

A systematic review and meta-analysis of individual patient data on the impact of the BIM deletion polymorphism on treatment outcomes in epidermal growth factor receptor mutant lung cancer

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ABSTRACT

Background: A germline deletion in the *BIM (BCL2L11)* gene has been shown to impair the apoptotic response to tyrosine kinase inhibitors (TKIs) *in vitro* but its association with poor outcomes in TKI-treated non-small cell lung cancer (NSCLC)

patients remains unclear. We conducted a systematic review and meta-analysis on both aggregate and individual patient data to address this issue.

Results: In an aggregate data meta-analysis ($n = 1429$), the *BIM* deletion was associated with inferior PFS (HR = 1.51, 95%CI = 1.06–2.13, $P = 0.02$). Using individual patient data ($n = 1200$), we found a significant interaction between the deletion and ethnicity. Amongst non-Koreans, the deletion was an independent predictor of shorter PFS (Chinese: HR = 1.607, 95%CI = 1.251–2.065, $P = 0.0002$; Japanese: HR = 2.636, 95%CI = 1.603–4.335, $P = 0.0001$), and OS (HR = 1.457, 95% CI = 1.063–1.997, $P = 0.019$). In Kaplan-Meier analyses, the *BIM* deletion was associated with shorter survival in non-Koreans (PFS: 8.0 months v 11.1 months, $P < 0.0005$; OS: 25.7 v 30.0 months, $P = 0.042$). In Koreans, the *BIM* deletion was not predictive of PFS or OS.

Materials and Methods: 10 published and 3 unpublished studies that reported survival outcomes in NSCLC patients stratified according to *BIM* deletion were identified from PubMed and Embase. Summary risk estimates were calculated from aggregate patient data using a random-effects model. For individual patient data, Kaplan-Meier analyses were supported by multivariate Cox regression to estimate hazard ratios (HRs) for PFS and OS.

Conclusions: In selected populations, the *BIM* deletion is a significant predictor of shorter PFS and OS on EGFR-TKIs. Further studies to determine its effect on response to other *BIM*-dependent therapeutic agents are needed, so that alternative treatment strategies may be devised.

INTRODUCTION

While sensitizing mutations in the *EGFR* gene predict very high response rates among patients with NSCLC [1–3], up to 30% of such patients fail to experience optimum responses [4, 5]. The identification of factors which predict patient response is important for both clinical and economic reasons [6–8], and is especially relevant for East Asian populations where the incidence of *EGFR*-mutated NSCLC is at least 4-fold higher than among non-East Asian populations [9].

Several mechanisms to explain the heterogeneity of TKI responses have been previously described [10–13]. In particular, impaired expression/function of BIM (BCL2L11), resulting in failure of TKI-induced apoptosis has been associated with TKI resistance in preclinical models [14–18]. BIM is a pro-apoptotic protein that is upregulated upon TKI treatment and binds to pro-survival BCL-2 family members via its BH3 domain, thereby initiating programmed cell death [19]. Accordingly, BIM expression has been found to represent a key node in determining responses to cancer drug therapy in general [20]. Of note, recent data from the European Tarceva (EURTAC) trial demonstrated poorer outcomes in *EGFR*-mutant NSCLC patients with low *BIM* expression, further supporting the importance of intact apoptotic machinery in such populations [21]. Together, these studies highlight BIM function as a critical factor in determining patient responses to cancer therapies.

Recently, a 2.9kb intronic deletion in the *BIM* gene that biases splicing towards non-apoptotic isoforms lacking the BH3 domain was discovered [22]. This deletion

polymorphism appeared to occur at a frequency of 12.3% in East Asians but was absent in Caucasian populations [22]. When introduced into NSCLC cell lines, the deletion resulted in an increase in the production of non-apoptotic isoforms at the expense of apoptotic isoforms, and relative resistance to EGFR-TKIs [22]. Importantly, it has also been shown that resistance conferred by the deletion could be overcome *in vitro* with HDAC inhibitors [23], and BH3 mimetics [22]. It was therefore hypothesised that the *BIM* deletion would have an impact on the sensitivity of EGFR-addicted tumors to EGFR-TKIs, and indeed the polymorphism was associated with shorter progression-free survival (PFS) in a retrospective EGFR-TKI-treated NSCLC cohort from Singapore and Japan [22].

Since then, other centers across East Asia, including China and Taiwan, have investigated the association between the *BIM* polymorphism and treatment outcome in both prospective and retrospective TKI-treated NSCLC patient cohorts, with conflicting results [24–30]. Notably, the two studies that reported a lack of association originated from South Korea, raising the question if the effect of the deletion may be modulated by ethnicity. Furthermore, although the *BIM* deletion was initially thought to be exclusive to East Asian populations, it has been recently reported in a Columbian population [31]. Hence, an understanding of the true effect of the deletion on TKI treatment outcomes in different ethnic populations is important for predicting response and guiding treatment strategies in East Asian populations, and potentially beyond East Asian populations.

A number of aggregate data meta-analyses that have been published have suggested that the *BIM* polymorphism

is indeed associated with shorter PFS on EGFR-TKIs [32–35]. However, these meta-analyses were limited by the use of summary statistics from published studies. The alternative approach, based on individual patient data, offers several clinical and statistical advantages [36]. In this collaborative study, we used individual patient data from published and unpublished studies to perform a meta-analysis on the effect of the *BIM* polymorphism on PFS and OS in EGFR-TKI-treated NSCLC patients as the primary aim.

RESULTS

Search strategy

Figure 1 illustrates the search strategy used to identify studies that investigated the association of the *BIM* deletion with responses among individuals with *EGFR*-mutant NSCLC. A total of 2187 unique articles were identified from PubMed and Embase after removing duplicate abstracts. 13 articles were selected for full-text review after inclusion and exclusion criteria were applied. 10 studies were eventually identified and the corresponding authors were contacted. We received individual patient data for 7 of these studies [22, 25–28, 30, 37]. Authors of 3 studies did not reply ($n = 502$) [24, 29, 31]. Published data was supplemented with 3 additional unpublished datasets from China, Japan and Singapore (SL, SY, WTL).

Table 1 summarizes the characteristics of the 13 studies we identified. In total, individual patient data from 1324 patients was received. We excluded patients if they were not treated with TKIs, the treatment period was < 2 weeks, or *BIM* deletion status was unknown. Duplicate patients were also excluded (Supplementary Table 1). Eventually, data from 1200 NSCLC patients (representing 90.6% of the total number of patients) was used for a pooled individual patient data meta-analysis, to determine PFS and OS on EGFR-TKIs in the whole cohort.

Baseline characteristics of patients with and without the *BIM* deletion

Baseline characteristics of the cohort as a whole, and segregated according to *BIM* deletion status, are detailed in Supplementary Table 2. Patients with or without the *BIM* deletion did not differ significantly (defined as $p > 0.05$) in terms of the characteristics examined, including established prognostic factors for *EGFR*-mutant NSCLC [38]. All the studies used for this pooled analysis used data from stage III/IV NSCLC patients on EGFR-TKI monotherapy. The majority of patients (96.4%) were treated with standard doses of either gefitinib or erlotinib. Although the TKI used differed according to each country's practices, similar outcomes have been reported in earlier studies that compared gefitinib to erlotinib [39, 40].

Progression-free survival on EGFR-TKIs in patients with and without the *BIM* deletion

We first performed a Kaplan-Meier analysis on the pooled individual patient dataset ($n = 1200$) to determine the PFS on EGFR-TKIs of patients segregated according to previously reported prognostic factors, in particular, the *BIM* deletion. We found that patients with the *BIM* deletion had a significantly shorter median PFS of 9.2 months compared to 11.0 months in those without the deletion ($P = 0.002$) (Figure 2A). As expected, log-rank p values for Kaplan-Meier analyses were also significant for well-validated predictors of survival such as histology ($P = 0.001$), *EGFR* phenotype ($P < 0.0005$), gender ($P < 0.0005$), smoking history ($P = 0.024$), and stage ($P < 0.005$). In addition, country ($P < 0.0005$), center ($P < 0.0005$), ethnicity ($P < 0.0005$) also emerged as significant predictors of PFS (data not shown).

Considering that ethnicity subsumes center and country, we focussed on investigating the effect of the *BIM* deletion stratified by ethnicity (Supplementary Figure 1), and found that Korean patients with and without the deletion—contrary to the other ethnicities—had similar PFS (11.9 v 10.9 months; $P = 0.474$) (Figure 2C). In contrast, an analysis of non-Korean patients showed a significant difference in PFS in patients with and without the *BIM* deletion (8.0 v 11.1 months; $P < 0.0005$) (Figure 2E).

To support our findings from the Kaplan-Meier analysis, we used Cox regression analysis to identify significant factors associated with shorter PFS in patients on EGFR-TKI treatment. Here, the *BIM* deletion emerged as a significant risk factor for reduced PFS in both the univariate (HR = 1.323, 95% CI = 1.108–1.581, $P = 0.002$) and stepwise multivariate Cox regression analyses (HR = 1.406, 95% CI = 1.174–1.684, $P < 0.0005$) (Table 2). To explore the extent to which ethnicity moderated the effect of the *BIM* deletion on PFS, an interaction term (*BIM* deletion x ethnicity) was incorporated into the Cox regression model (Table 3). This analysis yielded significant main effects for the *BIM* deletion ($P = 0.0002$) and ethnicity ($P = 0.0020$), as well as a significant interaction between *BIM* deletion and ethnicity ($P = 0.0023$). The effect of the *BIM* deletion differed according to ethnicity, as it was a significant risk factor for reduced PFS in Chinese (HR = 1.607, 95% CI = 1.251–2.065, $P = 0.0002$) and Japanese (HR = 2.636, 95% CI = 1.603–4.335, $P = 0.0001$), but not Korean patients (HR = 0.919, 95% CI = 0.668–1.264, $P = 0.603$) (Table 3).

Baseline characteristics of patients of Korean and non-Korean ethnicity

To exclude differences in patient population as a reason for the discordant results, we compared the baseline characteristics of patients of Korean and non-

Korean ethnicity. We found that the Korean and non-Korean cohorts differed in terms of ECOG status, histology, *EGFR* mutation, TKI used and line of treatment (Supplementary Table 3). We then performed subgroup Kaplan-Meier analyses using only data from patients who had 1) ECOG status 0 or 1, 2) adenocarcinoma histology, 3) TKI-sensitizing *EGFR* mutations, 4) gefitinib and/or erlotinib treatment or 5) first-line treatment with TKIs (Supplementary Table 4). In these subgroup analyses, the *BIM* deletion remained a significant predictor of shorter PFS in only the non-Korean patients, and not the Korean patients. Hence, it is unlikely that these differences in baseline characteristics contribute to the discordant effects of the deletion on PFS in Korean and non-Korean patients.

Overall survival on EGFR-TKIs in patients with and without the BIM deletion

In earlier studies, there had been conflicting results as to whether the *BIM* deletion predicted poorer overall survival [27, 28, 30]. Here, for the cohort as a whole, there was no significant difference in OS in patients with the *BIM* deletion compared to those without for whom overall survival data was available ($n = 830$) (26.1 v 28.9 months, $P = 0.115$) (Figure 2B). However, when stratified by Korean ethnicity, there was a significant difference in OS amongst non-Koreans with and without the *BIM* deletion (25.7 v 30.5 months, $P = 0.042$) (Figure 2F) but not amongst Koreans (28.2 v 27.2 months, $P = 0.585$) (Figure 2D). In a multivariate Cox regression analysis, the

BIM deletion emerged as a significant predictor of shorter OS (HR = 1.457, 95% CI = 1.063–1.997, $P = 0.019$) in non-Korean patients ($n = 549$) (Table 4).

Aggregate data meta-analysis

In view of potential study heterogeneity, and in order to include as many studies as possible (including the authors who did not reply to requests for IPD), we performed a meta-analysis using a random effects model based on aggregate patient data ($n = 1429$). Consistent with results from the pooled IPD analysis, the presence of the *BIM* deletion was associated with a significant overall increase in the risk of progression compared to individuals without the *BIM* deletion (HR = 1.51, 95% CI = 1.06–2.13, $P = 0.02$) in an aggregate data meta-analysis (Figure 3).

However, because there was significant heterogeneity within the combined group ($I^2 = 70%$, $P = 0.0001$) (Figure 3), and because the 2 studies to date that reported a lack of association between the *BIM* deletion and treatment outcome originated from Korean centers, we tested the possibility that the heterogeneity arose from differences in non-Korean v Korean groups. Consistent with this notion, the non-Korean groups were found to be homogenous when analyzed separately ($I^2 = 41%$, $P = 0.10$) (Figure 3). Importantly, and consistent with the results from the individual patient data-based analysis, we also found that the summary HR for risk of shorter PFS was significant in patients with the deletion in the non-Korean studies (HR = 1.88, 95% CI: 1.42–2.50,

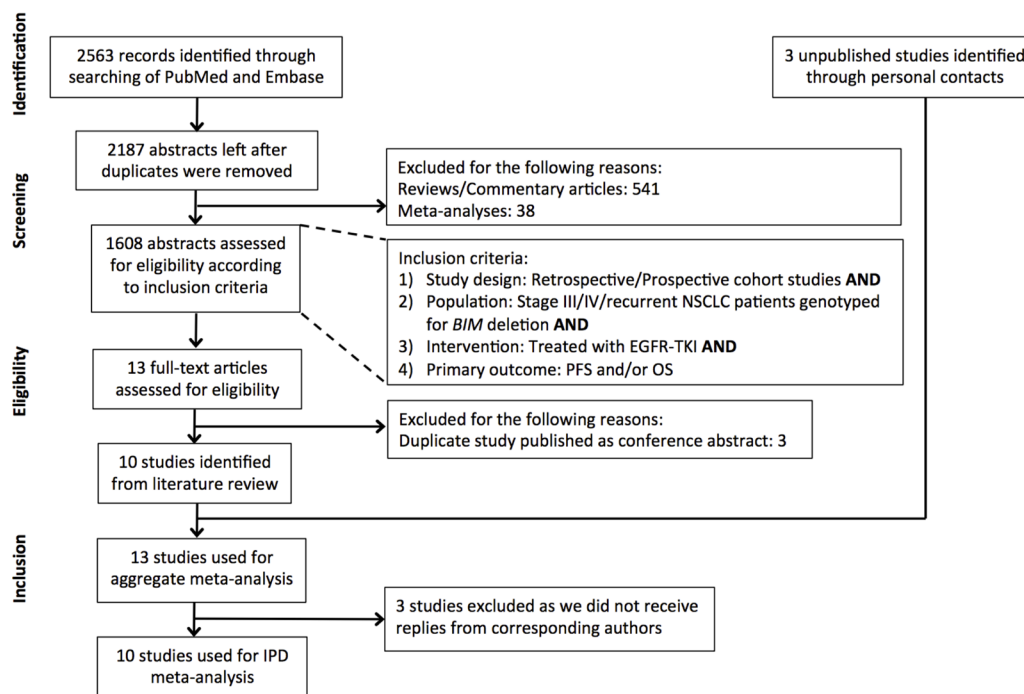


Figure 1: Flowchart of study identification, inclusion and exclusion. Studies identified from a PubMed and Embase search were filtered according to the exclusion and inclusion criteria listed in the Figure. In total, 10 studies were available for individual patient data (IPD) analysis.

Table 1: Characteristics of studies identified for the meta-analysis

Author	Year	Country	Sample size	Patients with <i>BIM</i> deletion (%)	Line of TKI treatment
Ng KP et al.	2012	Singapore, Japan	141	26 (18.4%)	First-line or later
Lee JK et al.	2013	South Korea	197	21 (10.7%)	First-line or later
Zheng L et al.*	2013	China	123	21 (17.1%)	Second-line or later
Isobe K et al.	2014	Japan	70	13 (18.6%)	First-line or later
Lee JH et al.†	2014	Taiwan	153	27 (17.6%)	First-line only
Zhao MC et al.	2014	China	166	16 (9.6%)	First-line or later
Zhong J et al.*	2014	China	290	45 (15.5%)	First-line or later
Cardona AF et al.*	2014	Colombia	89	14 (15.7%)	Not reported
Kim GW et al.	2015	South Korea	21	4 (19%)	First-line or later
Lee JY et al.	2015	South Korea	205	32 (15.6%)	First-line or later
Yano S et al.	Unpublished	Japan	39	6 (15.4%)	First-line or later
Lu S et al.	Unpublished	China	55	9 (16.4%)	First-line or later
Lim WT et al.	Unpublished	Singapore	178	22 (12.4%)	First-line or later

Legend:

*Authors did not respond to requests for data.

†Prospective study.

Table 2: Results of univariate and multivariate analyses for predictors of PFS, whole cohort

	Univariate Analysis			Multivariate Analysis		
	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value		
Age at diagnosis	0.992 (0.987–0.998)	0.012	0.989 (0.983–0.995)	< 0.0005		
Gender (Ref = Female)						
Male	1.359 (1.190–1.550)	< 0.0005	1.355 (1.186–1.547)	< 0.0005		
Ethnicity (Ref = Others)						
Chinese	1.048 (0.72–1.525)	0.806	1.047 (0.717–1.529)	0.810		
Japanese	0.678 (0.448–1.026)	0.066	0.796 (0.525–1.209)	0.285		
Korean	0.952 (0.651–1.392)	0.800	1.162 (0.79–1.708)	0.446		
Smoking history (Ref = Never smoker)						
Ever smoker	1.228 (1.059–1.425)	0.007	–	–		
Stage (Ref = Relapse)						
IIIA/B	1.583 (1.201–2.087)	0.001	1.523 (1.147–2.022)	0.004		
IV	1.412 (1.175–1.699)	< 0.0005	1.455 (1.201–1.762)	< 0.0005		
Histology (Ref = Adenocarcinoma)						
Non-adenocarcinoma	1.590 (1.203–2.101)	0.001	1.832 (1.381–2.433)	< 0.0005		
EGFR phenotype (Ref = Sensitizing)						
Resistant	2.551 (1.972–3.300)	< 0.0005	2.705 (2.067–3.542)	< 0.0005		
Line of treatment (Ref = Later)						
First-line	1.152 (1.010–1.313)	0.035	–	–		
ECOG status (Ref = 0 or 1)						
≥ 2	1.284 (0.997–1.653)	0.053	1.408 (0.898–2.209)	0.136		
<i>BIM</i> deletion (Ref = Absent)						
Present	1.323 (1.108–1.581)	0.002	1.406 (1.174–1.684)	< 0.0005		

Table 3: Significant predictors of PFS on EGFR-TKIs, whole cohort, using a *BIM* deletion x ethnicity interaction term

	Multivariate Analysis	
	HR (95% CI)	<i>p</i> value
Age at diagnosis	0.989 (0.983–0.995)	0.0002
Gender (Ref = Female)		
Male	1.325 (1.160–1.513)	< 0.0001
Stage (Ref = Relapse)		
IIIA/B	1.515 (1.141–2.012)	0.0041
IV	1.408 (1.163–1.705)	0.0005
Histology (Ref = Adenocarcinoma)		
Non-adenocarcinoma	1.858 (1.399–2.468)	< 0.0001
EGFR phenotype (Ref = Sensitising)		
Resistant	2.739 (2.093–3.585)	< 0.0001
ECOG status (Ref = 0 or 1)		
≥ 2	1.428 (1.106–1.844)	0.0063
<i>BIM</i> deletion x ethnicity (Ref = Absent)		
Chinese	1.607 (1.251–2.065)	0.0002
Japanese	2.636 (1.603–4.335)	0.0001
Korean	0.919 (0.668–1.264)	0.6030
Others	2.115 (0.804–5.564)	0.1290

$P < 0.0001$), whereas the deletion was not a significant risk factor for shorter PFS in Koreans (HR = 0.68, 95% CI = 0.30–1.53, $P = 0.35$) (Figure 3).

DISCUSSION

In the current work, we have used both pooled individual patient data as well as aggregate patient data to show that the germline *BIM* deletion, like TKI-resistant *EGFR* mutations, predicts inferior outcomes in selected populations. Our findings indicate that Chinese and Japanese patients with *EGFR*-mutant NSCLC who carry the *BIM* deletion polymorphism have an inferior progression-free survival compared to those who do not (Chinese: HR = 1.607, 95% CI = 1.251–2.065, $P = 0.0002$; Japanese: HR = 2.636, 95% CI = 1.603–4.335, $P = 0.0001$). Importantly, this remains the only germline variant known to reproducibly affect clinical outcomes in *EGFR*-mutant NSCLC across multiple studies [41]. In contrast, several germline polymorphic variants in ABC transporter genes which may influence EGFR-TKI pharmacokinetics have not carried the same effect [42].

To assess the impact of the deletion in the Korean population, we combined individual patient data from two Korean studies [25, 30], as well as an additional cohort ($n = 18$) from another Korean center [37]. The lack of effect of the deletion in the Korean studies could account for some of the statistical heterogeneity seen in our

random-effects model (Figure 3), and the use of individual patient data has been invaluable in allowing a more accurate quantification of the effect of the *BIM* deletion on PFS according to ethnicity (Table 3).

Several possibilities may account for the discordant effects of the deletion between Korean and non-Korean populations. Firstly, the discordant effects seen in different ethnic populations may be a result of differences in treatment protocols between countries, rather than ethnicity (Korean *v* non-Korean) per se. However, this is unlikely, as to our knowledge, EGFR-TKI treatment protocols of Korean and non-Korean centers are relatively similar. Although the Korean and non-Korean populations differed in terms of other baseline characteristics other than ethnicity (Supplementary Table 3), subgroup analysis using only patients who had the same ECOG status, histology, sensitizing *EGFR* mutations, TKI used and line of treatment showed a significantly shorter PFS in non-Korean patients with the *BIM* deletion, but not Korean patients.

If the discordant effect of the deletion truly arises from differences in genetic makeup between non-Koreans and Koreans, a possible explanation is the existence of other genetic factors in linkage disequilibrium with the *BIM* deletion that has occurred only in Korean populations through the process of genetic drift and divergence from the other East Asian populations. Such factors may modulate the effect of the deletion on TKI

Table 4: Results of univariate and multivariate analyses for predictors of OS, non-Koreans

	Univariate Analysis			Multivariate Analysis	
	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value	
Age at diagnosis	1.009	(0.999–1.019)	0.095	–	–
Gender (Ref = Female)					
Male	1.453	(1.149–1.838)	0.002	1.490 (1.175–1.890)	0.001
Ethnicity (Ref = Others)					
Chinese	0.806	(0.438–1.484)	0.489	–	–
Japanese	0.628	(0.329–1.198)	0.158	–	–
Smoking history (Ref = Never smoker)					
Ever smoker	1.215	(0.940–1.570)	0.136	–	–
Stage (Ref = Relapse)					
IIIA/B	1.286	(0.754–2.193)	0.356	–	–
IV	1.285	(0.892–1.851)	0.178	–	–
Histology (Ref = Adenocarcinoma)					
Non-adenocarcinoma	2.160	(1.408–3.311)	< 0.0005	2.551 (1.653–3.937)	< 0.0005
EGFR phenotype (Ref = Sensitising)					
Resistant	1.881	(1.378–2.566)	< 0.0005	1.939 (1.413–2.661)	< 0.0005
Line of treatment (Ref = Later)					
First-line	1.453	(1.081–1.952)	0.013	1.409 (1.041–1.907)	0.026
ECOG status (Ref = 0 or 1)					
≥ 2	2.370	(31.538–3.650)	< 0.0005	2.744 (2.464–3.056)	0.004
<i>BIM</i> deletion (Ref = Absent)					
Present	1.378	(1.010–1.881)	0.043	1.457 (1.063–1.997)	0.019

responses, and may also include Korean-specific single nucleotide polymorphisms (SNPs) in *BIM* itself that counter the effects of the *BIM* deletion. Of relevance to this discussion, functional polymorphisms in *BIM* besides the deletion polymorphism have been reported in other populations [43, 44]. It is also possible that functional *BIM* SNPs may exist at varying frequencies between different East Asian populations, as has been reported for functional SNPs affecting genes other than *BIM* [45].

Another factor we could not correct for despite the availability of individual patient data is the frequency of radiological imaging used to determine tumor response or progression. In this instance, length time bias may be introduced by differences in the frequency of imaging, and can only be properly addressed through large prospective studies employing imaging at regular and pre-defined intervals [46].

In addition to kinase inhibitors, the effect of the *BIM* deletion could potentially extend to chemotherapeutics. This possibility is consistent with the superior overall survival we observed in non-Korean patients without the deletion (Figure 2F and Table 4) since patients would very likely have received chemotherapy during the course of their disease. Importantly, this clinical finding is supported by preclinical data demonstrating that sensitivity to

taxanes and vinca alkaloids, which are commonly used in the treatment of NSCLC patients, depends on *BIM* expression [47, 48]. It would therefore be interesting in future studies to examine the effect of the *BIM* deletion on PFS on chemotherapy. If the *BIM* deletion did indeed influence treatment outcomes beyond TKIs, genotyping for the *BIM* deletion may be a recommendation for any therapeutic strategy that depends on *BIM* function for efficacy. Furthermore, a recent Japanese study has highlighted a correlation between the *BIM* deletion and relapse-free survival in patients with early-stage resected lung cancer [49], suggesting possible implications of the *BIM* deletion on adjuvant therapy in early-stage lung cancer, where vinorelbine is widely used.

Knowledge about EGFR-TKI resistance has evolved since the discovery of the *BIM* deletion and the understanding that low frequency *MET* amplification (< 5%) [50] and *de novo* T790M mutations (1–2%) [51] contribute to a small percentage of intrinsic resistance. These areas are not addressed in this meta-analysis because presence of these resistance mechanisms in individual patients is not routinely assessed in clinical practice as yet. Nevertheless, apoptosis remains the final common pathway by which inhibitors of *MET* or EGFR work, hence assessment of the *BIM* deletion status in populations where it is common remains relevant. Indeed,

ongoing clinical trials are currently testing the hypothesis that combination therapy using EGFR TKIs and HDAC inhibitors will restore TKI sensitivity to patients with the *BIM* deletion polymorphism [23].

There is also preliminary evidence suggesting that the impact of our results may extend beyond East Asian populations. The *BIM* deletion has been reported to be

present in a South American population at a frequency of 15.3% [31]. Accordingly, the effect of the *BIM* deletion among the South American population should also be examined, particularly since the *EGFR* mutation rate and therefore proportion of EGFR-TKI-responsive lung cancers in South American populations is higher than reported in European studies [52].

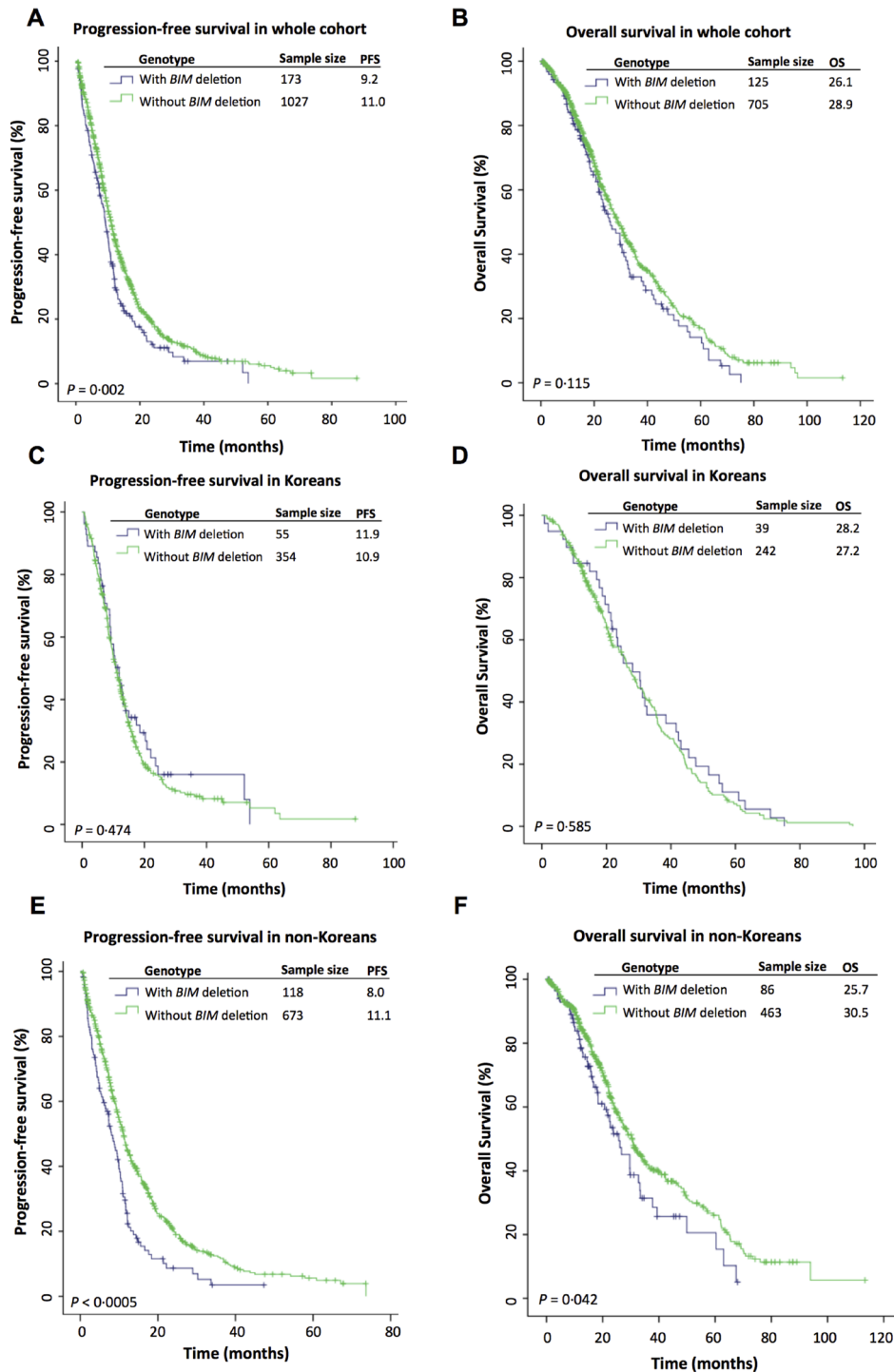


Figure 2: Kaplan-Meier curves comparing progression-free survival (PFS) and overall survival (OS) on EGFR-TKIs between patients with and without the *BIM* deletion in the (A, B) whole cohort, (C, D) Korean cohort and (E, F) non-Korean cohort.

In conclusion, our analysis confirms that patients with *EGFR*-mutant NSCLC harboring the *BIM* deletion are at higher risk for reduced progression-free survival compared to those without the deletion. In addition, the association is strongest among Chinese and Japanese individuals for whom the *BIM* deletion also predicts a reduction in overall survival. Accordingly, testing for the presence of the *BIM* deletion among patients of Chinese or Japanese ancestry with *EGFR*-mutant NSCLC may have prognostic utility. Finally, ongoing trials (clinicaltrials.gov/ct2/show/NCT02151721) are testing the hypothesis that combination therapy with HDAC inhibitors and EGFR-TKIs will overcome inferior outcomes conferred by the *BIM* deletion. If positive, such studies will support the use of the *BIM* deletion as a biomarker for combination therapy.

MATERIALS AND METHODS

Literature search and data extraction

A bibliographic search with no language restrictions was made on PubMed and Embase to identify eligible studies. As the first paper to describe the *BIM* polymorphism was published in 2012, the search was restricted to papers published in or after 2012, up to September 21, 2015. The search strategy was developed by FJS, and was run using terms related to lung cancer (“Lung Cancer” or “Carcinoma, Non-Small-Cell Lung” or “Lung Neoplasms” or “Lung Adenocarcinoma” or “EGFR lung cancer” or “EGFR mutant lung cancer”),

tyrosine kinase inhibitors (“tyrosine kinase inhibitor or “kinase inhibitor” or “TKI” or “Iressa” or “Tarceva” or “gefitinib” or “erlotinib” and *BIM* polymorphism (“*BIM*” or “*BCL2L1*” OR “*BCL-2*-like protein” or “*BIM* polymorphism” or “*BCL2L1* polymorphism”).

Two authors (SXS and WTL) independently screened the title and abstract of retrieved articles. Articles were included if they met the following criteria: 1) study design: prospective or retrospective cohort studies, 2) population: patients with advanced stage (IIIA/B/IV) or postoperative relapsed NSCLC and known *BIM* deletion status, 3) intervention: EGFR-TKI monotherapy and 4) primary outcome: PFS and/or OS. Meta-analyses, case studies, reviews, commentary articles and replies were excluded from the analysis. Studies that cleared the title and abstract screening stages were selected for full-text review. Disagreements were resolved by discussion between the two reviewers.

Quality and bias assessment

We used the Cochrane Risk of Bias Tool to assess the quality of the studies included (Supplementary Table 5). The quality of the study was regarded as high if all aspects were assessed favorably.

To minimize publication bias, and as suggested by the Cochrane Handbook of Systemic Reviews of Interventions [53], we have taken the following measures. First, we performed a comprehensive search on PubMed and Embase with no language restrictions, and used

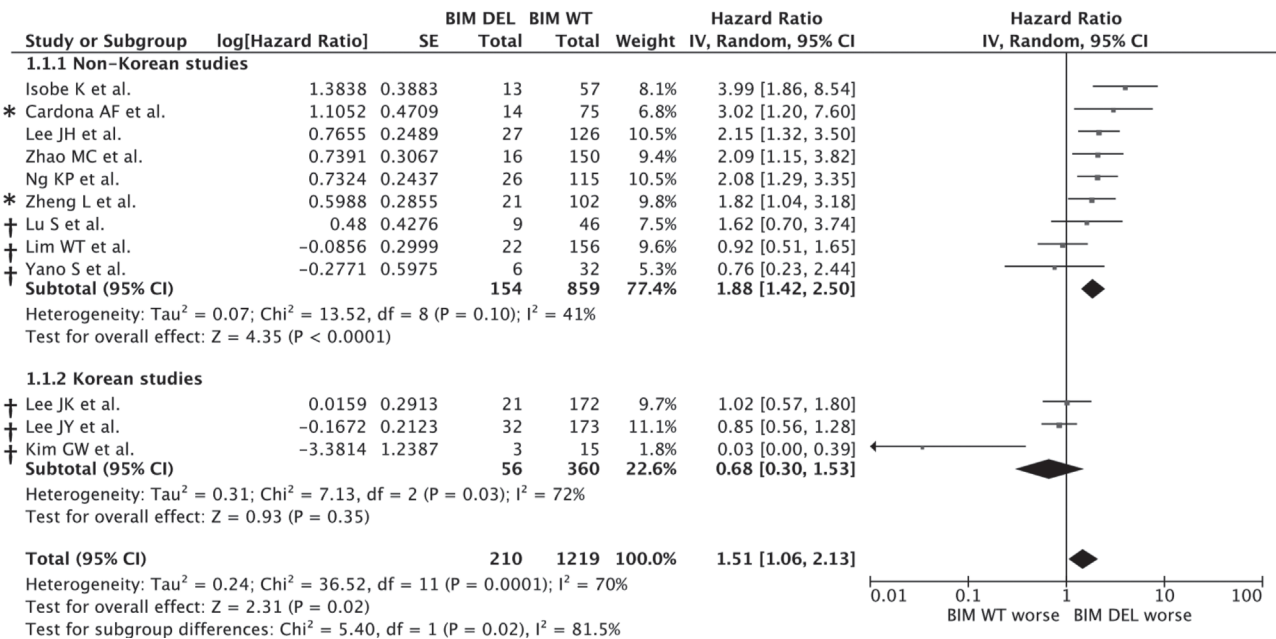


Figure 3: Cumulative meta-analysis of published and unpublished data of the association between the *BIM* deletion polymorphism and progression-free survival in EGFR-TKI-treated NSCLC patients. Estimates were adjusted for age, sex, smoking history, stage, ECOG status, histology, *EGFR* mutation and line of treatment. Zhong J *et al.* was omitted from this figure as both summary statistics and individual patient data (IPD) were not available. * indicates IPD was unavailable, † indicates summary HRs were calculated by the authors of this paper according to the methods described in the Methods section.

multiple synonyms of search terms. Secondly, and because the research topic here is a highly specialized one with only a small community of researchers in East Asia who are known to each other, we were able to obtain data from 3 unpublished studies for inclusion in the meta-analysis.

Individual patient data collection

Following ethics approval by the SingHealth Institutional Review Board (IRB), invitations to participate in the meta-analysis were sent to all investigators of the primary studies identified through the literature search. Corresponding authors were responsible for their individual IRB approvals. Each author was requested to submit anonymized individual patient data in a Microsoft Excel template which included the following fields: age at diagnosis, gender and ethnicity of the patient, smoking history, tumor stage at diagnosis, histology, *BIM* deletion status, *EGFR* mutation, EGFR-TKI used, line of treatment, ECOG status, date TKI was started/stopped, reason TKI was stopped, PFS, date of diagnosis, date of last follow-up, date of death, and OS. Any inconsistencies that were identified were resolved through email correspondence with the respective corresponding authors.

Definition of outcomes

The primary outcome, PFS, was defined as the length of time from the date TKI was started to either the date TKI was stopped or date of tumor progression, depending on availability of data. PFS data was considered 'mature' if treatment was stopped because of progression, and 'censored' if TKI treatment was stopped due to side-effects or was ongoing. The secondary outcome, OS, was defined as the length of time from the date first-line therapy (chemotherapy or TKI) was initiated for advanced disease until death from any cause. OS data was censored if the patient was still alive on the date of last follow-up.

Statistical analysis

The statistical analyses were carried out using SAS Version 9.3 (SAS® Cary, NC, USA), SPSS Version 20 (IBM, NY, USA) and Review Manager (RevMan) Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Differences in baseline characteristics of patients with and without the deletion, or with and without Korean ethnicity, were assessed using two-sample *t*-tests, Fisher's exact test and Pearson Chi-Square test where appropriate. Kaplan-Meier curves for factors reported in the literature to have an impact on survival were compared using the log-rank test. In addition, to evaluate potential differences possibly arising from including patients from different centers, survival was assessed among countries, centers, and ethnicities. As

we did not find evidence that the effect of *BIM* deletion on PFS was modulated by the type of TKI-sensitizing *EGFR* mutation in a Cox regression model incorporating type of *EGFR* mutation, *BIM* deletion and an interaction term *EGFR* mutation x *BIM* deletion ($P = 0.906$), for our analyses, we grouped the patients into those with sensitizing mutations, and those without. *EGFR* phenotype was defined as sensitizing if mutations in exon 18, 19 and 21 were present, and resistant if *EGFR* was wildtype or contained non-synonymous mutations in exon 20.

Univariate HRs were calculated for the following factors: age, gender, smoking history, stage of disease, histology, *EGFR* phenotype (sensitizing or resistant), line of treatment, ECOG status and *BIM* genotype. Owing to the exploratory nature of the analysis, significant factors ($P < 0.2$) were entered into a multivariate Cox regression analysis using a forward stepwise selection method and adjusted HRs and 95% CIs were obtained for the association between the *BIM* deletion and PFS.

To make the most of the available evidence in the absence of individual patient data for some of the studies, an aggregate data meta-analysis was also carried out. Adjusted HRs reported in the original papers were used when available. For the remaining studies, adjusted HRs for the *BIM* deletion were calculated using a Cox proportional hazards model that incorporated the following factors if significant ($P < 0.2$): age, gender, smoking history, stage of disease, histology, *EGFR* phenotype (sensitizing or resistant), line of treatment, and ECOG status (Supplementary Tables 6 to 11). In anticipation of heterogeneity in exposure and treatment-related variables, a random-effects model was employed to calculate summary HRs and 95% CIs for PFS.

Abbreviations

TKI: Tyrosine Kinase Inhibitor; ECOG: Eastern Cooperative Oncology Group; EGFR: Epidermal Growth Factor Receptor; BIM; Bcl-2 Interacting Mediator; NSCLC: Non-Small Cell Lung Cancer; PFS: Progression-free survival; OS: Overall survival; IPD: Individual Patient Data.

Authors' contributions

OST and W-TL conceived the study. OST, W-TL, FJS, JCA, SXS designed the study. SXS, OST, and W-TL identified studies. GWK, JCL, YY, MS, HM, RAS, T-MC, HE, SY, XN, SL, KI, J-HL, JCY, MZ, CZ, J-KL, S-HL, JYL, M-JA, TJT, DST, E-HT contributed patient data. SXS extracted and collated the individualized patient data sent by collaborators, and performed the analyses under supervision of FJS and JCA. SXS wrote the first draft of the report, which was revised and circulated to all collaborators for their comments and final approval.

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CONFLICTS OF INTEREST

We declare no relevant competing interests.

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