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 vs 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.

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

Jun Atsumi
Kimihiro Shimizu
Yoichi Ohtaki
Kyoichi Kaira
Seiichi Kakegawa
Toshiteru Nagashima
Yasuaki Enokida
Seshiru Nakazawa
Kai Obayashi
Yoshiaki Takase
Osamu Kawashima
Mitsuhiro Kamiyoshihara
Masayuki Sugano
Takashi Ibe
Hitoshi Igai
Izumi Takeyoshi

Authors’ disclosures of potential conflicts of interest and contributions are found at the end of this article.

Corresponding author: Kimihiro Shimizu, MD, PhD, Department of Thoracic and Visceral Organ Surgery, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan; e-mail: kmshimizu@gmail.com.
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. However, there have been no studies regarding the influence of the polymorphism on postoperative patients with lung cancer, including those with non-adenocarcinoma histology, or the impact of the polymorphism on postrecurrence treatment.

We hypothesized that the Bim deletion polymorphism affects survival among patients with postoperative recurrent NSCLC. 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, 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 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. 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.

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 χ² test. Continuous variables were compared by...
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, 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 $\alpha$ 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) | Wild Type (n = 349) No. (%) | Deletion Polymorphism (n = 61) No. (%) | P    |
|-----------------------------------------------|
| Median age, years                   | 67.6         | 67.9 (86.9)                  | 66.5 (13.1)                      | .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.
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 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% vs 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% vs 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 vs 13.9 months, respectively; P = .003; Fig 2A).

Table 2 – Multivariable Analysis of Predictors of OS and RFS

| Variable                          | OS HR (95% CI) | P       | RFS 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.
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.
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. However, only one report has examined the impact of the Bim deletion polymorphism on survival in patients with resectable NSCLC. 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, 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, 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. 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. Wang et al reported that BIM plays a role in paclitaxel-induced apoptosis in NSCLC cells and that the BIM protein is a potential target for paclitaxel-based chemotherapy.

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

| Variable                          | Wild Type (n = 78) | Deletion Polymorphism (n = 16) | P   |
|-----------------------------------|-------------------|-------------------------------|-----|
| Median age (years)                | 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                         |                   |                               | ![](image)
| Platinum-based chemotherapy       | 43                | 35 (81.4)                     | 8 (18.6)|
| Radiotherapy                      | 43                | 34 (79.1)                     | 9 (20.9)|
| EGFR-TKI                          | 33                | 30 (90.1)                     | 3 (9.1)|

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

*ADC v SQC and other.

Table 4 – Univariable and Multivariable Analyses of Predictors of PRS

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

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

radiation dose among the 22 patients who received radiotherapy alone (Table 5).
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, sensitivity to cytotoxic chemotherapy may be low in such patients. Consequently, as demonstrated here and in a previous study, 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. 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. 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, renal cell carcinoma, and colon carcinoma cells. 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. These studies support the suggestion that BIM protein plays an important role in suppressing tumor development. Merino et al 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 distant.

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 nonadenocarcinoma according to wild-type Bim (n = 22) or the Bim deletion polymorphism (n = 7).
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 IIIIB 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 indicator 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\(^{3,10}\) 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 analyses 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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**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).

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

| Type of Treatment         | Wild Type No. (%) | Deletion Polymorphism No. (%) | P  |
|---------------------------|-------------------|-------------------------------|----|
| Chemotherapy              | 19                | 4                             |    |
| Platinum                  | 12 (75.0)         | 4 (25.0)                      | .273|
|                           | 7 (100.0)         | 0 (0.0)                       |    |
| Taxane                    | 3 (75.0)          | 1 (25.0)                      | 1.000|
|                           | 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.
same standard. A prospective multicenter study is required to determine the clinical significance of the \textit{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 \textit{Bim} deletion polymorphism is validated, the results would not provide any benefit to non-Asian patients with NSCLC.

In conclusion, the \textit{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 \textit{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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AUTHOR CONTRIBUTIONS
Conception and design: Kimihiro Shimizu
Collection and assembly of data: Jun Atsumi, Yoichi Ohtaki, Seiichi Kakegawa, Toshiteru Nagashima, Yasuaki Enokida, Kai Obayashi, Yoshiaki Takase, Osamu Kawashima, Mitsuhiro Kamiyoshihara, Masayuki Sugano, Takashi Ibe, Hitoshi Igai, Izumi Takeyoshi
Data analysis and interpretation: Jun Atsumi, Kimihiro Shimizu, Yoichi Ohtaki, Kyoichi Kaira, Seshiru Nakazawa
Manuscript writing: All authors
Final approval of manuscript: All authors

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
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Jun Atsumi
No relationship to disclose
Kimihiro Shimizu
No relationship to disclose
Yoichi Ohtaki
No relationship to disclose
Kyoichi Kaira
No relationship to disclose
Seiichi Kakegawa
No relationship to disclose

Toshiteru Nagashima
No relationship to disclose
Yasuaki Enokida
No relationship to disclose
Seshiru Nakazawa
No relationship to disclose
Kai Obayashi
No relationship to disclose
Yoshiaki Takase
No relationship to disclose
Osamu Kawashima
No relationship to disclose
Mitsuhiro Kamiyoshihara
No relationship to disclose
Masayuki Sugano
No relationship to disclose
Takashi Ibe
No relationship to disclose
Hitoshi Igai
No relationship to disclose
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No relationship to disclose

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AFFILIATIONS
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