RESEARCH ARTICLE

High Expression of Bcl-2 Protein Predicts Favorable Outcome in Non-small Cell Lung Cancer: Evidence from a Systematic Review and Meta-analysis

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Abstract

Background: The prognostic value of Bcl-2 protein expression in non-small cell lung cancer (NSCLC) is under debate. We therefore systematically reviewed the evidence for Bcl-2 protein effects on NSCLC survival to elucidate this issue. Materials and Methods: An electronic search in Pubmed and Embase complemented by manual searches in article references were conducted to identify eligible studies to evaluate the association between Bcl-2 protein expression and overall survival (OS) as well as disease free survival (DFS) of NSCLC patients. Combined hazard ratios (HRs) with corresponding 95% confidence intervals (95%CIs) were pooled using the random-effects model. Results: A total of 50 trials (including 52 cohorts) encompassing 7,765 patients were pooled in the meta-analysis regarding Bcl-2 expression and OS of NSCLC patients. High expression of Bcl-2 protein had a favorable impact (HR=0.76, 95%CI=0.67-0.86). In the group of Bcl-2 expression and DFS, 11 studies including 2,634 patients were included. The synthesized result indicated high expression of Bcl-2 protein might predict good DFS (HR=0.85, 95%CI=0.75-0.95). Conclusions: Our present meta-analysis demonstrated favorable prognostic values of Bcl-2 expression in patients with NSCLC. Further prospective trails are welcomed to validate the utility of assessing Bcl-2 in NSCLC patient management.

Keywords: Bcl-2 - non-small cell lung cancer - prognosis - survival - systematic review - meta-analysis

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Introduction

Lung cancer is the most commonly diagnosed cancer as well as the leading cause of cancer death in males around the global and its incidence is steadily increasing in females (Jemal et al., 2011). Despite diagnostic and therapeutic improvements, the survival of lung cancer patients is still severe, with only 15% patients in US survive more than five years after diagnosis (Dela Cruz et al., 2011; Kumar, 2012). Among the pathological classification spectrum, the non-small cell lung cancer (NSCLC) which typically includes lung adenocarcinoma, lung squamous cell carcinoma and large cell lung carcinoma occupies more than70% of new incidences (Collins et al., 2007). Investigating on the diagnosis, treatment and management of NSCLC, therefore, is a steady hot-spot that will relieve the harmless of NSCLC.

Prognostic markers are biological markers or molecular biomarkers which are used alone or in combination to predict clinical outcomes at the time of diagnosis. To generate useful prognostic markers for NSCLC, one way is analyzing basic clinicopathological features by basic laboratory methods (Kaya et al., 2013) and another way is characterization of proteins and genes involve in tumor initiation and progression process at molecular level (Coate et al., 2009). Base on biological pathways, NSCLC prognostic markers are divided into several categories, including oncogenes or proto-oncogenes (e.g. RAS), tumor suppressor genes (e.g. P53, BRCA1, RRM1, ERCC1), markers of over-proliferation (e.g. EGFR) and markers of aggressive characteristics, such as angiogenesis (e.g. VEGF) (Mitsudomi et al., 2000; Mascaux et al., 2005; Bremnes et al., 2006; Rosell et al., 2007; Zheng et al., 2007; Coate et al., 2009; Pirker et al., 2012). Even though these prognostic markers are identified, their effects remain controversial in different cohorts and clinical utilities are rather limited. More prognostic markers evaluation will be helpful in promoting the translation of laboratory findings to clinical practices, strengthening and optimizing current personalized treatment strategies and thus is a dynamic research subject in NSCLC.

Apoptosis is a pathway in which cells activate enzymes that degrade the cells' own nuclear and cytoplasm to eliminate cells that are no longer needed and genetically altered or injured beyond repair, such as cancer cells. Apoptosis results from the activation of either the

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death receptor (extrinsic) pathway or the mitochondrial (intrinsic) pathway. The powers of death antagonists (e.g. Bcl-2, Bcl-XL, Bcl-W, Mcl-1) and agonists (e.g. Bax, Bak, Bcl-Xs, Bad, Bid) determine the fate of cells (Hockenbery et al., 1990; Chipuk et al., 2010; Hardwick and Soane, 2013). The Bcl-2 proto-oncogene is a 230kb gene that is originally discovered in a follicular B-cell lymphoma and now confirmed in various tumors. Its product, Bcl-2 protein, is located in the inner mitochondrial membrane and inhibits apoptosis to prolong cell survival by arresting cells in the G0/G1 phase of the cell cycle (Hockenbery et al., 1990; Chipuk et al., 2010; Hardwick and Soane, 2013). Envision of apoptosis is one of the most obvious hallmarks of cancers (Kumar, 2012). In several kinds of tumors, the biological functions of Bcl-2 protein have been linked with protecting tumor cells from apoptosis and drug induced death (Zhang and Zhang, 2013). As the consequence, Bcl-2 protein was evaluated in various cancers to investigate their prognostic and predictive significances, including NSCLC (Gascoyne et al., 1997; Anagnostou et al., 2010; Abd El-Hafez et al., 2013).

Although a large number of studies regarding Bcl-2 expression in predicting the survival of NSCLC patients emerged, its definite role remained controversial (Anagnostou et al., 2010; Graziano et al., 2010; Gao et al., 2012).To reconcile the contradiction, a systematic review and meta-analysis that synthesize current original trails are urgently needed. In present study, we performed this work to assess the prognostic values of Bcl-2 expression in NSCLC in an objective and impartial way.

Materials and Methods

Publication search strategy and selection criteria

Here, we reported the study following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISRMA) statement (Moher et al., 2009). To be eligible for inclusion, original studies must meet the following criteria: (i) hazard ratio (HR) and 95% confidence interval (CI) for overall survival (OS) or disease free survival(DFS) of NSCLC patients according to Bcl-2 expression (protein, DNA or RNA) dichotomic status (i.e. Bcl-2 positive *vs* Bcl-2 negative) either was reported or could be computed from the data presented; (ii) dealt with primary NSCLC only (not in metastatic tissue or tissue adjacent to tumor) and included more than 40 patients; (iii) when more than one study was confirmed to report results obtained from the same patient cohort, only the most informative one was included; (iv) full-length papers in English.

An electronic search on Pubmed and Embase, using the strategy in Table 1, complemented by manual search in articles identified by electronic search, was conducted to select the original studies. The search conducted on October 3rd 2013, and no chronological search criteria were used. Moreover, a review of European and American "grey literature" databases (National Technical Information Service and System for Information on Grey Literature in Europe) was conducted as well. The eligibility assessment was performed by two authors (XD Zhao and YY He) independently via two steps. The reviewers firstly screened titles and abstracts to determine possible eligible studies and then read the text for further validation. All reviewers were trained under the same standard and practiced using five articles for calibration. Disagreements between the two authors were resolved by consensus.

Data extraction

Data extraction was conducted by three authors (XD Zhao, YY He and HL Chen) via carefully reading the full-texts. The three authors got agreement via discussion when the extracted data were not uniform with each author. The extracted information included: first author's name; country; year of publication; inclusion and exclusion criteria; number of patient; age; gender; NSCLC stage; detection method; primary antibody information; cut-off value of dichotomic status; Bcl-2 positive rate; follow-up interval; survival data analysis method and HR and 95%CI of OS and DFS.

Methodological Assessments

To assess methodology, three investigators (XD Zhao, YY He and HL Chen) read each publication and scored them independently according to Newcastle-Ottawa scale (NOS) (Wells GA SB, 2000). Each item of the NOS scoring system was assessed using an ordinal scale (possible values 2, 1, 0 for item 5 and 1, 0 for other items of the NOS evaluation system). Final scores were reached in a meeting attended by all three evaluators via a consulting manner. Along with evaluation of original trails, the systematic review and meta-analysis itself was assessed. The widely used "Assessment of Multiple Systematic Reviews" (AMSTAR) checklist was performed for evaluating the current research (Shea et al., 2007).

Statistical analysis

In this systematic review and meta-analysis, a study was classed as "positive (+)" when high Bcl-2 expression level was a favorable OS/DFS predictor. Other situations,

Table 1. Search Strategy (up to October 3rd, 2013)

Search step	Search terms
#1	Bcl-2[Title/Abstract]
#2	Bcl2[Title/Abstract]
#3	Bcl 2[Title/Abstract]
#4	B-cell leukemia-2[Title/Abstract]
#5	B-cell lymphoma-2[Title/Abstract]
#6=#1 OR #2 OR #3 OR #4 OR #5	
#7	Prognos*[Title/Abstract]
#8	Predict*[Title/Abstract]
#9	Surviv*[Title/Abstract]
#10	Outcome*[Title/Abstract]
#11	Determine*[Title/Abstract]
#12=#7 OR #8 OR #9 OR #10 OR #11	
#13	NSCLC[Title/Abstract]
#14	NSCLCs[Title/Abstract]
#15	Non-Small-Cell Lung Carcinoma*[Title/Abstract]
#16	Non-Small-Cell Lung Cancer*[Title/Abstract]
#17	Non Small Cell Lung Carcinoma*[Title/Abstract]
#18	Non Small Cell Lung Cancer*[Title/Abstract]
#19	Nonsmall Cell Lung Carcinoma*[Title/Abstract]
#20	Nonsmall Cell Lung Cancer*[Title/Abstract]
#21	Lung Adenocarcinoma [Title/Abstract]
#22	Lung Squamous cell carcinoma [Title/Abstract]
#23	Large Cell Lung Carcinoma [Title/Abstract]
#24=#13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 #25=#6 AND #12 AND #24	

including the situation where high Bcl-2 expression level predicted poor OS/DFS or failed to predict OS/DFS were called "negative (-)". For the quantitative aggregation of the survival results, HRs and corresponding 95%CIs were used. We extracted the HR and 95%CI from each trail based on the results provided in the publication. We preferred to include data from multivariate Cox hazard regression analysis if were available as they were most accurate. Otherwise, we extracted data from univariate analysis instead. In cases where HRs and 95%CIs (Bcl-2 positive group *vs* Bcl-2 negative group) were not directly reported, we estimated them via loge hazard ratio (logHR) and standard error (SE) (logHR) and survival curves using the methods developed by Parmar et al. (1998), Williamson et al. (2002) and Tierney et al. (2007). The software used for calculating these values was designed by Tierney and his colleagues, published in *Trials*, 2007 (Tierney et al., 2007). If not available, we tried to connect with authors for unreported data.

Meta-analysis was performed using STATA 12.0 (Stata Corporation, College Station, TX, USA). In order to choose correct statistical model to summarize effect sizes of selected studies, we combined the consideration of heterogeneity testing (p-value and I^2-value), the differences between original trails and premises of statistic models (Borenstein M, 2009). When homogeneity testing showed significant heterogeneity ($p<0.1$ and $I^2>50\%$), random-effect model was chosen. Considering the obvious differences among original trails, such as different regions, disease stages, primary antibodies and inconsonant cut-off points, we preferred the random-effect model even homogeneity testing was not significant ($p\geq0.1$ or $I^2\leq50\%$). Forest plots were used to illustrate the HR and corresponding 95%CI of each included study and the synthesized results. An observed HR<1 indicated a better outcome for the high expression group and was considered statistically significant if corresponding 95%CI did not overlap 1. For subgroup analysis, five stratifying variables including publication year, cohort region, number of patients, disease stage and quality score were selected based on our review of original studies. We didn't use more than "study number/10"stratifying variables to avoid excessive data mining. Publication bias was assessed by visually evaluating the symmetry of funnel plot and formally with the Begg's tests. $p>0.05$ indicated no potential publication bias. Sensitivity analysis was performed by extraction of each single study to investigate the stability of the results.

Results

Study selection and quality assessment

Through the database search, a total of 542 articles were identified for initial evaluation after removing duplicates (Figure 1). Among the first round excluded articles, 360 articles belong to one of the following: basic studies in cell lines or animal models; review articles; articles not in English and abstracts. In the second round, 128 articles were excluded because of sample size lower than 40 patients and failing to provide enough survival data for extracting HRs and corresponding 95%CIs. After the two rounds of exclusion, 54 articles evaluating the prognostic value for OS or DFS of Bcl-2 levels in NSCLC were remained for further quality assessment in detail and data pooling.

The main characteristics of the 54 studies (56 independent cohorts) eligible for the systematic review and meta-analysis were showed in Table 2. A total of 31 studies reported HRs and corresponding 95%CIs. In the remaining 23 studies, we evaluated their HRs and corresponding 95%CIs based on reported data. The total study sample size of 54 studies was 8522 with a mean of 158 (range, 45-535 patients). 23 cohorts (Pezzella et al., 1993; Fontanini et al., 1995; Fontanini et al., 1996; O'Neill et al., 1996; Apolinario et al., 1997; Koukourakis et al., 1997; Pastorino et al., 1997; Fontanini et al., 1998; Cox et al., 2000; Moldvay et al., 2000; Nguyen et al., 2000; Cox et al., 2001; Laudanski et al., 2001; Rigau et al., 2002; Swinson et al., 2002; Grossi et al., 2003; Swinson et al., 2004; Fokkema et al., 2006; Yaren et al., 2006; Ludovini et al., 2008; Anagnostou et al., 2010; Grimminger et al., 2010; Karpathiou et al., 2013) evaluated patients from Europe, 12 cohorts (Ritter et al., 1995; Anton et al., 1997; Kwiatkowski et al., 1998; D'Amico et al., 1999; Mehdi et al., 1999; Carvalho et al., 2000; Han et al., 2002; Huang et al., 2003; Poleri et al., 2003; Renouf et al., 2009; Anagnostou et al., 2010; Graziano et al., 2010) from America and 21 cohorts (Ohsaki et al., 1996; Higashiyama et al., 1997; Ishida et al., 1997; Kim et al., 1998; Dosaka-Akita et al., 1999; Huang et al., 1999; Hwang et al., 2001; Hanaoka et al., 2002; Lai et al., 2002; Tomita et al., 2003; Shibata et al., 2004; Yoo et al., 2007; Liu et al., 2008; Lee et al., 2009; Ma, 2009; Shim et al., 2009; Zhu et al., 2009; Shi et al., 2011; Gao et al., 2012; Ko et al., 2013) from Asia. Methodological score of each selected study using NOS evaluation system was listed in Table 2. NOS scores of 1-3, 4-6 and 7-9 were defined as low, intermediate and high quality studies, respectively. All the included 54 studies had a median overall score of 7 (range 5 to 9), indicating the high quality of included original studies. The AMSTAR evaluation system was

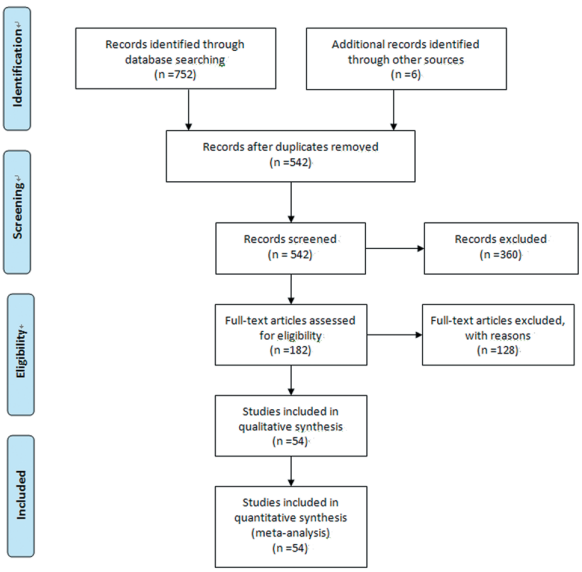

Figure 1. PRIMA Flow Diagram of Study Inclusion

Table 2. Main Characteristics of Included Studies

Study	Year	Region	Sample Number	Stage III&IV%	Follow-up Range (Month)	Follow-up Median (Month)	Method	Primary Antibody (Dilution)	Cut-off Value	Positive Rate%	Con-clusion	Quality Score
Pezzella	1993	UK	115	0	1.7-76	34	IHC	Clone100	PPC	21.7	+	8
Fontanini-1	1995	Italy	89	NR	2-41#	25#	IHC	Clone124 (1:20)	1%	67	+	7
Ritter	1995	USA	126	0	1-91	39	IHC	Clone124 (1:80)	5%	37	-	6
A.J.O'neill	1996	Ireland	66	0	1-47	NR	IHC	Clone124 (1:50)	1%	25*	-	7
Ohsaki	1996	Japan	96	53.5#	NR	NR	IHC	Clone124 (1:60)	20%	19.2*	+	8
Fontanini-2	1996	Italy	70	NR	32-51	46(Mean)	IHC	Clone124 (1:20)	1%	60	+	7
Ishida	1997	Japan	114	21.1	3-80	28.5	IHC	Clone124 (1:50)	10%	38	+	7
Higashiyama	1997	Japan	174	32.4#	1.1-75#	34.6#	IHC	Clone124 (1:50)	10%	19.8	+	7
Koukourakis	1997	UK	107	NR	NR	45	IHC	Clone100	PPC	18.7	-	7
Apolinario	1997	Netherlands	73	0	NR	NR	IHC	Clone100 (1:25)	0.50%	50.68	-	7
Anton	1997	USA	427	18.9	3-185	53.4	IHC	Clone124 (1:60)	PPC	46.8	-	5
Pastorino	1997	Italy	485	0	NR	64#	IHC	Clone100	10%	16*	-	7
Kwiatkowski	1998	USA	186	0	36-133#	65#	IHC	Clone124	NR	42	-	9
Fontanini-3	1998	Italy	107	NR	28-59	51	IHC	Clone124 (1:20)	NR	40.2	+	6
Kim	1998	Korea	238	77.7	1-73.9	21.8	IHC	Clone124	PPC	71.8	-	8
Huang-1	1999	Japan	203	32	18.2-65.4	41.8	IHC	Clone124 (1:50)	Score=50	38.9	-	8
Dosaka-Akita	1999	Japan	89	34.5#	NR	NR	IHC	Clone124	10%	34*	-	8
Mehdi	1999	USA	241	0	NR	NR	IHC	Clone124	Score=2	34	-	7
D'Amico	1999	USA	408	0	UB>60	NR	IHC	Clone120	50%	23	-	8
Cox-1	2000	UK	178	23.6	24-108	39.9	IHC	Clone124 (1:25)	20%	34.8	+	8
Moldvay	2000	France	227	44.1	18-109	NR	IHC	Clone124 (1:40)	PPC	25.1	+	8
Carvalho	2000	Brazil	45	44	4-90	22	IHC	Clone124 (1:400)	Score=17.4	33.3	+	7
Nguyen	2000	Czech	49	NR	LB>24	NR	IHC	Clone124	PPC	29.2*	-	6
Cox-2	2001	UK	167	21.6	24-108	39.8	IHC	Clone124 (1:25)	20%/ ++~+++	36.1	+	7
Laudanski	2001	Poland	100	52.9#	6.9-42.7	28	IHC	Clone124 (1:100)	20%	48*	-	8
Hwang	2001	Korea	53	98.5#	NR	NR	IHC	Clone124	50%	41.5	-	6
Han	2002	USA	85	0	32-44	39	IHC	Clone124 (1:60)	10%	46	-	8
Swinson-1	2002	UK	178	22.5	NR	NR	IHC	Clone124	NR	40.5	-	8
Lai	2002	Taiwan Area	100	25.4#	NR	34#	IHC	Clone100 (1:40)	10%	22.8*	-	7
Hanaoka	2002	Japan	70	21.4	2-67	33 (Mean)	IHC	Clone124 (1:30)	Score=2.6	58.6	-	7
Rigau	2002	France	86	41.9	85-125	107	IHC	Clone124 (1:50)	5%	52	-	6
Poleri	2003	Argentina	53	0	9-168	59	IHC	Clone100	33%	30	-	8
Huang-2	2003	USA	91	23	NR	NR	WB	Clone100 (1:500)	Present Band	52.7	-	6
Grossi	2003	Italy	213	30#	NR	NR	IHC	Clone124 (1:20)	50% or Strong Intensity	32.4	-	7
Tomita	2003	Japan	60	100	UB>60	NR	IHC	Clone124 (1:100)	10%	20	+	7
Swinson-2	2004	UK	172	21.5	61.7-130 (Alive)	90.6 (Alive)	IHC	Clone124	20%	34.3	-	7
Shibata	2004	Japan	120	24.2	NR	38.2	IHC	Clone124	10%	29.7	+	6
Yaren	2006	Turkey	69	42	3-102	34.7(Mean)	IHC	Clone100 (1:50)	Score=4	36.2	-	5
Fokkema	2006	Netherlands	84	100	NR	NR	IHC	Clone124 (1:50)	10% or Staining Intensity=1	58	+	5
Yoo	2007	Korea	219	26.9	1.6-117.8	38.9	IHC	Clone100 (1:50)	10%	11.4	-	9
Liu	2008	China	159	100	UB>60	NR	IHC	Clone124 (1:50)	NR	66.7	-	7
Ludovini	2008	Italy	136	25.7	NR	37	IHC	Clone100 (1:50)	10%	27.9	-	7
Renouf	2009	Canada	535	0	1.1-323.2	42.24	IHC	Clone124 (1:20)	5%	27.9	+	8
Ma	2009	China	78	100	3/22/14	11	IHC	Clone124 (1:40)	10% Moderate Staining	48.7	-	6
Lee	2009	Korea	50	100	1-47	11	IHC	Clone100 (1:50)	Median Score	16	-	9
Zhu	2009	China (Training cohort)	73	0	5-161.4	97.3	IHC	Clone100 (1:100)	Score=4	NR	-	8
	2009	China (Validating cohort)	75	0	3-83.1	61	IHC	Clone100 (1:100)	Score=4	NR	-	8
Shim	2009	Korea	49	100	NR	NR	IHC	Clone100 (1:50)	5%	44.9	-	5
Grimminger	2010	Germany	91	29.7	63-105	85.9	RT-PCR	No Antibody	16%	44	+	7
Graziano	2010	USA	222	0	UB>120	NR	IHC	Clone124	Score=2	NR	-	6
Anagnostou	2010	USA (Training cohort)	180	29.4	0.1-182	27.3	IHC	Clone124	AQUA Score=18.8	50	+	8
	2010	Greece (Validating cohort)	354	37.6	0.1-223	20	IHC	Clone124	AQUA Score=18.8	52	+	5
Shi	2011	China	144	34	16.4-63.7	35.8	IHC	Clone E17	Median Score	30.6	+	8
Gao	2012	China	62	46.8	3-120	NR	IHC	NR	Score=1	51.6	-	8
Karpathiou	2012	Greece	113	28.7#	2-102	32	IHC	Clone100 (1:50)	Strong Positive Cell or 50% Weak Positive	18*	-	7
Ko	2013	Korea	374	0	UB>150	65#	IHC	Clone100 (1:00)	Score=2	14.2*	+	7

*UB: Upper Bound; LB: Lower Bound; IHC: Immunohistochemistry; WB: Western-blot; RT-PCR: Reverse Transcription-polymerase Chain Reaction; PPC: Present Positive Cell; AQUA: Automated Quantitative Analysis; # indicates the data is from whole patients cohort which includes patients in survival analysis

performed to assess the quality of this systematic review and meta-analysis and our research fulfilled more than 9 of 11 items (except item 1 and 5) in AMSTAR evaluation system, indicating a good quality was reached.

Effects of Bcl-2 expression on OS of NSCLC

Totally, 50 studies (52 independent cohorts) that included 7765 patients reported the OS predictive value of Bcl-2 in NSCLC. Figure 2A demonstrated the forest plot of individual HR and corresponding 95%CI and results from the meta-analysis regarding Bcl-2 expression and OS of NSCLC patients. Certain degree of heterogeneity was observed in this group (I^2=56.2%, p=0.00). Overall, the pooled HR and 95%CI for all studies showed a significant decreased risk of death in NSCLC patients with high Bcl-2 expression level (HR=0.76, 95%CI=0.67-0.86,random-effect model). Sensitivity analysis via omitting original investigations in order validated stability of overall analysis result (Figure 3A). The funnel plot (Figure 4A) for publication bias indicated a good degree of symmetry, demonstrating no obvious publication bias existed. Begg's test showed no significant publication bias as well (p=0.84).

Subgroup analysis by publication year, cohort region, number of patients, disease stage and quality score was performed and the results are summarized in Table 3. Particularly, very good homogeneity was observed in subsets of American studies and studies contain only early stage patients (Both I^2=0.00%). Nearly in all subsets, the

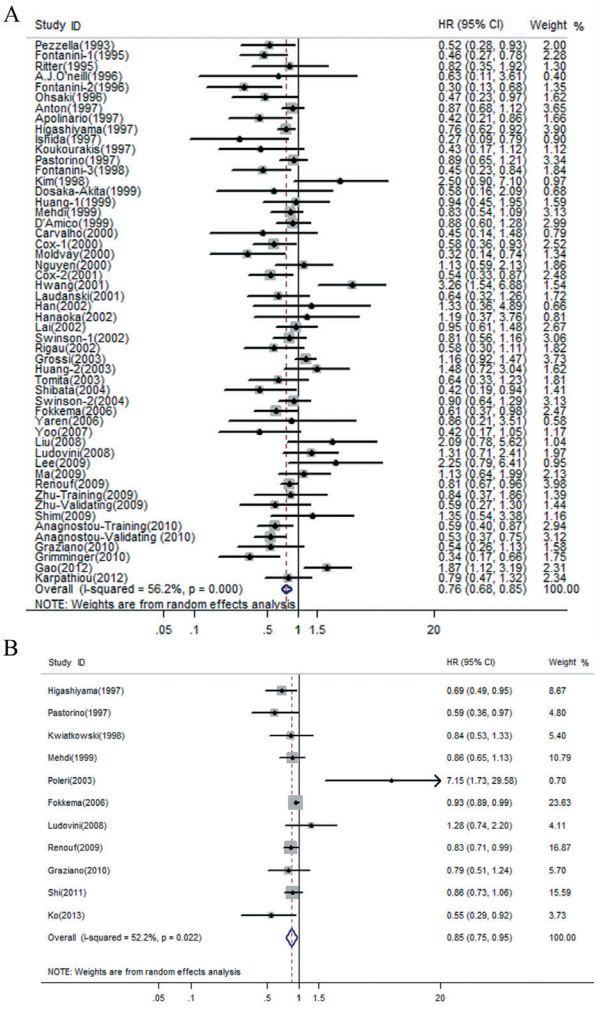

Figure 2. Meta-analysis of Impact of Bcl-2 Expression on OS (A) and DFS (B) of Patients with NSCLC. Results are presented as individual and pooled HR, and corresponding 95%CI

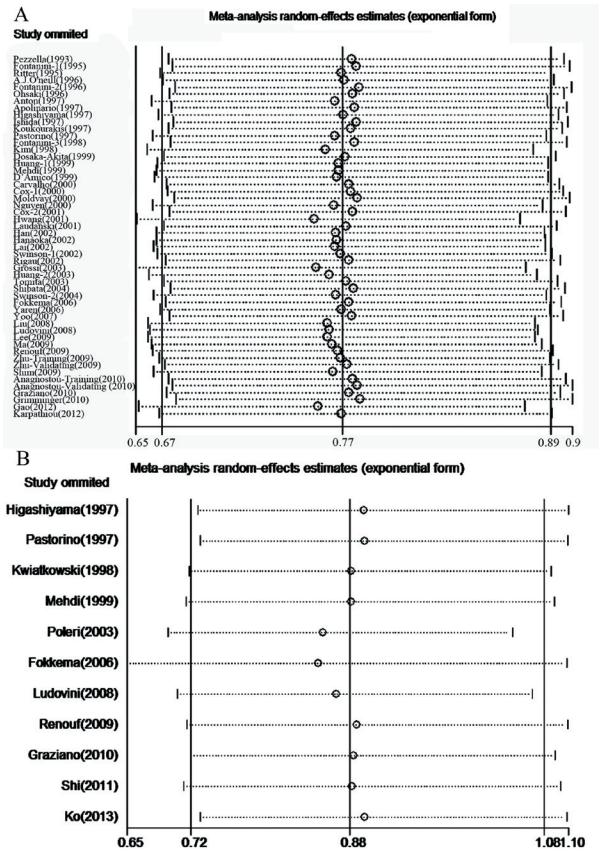

Figure 3. Result of Sensitivity Analysis of Aggregated Result of Bcl-2 Expression on OS (A) and DFS (B) of Patients with NSCLC

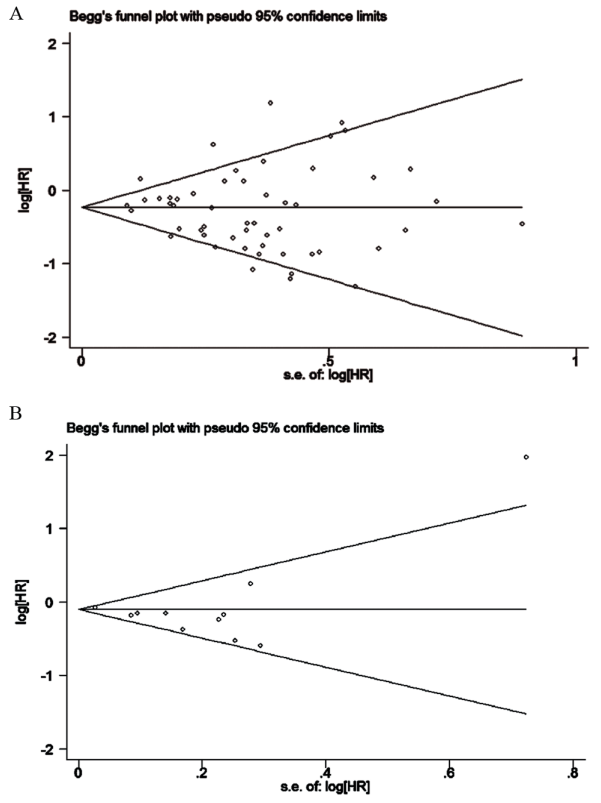

Figure 4. Funnel Plots of Included Trails Reporting Prognostic Value of Bcl-2 Expression in OS (A) and DFS (B) of Patients with NSCLC

Table 3. Results of Subgroup Analysis for effects of Bcl-2 Expression on NSCLC Patients' OS (52 cohorts)

Stratified analysis		No. of cohorts	No. of patients	Pooled HR (95%CI)		Heterogeneity
				Fixed-model	Random-model	testing (I^2)
Cohort region	America	10	2360	0.81 (0.72-0.91)	0.81 (0.72-0.91)	0.00%
	Europe	23	3329	0.73 (0.66-0.81)	0.65 (0.54-0.77)	58.70%
	Asia	19	2076	0.88 (0.77-1.00)	0.95 (0.73-1.23)	63.50%
Publication year	≤2001	25	4041	0.74 (0.67-0.81)	0.69 (0.58-0.81)	55.70%
	>2002	27	3724	0.84 (0.77-0.92)	0.83 (0.71-0.98)	55.40%
Number of patients	≤200	40	3993	0.75 (0.69-0.82)	0.75 (0.64-0.87)	54.60%
	>200	12	3772	0.85 (0.77-0.93)	0.81 (0.68-0.97)	59.80%
Disease stage#	Stage III&IV=0%	12	2504	0.79 (0.70-0.89)	0.79 (0.70-0.89)	0.00%
	Stage III&IV=1%-99%	29	4359	0.81 (0.74-0.88)	0.77 (0.66-0.92)	65.50%
	Stage III&IV=100%	6	480	0.93 (0.71-1.24)	1.05 (0.68-1.61)	52.50%
Quality score	≤6	15	1929	0.82 (0.72-0.93)	0.87 (0.67-1.14)	69.40%
	>6	37	5836	0.78 (0.73-0.85)	0.73 (0.64-0.83)	48.80%

#5 cohorts didn't report disease stages of patients

pooled results supported a favorable predictive value of Bcl-2 expression excepted subsets of studies in Asia (HR=0.95, 95%CI=0.73-1.23, random-effect model), studies contain only late stage patients (HR=0.93, 95%CI=0.71-1.24, random-effect model; HR=1.05, 95%CI=0.68-1.61, random-effect model) and studies with low quality score (HR=0.87, 95%CI=0.67-1.14, random-effect model).

Effects of Bcl-2 expression on DFS of NSCLC

As indicated in Figure 2B, 11 studies including 2634 patients reported the DFS predictive value of Bcl-2 in NSCLC. The pooled result reached statistical significance that high Bcl-2 expression level predicted good DFS (HR=0.85, 95%CI=0.75-0.95, random-effect model). Obvious heterogeneity was observed in this group (p=0.02 or I^2=52.2%). Sensitivity analysis via omitting individual investigation orderly indicated a certain degree of unstable of the aggregated result when some studies were deleted (Figure 3B). Funnel plot and Begg's test were performed to detect publication bias. The result of Begg's text didn't reach significant (p=0.592), however, the funnel plot indicated a certain degree of asymmetry (Figure 4B).

Discussion

Lung cancer, with a high incidence and mortality rate, is regarded as a serious health threaten around the global and NSCLC is the most common pathological type (Collins et al., 2007; Jemal et al., 2011). Even though a few prognostic and predictive markers have been validated, such as EGFR, identifying more established markers possessing the predictive value for survival of NSCLC patients remains a topic for exploration. Bcl-2, an inhibitor of intrinsic cell apoptosis, has important functions in tumor initiation and progression (Hockenbery et al., 1990; Chipuk et al., 2010; Hardwick and Soane, 2013). In past twenty years, increasing number of studies were performed to evaluate prognostic value of Bcl-2 expression in NSCLC, however, the controversy still retain. A systematic review and meta-analysis is an effective way to reconcile the contradiction and lead to a relative confirmed conclusion. We thus conducted this work to aggregate current original results to elucidate the outcome predictive values of Bcl-2 in NSCLC.

Our systematic review and meta-analysis based on the outcomes of 7765 patients from 50 individual trails (52 independent cohorts), revealing that high expression of Bcl-2 protein is a favorable OS predictive marker in patients with NSCLC (HR=0.76, 95%CI=0.67-0.86). This result keeps stable in sensitivity analysis, indicating the real and steady effect of Bcl-2 expression on NSCLC OS prediction. Similarly, the pooled data based on 2634 patients, suggested high expression of Bcl-2 protein predicted good DFS in patients with NSCLC (HR=0.85, 95%CI=0.75-0.95). Unfortunately, the current conclusion regarding DFS is not stable enough as showed in Figure 3B.

Although we strived to validate our aggregated results by several inclusion/exclusion criteria and analysis methods, our approach didn't eliminate all potential biases. Funnel plot and Begg's test didn't present significant publication bias; however, the potential publication bias in our study couldn't be totally excluded. To utmost make sure that we can get full information in original studies, our systematic review and meta-analysis only took into account fully published studies. Though this inclusion criterion ensured us to get sufficient information about each trails and analyzing heterogeneities in different trails, it increased the risk of false positive results as well. As we know, studies that do not possess statistically significant results are less frequently published in full papers, but probably in abstracts that were ruled out of our analysis. It should be also note that our analysis only searched original articles published in English. As Egger M et al. (1997) indicated, positive results are more frequently published in English, while those negative ones tend to be more often published in native languages. This limitation will potentially lead to favor of positive original studies in our analysis and influence the reliability of synthesized conclusions.

Another potential source of bias derived from the method of extracting HRs and corresponding 95%CIs. If the original articles didn't report the HR and 95%CI, we had to estimate them based on data available in the article and the survival curve. Since the method established by Parmar et al. (1998), Williamson et al. (2002) and Tierney et al. (2007) cannot thoroughly restore all primary data for calculating HRs and 95%CIs, random errors existed in this process without doubt. Besides, the use of same cohort of patients for different publications couldn't be totally excluded. If the patient number was not totally the

same in two papers, we assumed that the authors were honest enough not to re-report the results from the same cohort of patients. Thus, we couldn't rule out same cohorts were included twice or more in this meta-analysis, that would give higher weighting to a particular positive or negative trend.

The heterogeneity within different studies is of concern when interpreting the clinical utility of the current conclusion that high expression of Bcl-2 predicts good survival in patients with NSCLC. As showed in Figure 2A, heterogeneity testing detected certain degree of inter study heterogeneity. Information in Table 2 visually displayed the difference between original trails, such as the primary anti-body dilution, cut-off point of positive and negative (high and low) and survival data analysis method. Therefore, more well-designed retrospective and prospective trails that aim to promote its clinical utilities via validating the most suitable disease stage, best cut-off point and so on are still highly welcomed.

According to our aggregated result, patients with Bcl-2-positive tumors had significantly better survival than those with Bcl-2-negative tumors. It seems that this conclusion is controversial with biological functions of Bcl-2 protein. Originally, the high expression of Bcl-2 gene product is implicated in tumorigenesis because of its ability to prolong cell survival through the inhibition of apoptosis (Hockenbery et al., 1990). The process of apoptosis involves both the anti-apoptotic proteins (such as Bcl-2, Bcl-X, and Bfl-1) and the pro-apoptotic proteins (such as Bax, Bak and Bad), which can interact collaboratively or antagonistically to regulate cellular apoptosis (Kroemer, 1997; Chipuk et al., 2010; Hardwick and Soane, 2013). Thus, the study of only one apoptotic protein leads to a partial appraisal of apoptosis and this may partly reconcile the above paradox. Here, we may suppose that evaluating the anti-apoptotic and pro-apoptotic proteins in combination in NSCLC tissues is possibly an interesting and worthwhile research topic. Furthermore, the cell cycle entry inhibition role and carcinogenesis inhibition phenomenon of Bcl-2 as demonstrated in some articles (Pierce et al., 2002; Kirkin et al., 2004) also suggested the possibility and rationality of high Bcl-2 expression is a good prognosticator. Of course, more direct biological evidences are highly needed to illustrate the molecular mechanisms and support the current conclusion.

To sum up, this meta-analysis got a rather safe conclusion that high expression of Bcl-2 protein predicted good OS in NSCLC patients. However, as inter study heterogeneity exists in current trails, the fittest cut-off value, disease stage and other clinical practice relevant parameters remain undetermined. For further research, more high quality prospective clinical *trials* and high quality retrospective cohort studies are worthwhile to be performed and highly needed.

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References

Abd El-Hafez A, Shawky Mohamed Ael A, Elesawy BH (2013). Different prognostic factors correlate with Bcl-2 expression among triple negative and non-triple negative breast cancers. *Asian Pac J Cancer Prev*, **14**, 1037-41.

Anagnostou VK, Lowery FJ, Zolota V, et al (2010). High expression of BCL-2 predicts favorable outcome in non-small cell lung cancer patients with non squamous histology. *BMC Cancer*, **10**, 186.

Anton RC, Brown RW, Younes M, et al (1997). Absence of prognostic significance of bcl-2 immunopositivity in non-small cell lung cancer: analysis of 427 cases. *Hum Pathol*, **28**, 1079-82.

Apolinario RM, van der Valk P, de Jong JS, et al (1997). Prognostic value of the expression of p53, bcl-2, and bax oncoproteins, and neovascularization in patients with radically resected non-small-cell lung cancer. *J Clin Oncol*, **15**, 2456-66.

Borenstein M HL, Higgins JPT, Rothstein HR 2009. Fixed-effect versus random-effects models. introduction to meta-analysis, John Wiley & Sons, Ltd.

Bremnes RM, Camps C, Sirera R (2006). Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood. *Lung Cancer*, **51**, 143-58.

Carvalho PE, Antonangelo L, Bernardi FD, et al (2000). Useful prognostic panel markers to express the biological tumor status in resected lung adenocarcinomas. *Jpn J Clin Oncol*, **30**, 478-86.

Chipuk JE, Moldoveanu T, Llambi F, et al (2010). The BCL-2 family reunion. *Mol Cell*, **37**, 299-310.

Coate LE, John T, Tsao MS, et al (2009). Molecular predictive and prognostic markers in non-small-cell lung cancer. *Lancet Oncol*, **10**, 1001-10.

Collins LG, Haines C, Perkel R, et al (2007). Lung cancer: diagnosis and management. *Am Fam Physician*, **75**, 56-63.

Cox G, Louise Jones J, Andi A, et al (2001). Bcl-2 is an independent prognostic factor and adds to a biological model for predicting outcome in operable non-small cell lung cancer. *Lung Cancer*, **34**, 417-26.

Cox G, Walker RA, Muller S, et al (2000). Does immunointensity account for the differences in prognostic significance of Bcl-2 expression in non-small cell lung cancer? *Pathol Oncol Res*, **6**, 87-92.

D'Amico TA, Massey M, Herndon JE, 2nd, et al (1999). A biologic risk model for stage I lung cancer: immunohistochemical analysis of 408 patients with the use of ten molecular markers. *J Thorac Cardiovasc Surg*, **117**, 736-43.

Dela Cruz CS, Tanoue LT, Matthay RA (2011). Lung cancer: epidemiology, etiology, and prevention. *Clin Chest Med*, **32**, 605-44.

Dosaka-Akita H, Katabami M, Hommura H, et al (1999). Bcl-2 expression in non-small cell lung cancers: higher frequency of expression in squamous cell carcinomas with earlier pT

status. *Oncology*, **56**, 259-64.
Egger M, Zellweger-Zahner T, Schneider M, et al (1997). Language bias in randomised controlled *trials* published in English and German. *Lancet*, **350**, 326-9.
Fokkema E, Timens W, de Vries EG, et al (2006). Expression and prognostic implications of apoptosis-related proteins in locally unresectable non-small cell lung cancers. *Lung Cancer*, **52**, 241-7.
Fontanini G, Boldrini L, Vignati S, et al (1998). Bcl2 and p53 regulate vascular endothelial growth factor (VEGF)-mediated angiogenesis in non-small cell lung carcinoma. *Eur J Cancer*, **34**, 718-23.
Fontanini G, Vignati S, Bigini D, et al (1995). Bcl-2 protein: a prognostic factor inversely correlated to p53 in non-small-cell lung cancer. *Br J Cancer*, **71**, 1003-7.
Fontanini G, Vignati S, Bigini D, et al (1996). Recurrence and death in non-small cell lung carcinomas: a prognostic model using pathological parameters, microvessel count, and gene protein products. *Clin Cancer Res*, **2**, 1067-75.
Gao Q, Yang S, Kang MQ (2012). Influence of survivin and Bcl-2 expression on the biological behavior of non-small cell lung cancer. *Mol Med Rep*, **5**, 1409-14.
Gascoyne RD, Adomat SA, Krajewski S, et al (1997). Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. *Blood*, **90**, 244-51.
Graziano SL, Gu L, Wang X, et al (2010). Prognostic significance of mucin and p53 expression in stage IB non-small cell lung cancer: a laboratory companion study to CALGB 9633. *J Thorac Oncol*, **5**, 810-7.
Grimminger PP, Schneider PM, Metzger R, et al (2010). The prognostic role of Bcl-2 mRNA expression in curatively resected non-small cell lung cancer (NSCLC). *Lung Cancer*, **70**, 82-7.
Grossi F, Loprevite M, Chiaramondia M, et al (2003). Prognostic significance of K-ras, p53, bcl-2, PCNA, CD34 in radically resected non-small cell lung cancers. *Eur J Cancer*, **39**, 1242-50.
Han H, Landreneau RJ, Santucci TS, et al (2002). Prognostic value of immunohistochemical expressions of p53, HER-2/neu, and bcl-2 in stage I non-small-cell lung cancer. *Hum Pathol*, **33**, 105-10.
Hanaoka T, Nakayama J, Haniuda M, et al (2002). Immunohistochemical demonstration of apoptosis-regulated proteins, Bcl-2 and Bax, in resected non-small-cell lung cancers. *Int J Clin Oncol*, **7**, 152-8.
Hardwick JM, Soane L (2013). Multiple functions of BCL-2 family proteins. *Cold Spring Harb Perspect Biol*, 5.
Higashiyama M, Doi O, Kodama K, et al (1997). bcl-2 oncoprotein in surgically resected non-small cell lung cancer: possibly favorable prognostic factor in association with low incidence of distant metastasis. *J Surg Oncol*, **64**, 48-54.
Hockenbery D, Nunez G, Milliman C, et al (1990). Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*, **348**, 334-6.
Huang C, Kohno N, Inufusa H, et al (1999). Overexpression of bax associated with mutations in the loop-sheet-helix motif of p53. *Am J Pathol*, **155**, 955-65.
Huang CI, Neuberg D, Johnson BE, et al (2003). Expression of bcl-2 protein is associated with shorter survival in nonsmall cell lung carcinoma. *Cancer*, **98**, 135-43.
Hwang JH, Lim SC, Kim YC, et al (2001). Apoptosis and bcl-2 expression as predictors of survival in radiation-treated non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys*, **50**, 13-8.
Ishida H, Irie K, Itoh T, et al (1997). The prognostic significance of p53 and bcl-2 expression in lung adenocarcinoma and its correlation with Ki-67 growth fraction. *Cancer*, **80**, 1034-45.
Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.
Karpathiou G, Sivridis E, Koukourakis M, et al (2013). Autophagy and Bcl-2/BNIP3 death regulatory pathway in non-small cell lung carcinomas. *APMIS*, **121**, 592-604.
Kim YC, Park KO, Kern JA, et al (1998). The interactive effect of Ras, HER2, P53 and Bcl-2 expression in predicting the survival of non-small cell lung cancer patients. *Lung Cancer*, **22**, 181-90.
Kaya V, Yildirim M, Demirpence O, et al (2013). Prognostic significance of basic laboratory methods in non- small-cell-lung cancer. *Asian Pac J Cancer Prev,* **14**, 5473-6.
Kirkin V, Joos S, Zornig M (2004). The role of Bcl-2 family members in tumorigenesis. *Biochim Biophys Acta*, **1644**, 229-49.
Ko E, Kim Y, Cho EY, et al (2013). Synergistic effect of Bcl-2 and cyclin A2 on adverse recurrence-free survival in stage I non-small cell lung cancer. *Ann Surg Oncol*, **20**, 1005-12.
Koukourakis MI, Giatromanolaki A, O'Byrne KJ, et al (1997). Potential role of bcl-2 as a suppressor of tumour angiogenesis in non-small-cell lung cancer. *Int J Cancer*, **74**, 565-70.
Kroemer G (1997). The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nat Med*, **3**, 614-20.
Kumar VA, AK. Aster, JC 2012. Robbins basic pathology, 9th edition, Saunders.
Kwiatkowski DJ, Harpole DH Jr., Godleski J, et al (1998). Molecular pathologic substaging in 244 stage I non-small-cell lung cancer patients: clinical implications. *J Clin Oncol*, **16**, 2468-77.
Lai RS, Wang JS, Hsu HK, et al (2002). Prognostic evaluation of the expression of p53 and bcl-2 oncoproteins in patients with surgically resected non-small cell lung cancer. *Jpn J Clin Oncol*, **32**, 393-7.
Laudanski J, Niklinska W, Burzykowski T, et al (2001). Prognostic significance of p53 and bcl-2 abnormalities in operable nonsmall cell lung cancer. *Eur Respir J*, **17**, 660-6.
Lee HW, Choi YW, Han JH, et al (2009). Expression of excision repair cross-complementation group 1 protein predicts poor outcome in advanced non-small cell lung cancer patients treated with platinum-based doublet chemotherapy. *Lung Cancer*, **65**, 377-82.
Liu H, Zhang T, Li X, et al (2008). Predictive value of MMP-7 expression for response to chemotherapy and survival in patients with non-small cell lung cancer. *Cancer Sci*, **99**, 2185-92.
Ludovini V, Pistola L, Gregorc V, et al (2008). Biological markers and DNA flow cytometric analysis in radically resected patients with non-small cell lung cancer. A study of the Perugia Multidisciplinary Team for Thoracic Tumors. *Tumori*, **94**, 398-405.
Ma HS H, Huang F, Li J, Cao X, Jiang W (2009). Expression of ERCC1, Bcl-2, MT and their clinical significance in advanced non-small-cell lung cancer treated with cisplatin-based chemotherapy. *Latin Am J Pharmacy*, **28**, 827-34.
Mascaux C, Iannino N, Martin B, et al (2005). The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer*, **92**, 131-9.
Mehdi SA, Tatum AH, Newman NB, et al (1999). Prognostic markers in resected stage I and II non small-cell lung cancer: an analysis of 260 patients with 5 year follow-up. *Clin Lung Cancer*, **1**, 59-67.
Mitsudomi T, Hamajima N, Ogawa M, et al (2000). Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analysis. *Clin Cancer Res*, **6**, 4055-63.
Moher D, Liberati A, Tetzlaff J, et al (2009). Preferred reporting

items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*, **6**, 1000097.

Moldvay J, Scheid P, Wild P, et al (2000). Predictive survival markers in patients with surgically resected non-small cell lung carcinoma. *Clin Cancer Res*, **6**, 1125-34.

Nguyen VN, Mirejovsky P, Mirejovsky T, et al (2000). Expression of cyclin D1, Ki-67 and PCNA in non-small cell lung cancer: prognostic significance and comparison with p53 and bcl-2. *Acta Histochem*, **102**, 323-38.

O'Neill AJ, Staunton MJ, Gaffney EF (1996). Apoptosis occurs independently of bcl-2 and p53 over-expression in non-small cell lung carcinoma. *Histopathology*, **29**, 45-50.

Ohsaki Y, Toyoshima E, Fujiuchi S, et al (1996). bcl-2 and p53 protein expression in non-small cell lung cancers: correlation with survival time. *Clin Cancer Res*, **2**, 915-20.

Parmar MK, Torri V, Stewart L (1998). Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*, **17**, 2815-34.

Pastorino U, Andreola S, Tagliabue E, et al (1997). Immunocytochemical markers in stage I lung cancer: relevance to prognosis. *J Clin Oncol*, **15**, 2858-65.

Pezzella F, Turley H, Kuzu I, et al (1993). bcl-2 protein in non-small-cell lung carcinoma. *N Engl J Med*, **329**, 690-4.

Pierce RH, Vail ME, Ralph L, et al (2002). Bcl-2 expression inhibits liver carcinogenesis and delays the development of proliferating foci. *Am J Pathol*, **160**, 1555-60.

Pirker R, Pereira JR, von Pawel J, et al (2012). EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. *Lancet Oncol*, **13**, 33-42.

Poleri C, Morero JL, Nieva B, et al (2003). Risk of recurrence in patients with surgically resected stage I non-small cell lung carcinoma: histopathologic and immunohistochemical analysis. *Chest*, **123**, 1858-67.

Renouf DJ, Wood-Baker R, Ionescu DN, et al (2009). BCL-2 expression is prognostic for improved survival in non-small cell lung cancer. *J Thorac Oncol*, **4**, 486-91.

Rigau V, Molina TJ, Chaffaud C, et al (2002). Blood vessel invasion in resected non small cell lung carcinomas is predictive of metastatic occurrence. *Lung Cancer*, **38**, 169-76.

Ritter JH, Dresler CM, Wick MR (1995). Expression of bcl-2 protein in stage T1N0M0 non-small cell lung carcinoma. *Hum Pathol*, **26**, 1227-32.

Rosell R, Skrzypski M, Jassem E, et al (2007). BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. *PLoS One*, **2**, 1129.

Shea BJ, Grimshaw JM, Wells GA, et al (2007). Development of AMSTAR: a measurement tool to assess the methodological quality of systematic reviews. *BMC Med Res Methodol*, **7**, 10.

Shi Y, Chen L, Li J, et al (2011). Prognostic and predictive values of pERK1/2 and pAkt-1 expression in non-small cell lung cancer patients treated with adjuvant chemotherapy. *Tumour Biol*, **32**, 381-90.

Shibata Y, Hidaka S, Tagawa Y, et al (2004). Bcl-2 protein expression correlates with better prognosis in patients with advanced non-small cell lung cancer. *Anticancer Res*, **24**, 1925-8.

Shim BY, Kim CH, Ahn MI, et al (2009). HER2 and tau expression as potential markers for response and survival to first line taxane plus cisplatin therapy in non-small cell lung cancer. *Asian Pac J Clin Oncol*, **5**, 232-41.

Swinson DE, Jones JL, Cox G, et al (2004). Hypoxia-inducible factor-1 alpha in non small cell lung cancer: relation to growth factor, protease and apoptosis pathways. *Int J Cancer*, **111**, 43-50.

Swinson DE, Jones JL, Richardson D, et al (2002). Tumour necrosis is an independent prognostic marker in non-small cell lung cancer: correlation with biological variables. *Lung Cancer*, **37**, 235-40.

Tierney JF, Stewart LA, Ghersi D, et al (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*, **8**, 16.

Tomita M, Matsuzaki Y, Edagawa M, et al (2003). Prognostic significance of bcl-2 expression in resected pN2 non-small cell lung cancer. *Eur J Surg Oncol*, **29**, 654-7.

Wells GA SB OCD, Peterson J, Welch V, Losos M, Tugwell P (2000). The newcastle-ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [Online]. Available: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

Williamson PR, Smith CT, Hutton JL, et al (2002). Aggregate data meta-analysis with time-to-event outcomes. *Stat Med*, **21**, 3337-51.

Yaren A, Oztop I, Kargi A, et al (2006). Bax, bcl-2 and c-kit expression in non-small-cell lung cancer and their effects on prognosis. *Int J Clin Pract*, **60**, 675-82.

Yoo J, Jung JH, Lee MA, et al (2007). Immunohistochemical analysis of non-small cell lung cancer: correlation with clinical parameters and prognosis. *J Korean Med Sci*, **22**, 318-25.

Zhang GJ, Zhang Z (2013). Effect of Bcl-2 on apoptosis and transcription factor NF-kappaB activation induced by adriamycin in bladder carcinoma BIU87 cells. *Asian Pac J Cancer Prev*, **14**, 2387-91.

Zheng Z, Chen T, Li X, et al (2007). DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. *N Engl J Med*, **356**, 800-8.

Zhu ZH, Sun BY, Ma Y, et al (2009). Three immunomarker support vector machines-based prognostic classifiers for stage IB non-small-cell lung cancer. *J Clin Oncol*, **27**, 1091-9.