to EGFR-TKIs and chemotherapy in the Asian population.[10-13,19] However, only one report has examined the impact of the *Bim* deletion polymorphism on survival in patients with resectable NSCLC.[18] In this study, we demonstrated the impact of the *Bim* deletion polymorphism on NSCLC outcomes (survival) after complete tumor resection. The *Bim* deletion polymorphism was an independent unfavorable prognostic factor of OS in all patients with NSCLC who received complete resection, which was the result of shorter RFS and PRS associated with the *Bim* deletion polymorphism among those who developed recurrent disease. Furthermore, the PRS trend was consistent in subgroup analyses stratified by *EGFR* mutation status, histology, and therapeutic modality. On the basis of the results of this study, we suggest that the *Bim* deletion polymorphism has a positive impact on early emergence of metastasis and a negative impact on anticancer treatment in recurrent NSCLC.

There have been few studies regarding the biologic characteristics associated with *Bim* deletion polymorphism,[10] but several basic studies demonstrated that the BIM protein is essential for anticancer therapy-induced apoptosis. EGFR-TKI–induced apoptosis requires BIM protein expression in *EGFR*-mutated NSCLC cell lines,[9] and clinical studies have focused on the relationship between the *Bim* deletion polymorphism or *Bim* messenger RNA expression and *EGFR*-mutated NSCLC treated with EGFR-TKIs.[10-13,20] Our results support the notion that the *Bim* deletion polymorphism is an indicator of significantly poorer outcomes for EGFR-TKI therapy against *EGFR*-mutated NSCLC (Fig 4A). In terms of cytotoxic chemotherapy, BIM protein was shown to mediate apoptosis induced by paclitaxel in NSCLC cells and to be a major determinant in the response of tumors to paclitaxel.[21,22] Wang et al[23] reported that BIM plays a

Table 4 – Univariable and Multivariable Analyses of Predictors of PRS

Variable	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
RFS < 12 months	1.990 (1.172 to 3.380)	.011	1.804 (1.045 to 3.117)	.034
Bim deletion polymorphism	3.645 (1.925 to 6.901)	< .001	3.363 (1.747 to 6.474)	< .001
EGFR mutated	0.356 (0.189 to 0.668)	.001	0.344 (0.183 to 0.647)	.001

Abbreviations: *EGFR*, epidermal growth factor receptor; HR, hazard ratio; PRS, postrecurrence survival; RFS, recurrence-free survival.

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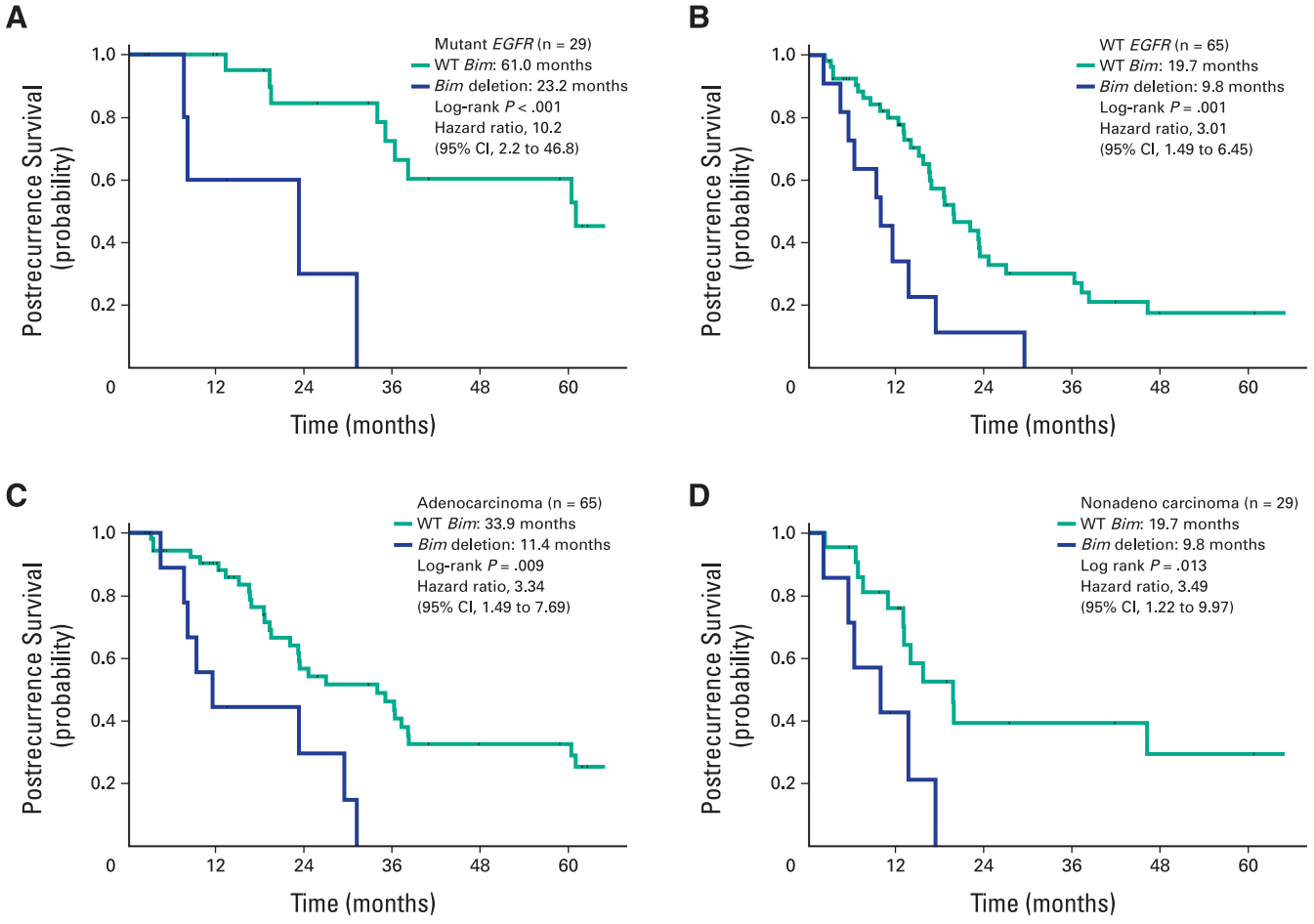

Figure 3 – Kaplan-Meier curves for postrecurrence survival (PRS) according to *EGFR* gene status and histology. (A) PRS in *EGFR*-mutated tumors according to wild-type (WT) *Bim* (n = 24) or the *Bim* deletion polymorphism (n = 5). (B) PRS in wild-type *EGFR* tumors according to wild-type *Bim* (n = 54) or the *Bim* deletion polymorphism (n = 11). (C) PRS in adenocarcinoma according to wild-type *Bim* (n = 56) or the *Bim* deletion polymorphism (n = 9). (D) PRS in nonadeno carcinoma according to wild-type *Bim* (n = 22) or the *Bim* deletion polymorphism (n = 7).

critical role in cisplatin resistance, demonstrating that BIM protein is degenerated in cisplatin-resistant but not in cisplatin-sensitive cells, and inhibition of BIM degeneration can effectively induce cancer cell death. Because expression of the proapoptotic BH3 domain in BIM is suppressed in individuals with the *Bim* deletion polymorphism,[10] sensitivity to cytotoxic chemotherapy may be low in such patients. Consequently, as demonstrated here and in a previous study,[13] patients with the *Bim* deletion polymorphism tend to have shorter survival periods than those with wild-type *Bim* after cytotoxic chemotherapy (Fig 4B).

With regard to radiotherapy-induced apoptosis, it has been reported that radiation increases FOXO3a protein expression, leading to upregulation of BIM expression and apoptotic induction, a reaction that is downstream of the PI3K/AKT signaling pathway and independent of the p53 pathway.[24,25] The PI3K/AKT pathway, which regulates BIM expression, is expected to contribute to radiotherapy resistance, and blockade of the pathway may enhance cancer cell radiotherapy sensitivity.[25,26] Our results indicate that the *Bim* deletion polymorphism is an indicator of poorer radiotherapy outcomes in recurrent NSCLC after complete resection (Fig 4C). Taken together, these findings suggest that the *Bim* deletion polymorphism confers resistance against treatment with EGFR-TKIs, chemotherapy, and radiotherapy.

The relationship between BIM and tumor development has been investigated in several solid tumors. Comparison of BIM levels in primary and metastatic tumors revealed progressive decreases in BIM expression in melanoma,[27] renal cell carcinoma,[28] and colon carcinoma cells.[29] In NSCLC cells, low BIM expression was observed more frequently in cases of advanced pathologic stage, poorer differentiation, and squamous histology, although no impact on survival was observed.[30] These studies support the suggestion that BIM protein plays an important role in suppressing tumor development. Merino et al[31] recently reported that *Bim* loss does not affect proliferation or the expression of epithelial-mesenchymal transition markers but does increase the number of lung metastases in breast cancers. They suggested that the loss of *Bim* may be responsible for dissemination of tumor cells and their colonization of distant

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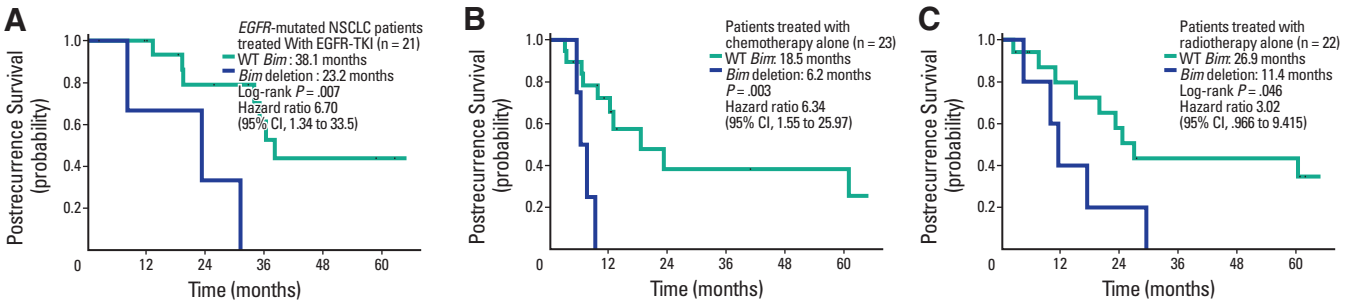

Figure 4 – Kaplan-Meier curves for postrecurrence survival (PRS) according to therapeutic modality. (A) PRS in patients with *EGFR*-mutated non–small-cell lung cancer (NSCLC) who received epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) according to wild-type (WT) *Bim* (n = 18) or the *Bim* deletion polymorphism (n = 3). (B) PRS in patients treated with cytotoxic chemotherapy alone according to wild-type *Bim* (n = 19) or the *Bim* deletion polymorphism (n = 4). (C) PRS in patients treated with radiotherapy alone according to wild-type *Bim* (n = 17) or the *Bim* deletion polymorphism (n = 5).

tissues.[31] Subgroup analyses in our study revealed no significant differences between patients with stage I NSCLC with or without the *Bim* deletion polymorphism (n = 275) with respect to the percentage of patients with lymphatic invasion (27.6% *v* 23.6%, respectively; *P* = .648), to the 5-year OS rate (81.8% *v* 90.4%, respectively; *P* = .402), or to the 5-year RFS rate (83.1% *v* 70.0%, respectively; *P* = .806), consistent with a previous report.[18] Furthermore, our study demonstrated that the *Bim* polymorphism was a significant predictor of RFS only for patients with recurrence (Data Supplement). These results suggest that the *Bim* deletion polymorphism is associated with tumor development in disseminated or metastatic lesions, whereas it has little influence on the primary lesion. Patients with recurrence are likely to have micrometastases at the time of surgery, and therefore *Bim* deletion polymorphism may somehow be involved in growth of these metastatic lesions. Taken together, the *Bim* deletion polymorphism has little effect on tumor aggressiveness or survival in early and/or primary NSCLC but may have an impact on tumor survival in metastatic NSCLC.

Table 5 – Therapeutic Background of Patients Who Received Cytotoxic Chemotherapy or Radiotherapy Alone

Type of Treatment	*Bim* Status		*P*
	Wild Type No. (%)	Deletion Polymorphism No. (%)	
Chemotherapy	19	4	
Platinum			
Yes	12 (75.0)	4 (25.0)	.273
No	7 (100.0)	0 (0.0)	
Taxane			
Yes	3 (75.0)	1 (25.0)	1.000
No	16 (84.2)	3 (15.8)	
Radiation*	17	5	
Conventional	15 (88.2)	4 (11.8)	.637
Cyberknife	2 (66.7)	1 (33.3)	

*Total dose average: patients with wild-type sequence, 66.0 Gy (range, 39-100 Gy); patients with deletion polymorphism, 52.0 Gy (range, 30-104 Gy); *P* = .217.

To the best of our knowledge, this is the first investigation of the impact of *Bim* deletion polymorphism on PRS in patients with NSCLC. Previous studies[10-12,14] demonstrated that the *Bim* deletion polymorphism is a prognostic factor for progression-free survival in patients with stage IIIB or IV NSCLC who received EGFR-TKIs and chemotherapy, although all but one study[13] showed no obvious impact on OS. The reasons underlying these inconsistencies regarding the impact of the *Bim* deletion polymorphism in this and previous studies are unclear. However, previous studies indicated that patients with NSCLC who had recurrence after curative surgery had a favorable prognosis compared with those with advanced-stage disease at initial presentation.[15,32] These results suggest that although both patient groups can be classified as advanced NSCLC, biologic characteristics, such as EGFR-TKI and/or chemotherapy treatment outcome, may be distinct.

The *Bim* polymorphism may be a novel germline biomarker for therapy resistance in patients with advanced NSCLC. The presence of the *Bim* deletion polymorphism may be a negative indication for standard therapies, with the exception of surgery, because such patients are at risk of developing aggressive cancer refractory to EGFR-TKI, chemotherapy, and radiotherapy. Thus, patients with unresectable or recurrent NSCLC who harbor the *Bim* deletion may benefit from treatment with a BH3-mimetic drug[9,10] or histone deacetylase inhibitor[33] to overcome therapy resistance.

This study had several limitations. The first and most important one was the small sample size. The survival analysis included heterogeneous patient backgrounds. Because the subset analyses according to histology or therapy modality were performed by using small sample sizes, this study lacked statistical power, and further investigation is required with a larger sample. Second, this was a retrospective study. Although the indications and therapeutic strategies for recurrent disease were reviewed by the cancer board of our department, not all patients received treatment according to the

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same standard. A prospective multicenter study is required to determine the clinical significance of the *Bim* deletion polymorphism with regard to therapy for advanced and recurrent NSCLC. Finally, this polymorphism is observed only in Asian populations. Even if the significance of the *Bim* deletion polymorphism is validated, the results would not provide any benefit to non-Asian patients with NSCLC.

In conclusion, the *Bim* deletion polymorphism was an indicator of poor RFS and PRS in patients with recurrence after complete resection and is consequently an independent unfavorable prognostic factor for OS in all patients with NSCLC who received complete resection. The polymorphism was associated with tumor aggressiveness and therapy resistance in metastatic disease. If validated, these results suggest that the *Bim* polymorphism may be a biomarker of poor outcome for multimodal therapies in treating recurrent or advanced NSCLC in the Asian population.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2012. CA Cancer J Clin 62:10-29, 2012
2. Sugimura H, Nichols FC, Yang P, et al: Survival after recurrent nonsmall-cell lung cancer after complete pulmonary resection. Ann Thorac Surg 83:409-417, 2007
3. Kelsey CR, Marks LB, Hollis D, et al: Local recurrence after surgery for early stage lung cancer: An 11-year experience with 975 patients. Cancer 115:5218-5227, 2009
4. Maemondo M, Inoue A, Kobayashi K, et al: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362:2380-2388, 2010

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5. Mok TS, Wu YL, Thongprasert S, et al: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947-957, 2009
6. Ohtaki Y, Shimizu K, Kakegawa S, et al: Postrecurrence survival of surgically resected pulmonary adenocarcinoma patients according to EGFR and KRAS mutation status. Mol Clin Oncol 2:187-196, 2014
7. Katayama T, Matsuo K, Kosaka T, et al: Effect of gefitinib on the survival of patients with recurrence of lung adenocarcinoma after surgery: A retrospective case-matching cohort study. Surg Oncol 19:e144-e149, 2010
8. Takenaka T, Takenoyama M, Yamaguchi M, et al: Impact of the epidermal growth factor receptor mutation status on the post-recurrence survival of patients with surgically resected non-small-cell lung cancer. Eur J Cardiothorac Surg 47:550-555, 2015
9. Cragg MS, Kuroda J, Puthalakath H, et al: Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med 4:1681-1690, 2007
10. Ng KP, Hillmer AM, Chuah CT, et al: A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 18:521-528, 2012
11. Zhao M, Zhang Y, Cai W, et al: The Bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non-small cell lung cancer. Cancer 120:2299-2307, 2014
12. Isobe K, Hata Y, Tochigi N, et al: Clinical significance of BIM deletion polymorphism in non-small-cell lung cancer with epidermal growth factor receptor mutation. J Thorac Oncol 9:483-487, 2014
13. Lee JH, Lin YL, Hsu WH, et al: Bcl-2-like protein 11 deletion polymorphism predicts survival in advanced non-small-cell lung cancer. J Thorac Oncol 9:1385-1392, 2014
14. Lee JK, Shin JY, Kim S, et al: Primary resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in patients with non-small-cell lung cancer harboring TKI-sensitive EGFR mutations: An exploratory study. Ann Oncol 24:2080-2087, 2013
15. Sekine I, Nokihara H, Yamamoto N, et al: Comparative chemotherapeutic efficacy in non-small cell lung cancer patients with postoperative recurrence and stage IV disease. J Thorac Oncol 4:518-521, 2009
16. Travis WD, Colby TV, Corrin B, et al: Histological Typing of Lung and Pleural Tumors (ed 3). Berlin, Germany, Springer-Verlag, 1999
17. Miyamae Y, Shimizu K, Mitani Y, et al: Mutation detection of epidermal growth factor receptor and KRAS genes using the smart amplification process version 2 from formalin-fixed, paraffin-embedded lung cancer tissue. J Mol Diagn 12:257-264, 2010
18. Ebi H, Oze I, Nakagawa T, et al: Lack of association between the BIM deletion polymorphism and the risk of lung cancer with and without EGFR mutations. J Thorac Oncol 10:59-66, 2015
19. Ong ST, Chuah CT, Ko TK, et al: Reply: The BIM deletion polymorphism cannot account for intrinsic TKI resistance of Chinese individuals with chronic myeloid leukemia. Nat Med 20:1090-1091, 2014
20. Costa C, Molina MA, Drozdowskyj A, et al: The impact of EGFR T790M mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EURTAC trial. Clin Cancer Res 20:2001-2010, 2014
21. Li R, Moudgil T, Ross HJ, et al: Apoptosis of non-small-cell lung cancer cell lines after paclitaxel treatment involves the BH3-only proapoptotic protein Bim. Cell Death Differ 12:292-303, 2005
22. Tan TT, Degenhardt K, Nelson DA, et al: Key roles of BIM-driven apoptosis in epithelial tumors and rational chemotherapy. Cancer Cell 7:227-238, 2005
23. Wang J, Zhou JY, Wu GS: Bim protein degradation contributes to cisplatin resistance. J Biol Chem 286: 22384-22392, 2011
24. Yang JY, Xia W, Hu MC: Ionizing radiation activates expression of FOXO3a, Fas ligand, and Bim, and induces cell apoptosis. Int J Oncol 29:643-648, 2006
25. Hein AL, Ouellette MM, Yan Y: Radiation-induced signaling pathways that promote cancer cell survival (review). Int J Oncol 45:1813-1819, 2014
26. Toulany M, Lee KJ, Fattah KR, et al: Akt promotes post-irradiation survival of human tumor cells through initiation, progression, and termination of DNA-PKcs-dependent DNA double-strand break repair. Mol Cancer Res 10:945-957, 2012
27. Dai DL, Wang Y, Liu M, et al: Bim expression is reduced in human cutaneous melanomas. J Invest Dermatol 128:403-407, 2008
28. Zantl N, Weirich G, Zall H, et al: Frequent loss of expression of the pro-apoptotic protein Bim in renal cell carcinoma: Evidence for contribution to apoptosis resistance. Oncogene 26:7038-7048, 2007
29. Sinicrope FA, Rego RL, Okumura K, et al: Prognostic impact of bim, puma, and noxa expression in human colon carcinomas. Clin Cancer Res 14:5810-5818, 2008
30. Sakakibara-Konishi J, Oizumi S, Kikuchi J, et al: Expression of Bim, Noxa, and Puma in non-small cell lung cancer. BMC Cancer 12:286, 2012

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31. Merino D, Best SA, Asselin-Labat ML, et al: Pro-apoptotic Bim suppresses breast tumor cell metastasis and is a target gene of SNAI2. Oncogene 34:3926-3934, 2015

32. Yoshioka H, Mitsudomi T, Morita S, et al: Final overall survival results of WJTOG 3405, a randomized phase 3 trial comparing gefitinib (G) with cisplatin plus docetaxel (CD) as the first-line treatment for patients with non-small cell lung cancer (NSCLC) harboring mutations of the epidermal growth factor receptor (EGFR). J Clin Oncol 32, 2014 (suppl 5s; abstr 8117)

33. Nakagawa T, Takeuchi S, Yamada T, et al: EGFR-TKI resistance due to BIM polymorphism can be circumvented in combination with HDAC inhibition. Cancer Res 73:2428-2434, 2013

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