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Prognostic Value of ki67 in Bacillus Calmette–Guérin-Treated Non-muscle-Invasive Bladder Cancer: a Meta-analysis and Systematic Review

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3 **Prognostic Value of ki67 in Bacillus Calmette–Guérin-Treated**
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6 **Non-muscle-Invasive Bladder Cancer: a Meta-analysis and**
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8 **Systematic Review**
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Abstract

Objectives: The aim of this study was to explore the prognostic value of ki67 as a marker in Bacillus Calmette–Guérin (BCG)-treated non-muscle-invasive bladder cancer (NMIBC).

Methods: Studies were systematically retrieved from the relevant databases (Web of Science, PubMed, Cochrane Library, and Embase), and the expiry date was May 2017. The research steps referred to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement.

Results: A total of 11 studies, including 1321 cases, complying with the inclusion criteria were enrolled. The results of the meta-analysis indicated that the expression of ki67 was not statistically significantly correlated with recurrence-free survival (RFS). No significant heterogeneity was found among all included studies. The expression of ki67 was statistically significantly correlated with progression-free survival (PFS), and the overexpression of ki67 was the risk factor for PFS. Statistical heterogeneity was noted among all the included studies. The studies that might cause heterogeneity were excluded using the Galbraith plot, and then the meta-analysis was performed again. The results showed that the expression of ki67 was still correlated with PFS. In the Caucasian subgroup, the overexpression of ki67 was the risk factor for RFS and PFS.

Conclusions: For the patients with NMIBC treated with BCG intravesical immunotherapy, the overexpression of ki67 was the risk factor for PFS, and the relationship between the expression of ki67 and RFS had no statistical significance. In Caucasians, the overexpression of ki67 was the risk factor for RFS and PFS. However, these findings still need well-designed, prospective, randomized controlled trials with a large sample size for validation.

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5 **Key words:** Ki67; meta-analysis; non-muscular-invasive bladder cancer; prognosis
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Strengths and limitations of this study

This meta-analysis and systematic review was performed by a strictly literature search and was the first meta-analysis to evaluate the prognostic value of ki67 on patients with NMIBC after transurethral resection and BCG intravesical immunotherapy.

The review found the Prognostic Value of ki67 in BCG-Treated NMIBC, which can guide the follow-up immunohistochemical markers research.

The review only included English published studies and did not consider the surgical skills from published studies.

Introduction

Bladder cancer is one of the most common clinical urological tumor and is a direct threat to the survival of patients with the disease. The incidence of bladder cancer varies across the world with the highest rate in the developed communities.¹ A total of 429,800 new cases of bladder cancer and 165,100 deaths occurred in 2012 worldwide. The majority of bladder cancer occurs in men, and about a tenfold variation has been reported in incidence rates internationally.² About 70% of these patients are non-muscle-invasive bladder cancer (NMIBC).³ Four major organizational guidelines on NMIBC, including the American Urological Association/Society of Urologic Oncology, European Association of Urology, National Comprehensive Cancer Network, and National Institute for Health and Care Excellence guidelines, recommend that the proper initial transurethral resection (TUR) of bladder tumor is a critical step in the initial management and staging of the disease.⁴ However, TUR surgery alone cannot solve the postoperative problems for NMIBC because of high recurrence rate and disease development.⁵ Postoperative TUR associated with Bacillus Calmette–Guérin (BCG) intravesical immunotherapy can prevent the postoperative recurrence of NMIBC and significantly reduce the moderate and high development risk of NMIBC.^{6, 7} However, the postoperative BCG intravesical immunotherapy still has some problems. The failure rate of BCG intravesical therapy in NMIBC is about 40%–50%.⁸ Furthermore, BCG also has toxic side effects, such as hepatitis, pneumonitis, epididymitis/orchitis, abscess formation, bladder contracture, ureteral obstruction, BCG sepsis, leukopenia, and hematuria.⁹ Therefore, BCG therapy should be individually performed, and the patients having no effectiveness on BCG therapy should be timely recognized. These patients or those with poor prognosis should receive radical cystectomy or other therapy in time to avoid futile

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3 treatment and alleviate pain. However, the recognition of patients with no effect of
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5 TUR postoperative BCG intravesical immunotherapy is still hard due to the
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7 heterogeneity of bladder cancer and individuality of patients.¹⁰ Therefore, finding the
8
9 prognostic factors for patients with NMIBC receiving TUR and BCG therapy is
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11 extremely necessary.

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13 The recurrence rate of bladder cancer treated with different therapies is between 50%
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15 and 80%, and about 15% of the low-grade tumor recurrence involves high-grade
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17 tumors.¹¹ The patients need periodical cystoscopy to find the recurrent focus in time.
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19 A reliable prognostic molecular marker can reduce the pain caused by cystoscopy.
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21 Because NMIBC does not have reliable prognostic markers, it is hard to decide
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23 postoperative therapy in the clinic,¹² which depends mainly on the clinical guidelines
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25 and physician's experience. Currently, some of the published studies about
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27 immunohistochemical markers have evaluated the prognostic value of BCG
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29 intravesical immunotherapy on the patients first receiving TUR. The main
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31 immunohistochemical markers include ki67, p53, p27, pRb, CD9, CD20, E2F1, and
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33 so forth.^{13, 14} However, no immunohistochemical marker has been confirmed so far.
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36 The prognostic value of ki67 antigen on the survival in patients with NMIBC
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38 receiving BCG intravesical immunotherapy has been controversial. For example,
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40 Kruger.¹⁵ reported that ki67 antigen was an independent predictive factor of
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42 recurrence in pT1 stage tumor, but Oderde¹⁶ believed that ki67 was an independent
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44 predictive factor for all the NMIBC recurrence. Zlotta¹⁷ reported that ki67 antigen had
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46 no independent prognostic value in patients receiving BCG therapy. Saint¹⁸
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48 retrospected the recent 25-year published studies and believed that the independent
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50 prognostic factor for bladder cancer on BCG response was not unclear. An
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52 international consensus group listed various bladder cancer prognostic indexes by
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3 reviewing PubMed and considered that although some markers (such as ki67 and p53)
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5 were possible to predict the recurrence and development of bladder cancer, the data
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7 still had heterogeneity. Thus, strict test criteria and clear statistical methods should be
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9 established for further evaluation.¹⁹

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11 A meta-analysis can enlarge the sample size by integrating independent studies with
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13 small sample size, further increase the statistical efficacy, and reduce the wrong
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15 conclusion caused by the small sample size.²⁰ The aim of this study was to explore the
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17 prognostic value of ki67 as a marker in BCG-treated NMIBC. Based on the literature
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19 search, this study was the first meta-analysis to evaluate the prognostic value of ki67
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21 on patients with NMIBC after BCG therapy.
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26 ***Methods***

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28 This meta-analysis was performed according to the Preferred Reporting Items for
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30 Systematic Reviews and Meta-Analyses (PRISMA) Statement (**Table S1**).²¹ Because
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32 all the enrolled published studies were approved by the ethics committee in the
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34 research institute, the present meta-analysis did not need the approval.
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37 ***Literature retrieval strategy***

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39 Published studies were retrieved from Web of Science, PubMed, Cochrane Library,
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41 and Embase databases. Free word retrieval strategy was used. The search terms were
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43 “bladder cancer or bladder carcinoma or bladder neoplasm or bladder tumor,”
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45 “Bacillus Calmette–Guérin or BCG,” and “ki67 antigen or ki-67 or ki67 or MBI-1.”
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47 The retrieval time was until May 24, 2017, assisted with manual retrieval. The
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49 enrolled contents included the reference and relevant suggestive references while
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51 searching.
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54 ***Inclusion and exclusion criteria***

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3 Inclusion criteria were as follows: (1) prospective studies or retrospective research
4 published studies evaluating the prognostic relationship between the expression of
5 ki67 and NMIBC treated with BCG; (2) the expression of ki67 in tissues detected by
6 immunohistochemistry; (3) hazard ratio (HR) and 95% confidence interval (95% CI)
7 directly obtained from the published studies; and (4) published English studies.

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13 Exclusion criteria were as follows: (1) review, systematic evaluation, case report,
14 editorial, and specialist experience; (2) no human subjects; and (3) published studies
15 in which data could not be extracted or those having wrong data.
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19 20 ***Data extraction and evaluation of literature quality***

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22 Based on the aforementioned criteria, two reviewers independently screened the
23 published studies by reading titles and abstracts and got preliminary conclusions. If
24 the conclusions were not consistent, the literature was discussed by all the authors to
25 decide its enrollment. The relevant information of the enrolled published studies was
26 extracted, such as first author, publication time, research country, sex, case number,
27 age, follow-up date, disease stage, cutoff values, RFS, and PFS. The Newcastle–
28 Ottawa Scale (NOS) was used to evaluate the quality of all the published studies,²²
29 scores 0–3, 4–5, and 6–8 were accepted as low, medium, and high quality.
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39 40 ***Statistical methods***

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42 The study effects of recurrence-free survival (RFS) and progression-free survival
43 (PFS) were reflected by 95% CI and HR. The influence of the expression of ki67 on
44 prognosis was expressed as 95% CI and HR. The values of HR and 95% CI were
45 directly obtained from the original published studies. Besides, the Parmar and
46 Tierney's²³ method was used to extract the data because some of the published studies
47 did not directly provide HR and 95% CI. For example, some studies provided only the
48 survival curve. In this meta-analysis, the DerSimonian–Laird random-effects model²⁴
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3 was used because when the heterogeneity was large, only the random-effects model
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5 could be suitable used. Similar to traditional methods, $HR > 1$ was considered as the
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7 prognostic risk factor for the overexpression of ki67, and $HR < 1$ was a protective
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9 factor. $95\% CI < 1$ indicated a statistical difference in the relationship between the
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11 overexpression of ki67 and prognosis.
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15 The heterogeneity was calculated according to chi-square-based Q test and I^2
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17 statistic.²⁵ The heterogeneity was judged by the I^2 value (low heterogeneity: $I^2 <$
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19 25% ; moderate heterogeneity: $I^2 = 25\%–50\%$; large heterogeneity: $I^2 > 50\%$).
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23 Besides, a P value >0.05 was also considered as low heterogeneity. Then, the
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25 subgroup analysis based on regions, sample size, follow-up period, tumor grading,
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27 cutoff value, publication time, and patient age was performed. A Galbraith plot was
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29 used to search published studies with heterogeneity,²⁶ and after excluding these
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31 published studies, the meta-analysis was performed again. Meanwhile, the factors
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33 causing heterogeneity were also explored by the residual maximum likelihood
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35 (REML)-based random-effects meta-regression analysis.²⁷ All the statistical analyses
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37 were performed using the Stata12.0 software (StataCorp, TX, USA), and the
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39 two-sided test was used to evaluate the P value.
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44 45 ***Evaluation of publication bias***

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47 Begg's plot and Egger's test method were used to find the possible publication bias. A
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49 P value <0.05 was believed to have publication bias.
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52 53 ***Results***

Literature screening

A total of 97 published studies were retrieved. Furthermore, 68 of them were excluded after duplicates removed and records screened, and 18 were excluded after reading the full text (10 published studies from which HR and 95% CI could not be obtained, 2 non-English studies, and 6 that did not use ki67 detection). Finally, 11 published studies were enrolled in the meta-analysis (**Figure 1**).

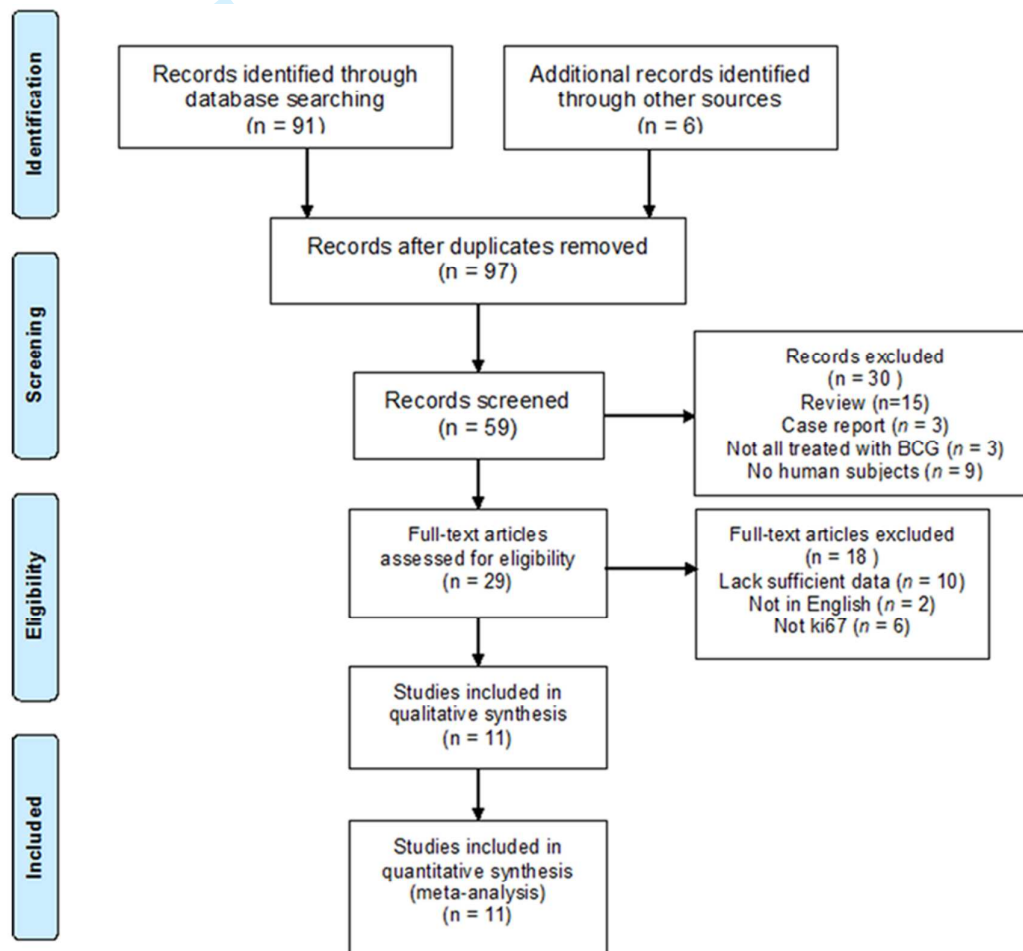


Figure 1. Flow diagram of study selection.

Basic characteristics and quality evaluation of enrolled published studies

The enrolled 11 published studies were published between 1997 and 2013, and the

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3 countries included Italy, South Korea, Spain, Germany, New Zealand, Canada,
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5 Portugal, and France. The largest sample size was 309, and the smallest one was 32. A
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7 total of 1321 patients were enrolled in this study. The follow-up period was beyond 36
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9 months, and the longest was 229 months. T1 was the main tumor grading, and the
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11 cutoff value ranged from 10.4% to 40%. Seven published studies reported patients'
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13 RFS, and nine reported PFS (**Table 1**). One literature was scored as 6 star by NOS,
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15 seven as 7 star, and three as 8 star, and the median of the NOS score was 7 (**Table 2**).
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Table 1. Main characteristics of all studies included in this meta-analysis

Study	Year	Country	Male/Female	No. of patients	Age (year)	Follow-up (month)	Stage	Cutoff	Survival analysis
Oderda ¹⁶	2013	Italy	166/26	192	73.2 (SD 11.9)	100 (2–229)	All NMIBC	20%	RFS
Park ¹⁴	2013	Korea	53/8	61	66 (31–85)	60 (6–217)	T1G3	10.4%	RFS/PFS
Quintero ²⁸	2013	Spain	143/21	164	61 (29–93)	75 (60–144)	Ta	13%	PFS
Bertz ¹²	2012	Germany	237/72	309	71.7 (38–87)	49 (5–172)	pT1	15%	RFS/PFS
van Rhijn ²⁹	2012	Netherlands, Canada	105/24	129	68.8 (SD 9.9)	78 (39.6–110.4)	T1	25%	RFS/PFS
Burger ³⁰	2007	Germany	45/21	71	71 (52–94)	39 (1–133)	T1/Ta	15%	RFS
Queipo-zaragoza ³¹	2007	Spain	71/12	83	68.1 (SD 8.5)	All >36	T1G3	40%	PFS
Lopez-Beltran ³²	2004	Spain	49/2	51	69.96 (49–89)	63.82 (60–144)	T1G3	13%	PFS
Santos ³³	2003	Portugal	115/44	159	66 (21–88)	46.5 (4–123)	pTa/pT1	18%	RFS/PFS
Blanchet ³⁴	2001	France	-	70	62.6 (21–84)	64 (12–111)	pT1/pTa	13%	PFS
Lee ³⁵	1997	Korea	28/4	32	57.1 (30–81)	All >24	T1G2-3	20%	RFS

No., number; NMIBC, non-muscle-invasive bladder cancer; RFS, recurrence-free survival; PFS, progression-free survival

Table 2. Quality of the included studies assessed by NOS

Study	Selection			Comparability		Exposure			Scores
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Ascertainment of exposure	Same method to ascertain for cases and controls	Non-response rate	
Oderda ¹⁶	—	☆	☆	☆	☆☆	☆	☆	☆	8
Park ¹⁴	—	☆	☆	—	☆☆	☆	☆	☆	7
Quintero ²⁸	—	☆	☆	—	☆☆	☆	☆	—	6
Bertz ¹²	—	☆	☆	—	☆☆	☆	☆	☆	7
van Rhijn ²⁹	—	☆	☆	—	☆☆	☆	☆	☆	7
Burger ³⁰	☆	☆	☆	—	☆☆	☆	☆	—	7
Queipo-zaragoza ³¹	—	☆	☆	—	☆☆	☆	☆	☆	7
Lopez-Beltran ³²	—	☆	☆	☆	☆☆	☆	☆	☆	8
Santos ³³	—	☆	☆	—	☆☆	☆	☆	☆	7
Blanchet ³⁴	—	☆	☆	☆	☆☆	☆	☆	☆	8
Lee ³⁵	—	☆	☆	—	☆☆	☆	☆	☆	7

Influence of the expression of ki67 on RFS

Seven published studies reported ki67 expression and PFS results of patients with NMIBC receiving BCG. The meta-analysis indicated that ki67 had no statistical significance with RFS (HR = 1.331, 95% CI: 0.980–1.809), and no heterogeneity among the enrolled studies was reported ($I^2 = 36.7%$, $P = 0.148$) (**Figure 2A**). The subgroup analysis was performed based on the regions, sample size, follow-up period, stage, cutoff value, publication time, and age. The results indicated that in the Caucasian subgroup, the overexpression of ki67 was the risk factor for RFS (HR = 1.441, 95% CI: 1.014–2.047). In the subgroup with a follow-up period shorter than 6 months, the overexpression of ki67 was the risk factor for RFS (HR = 1.853, 95% CI: 1.316–2.607) (**Table 3**).

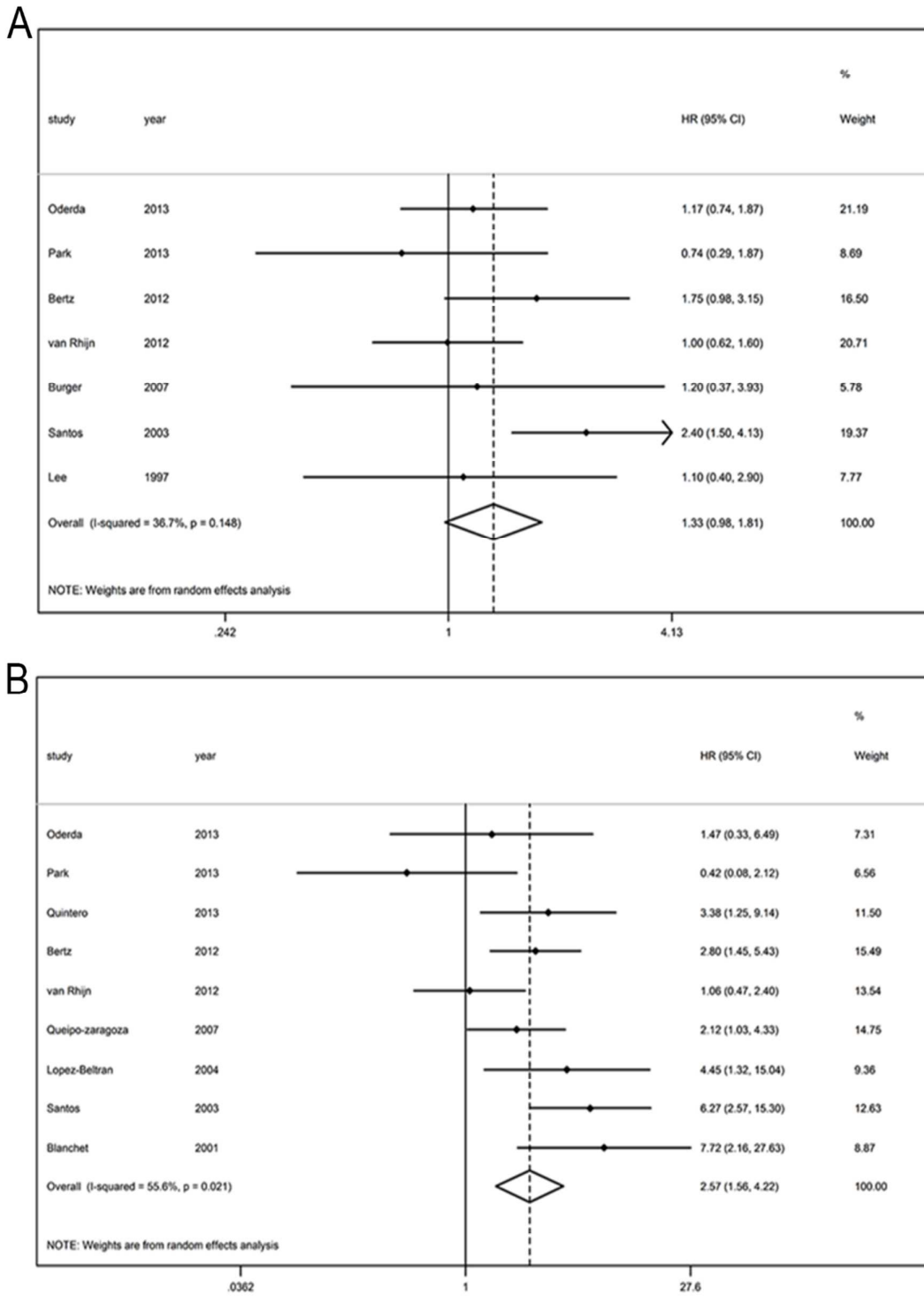


Figure 2. Forest plots of HRs estimated for the relationship between the expression of ki67 and RFS (A) or PFS (B) among patients with NMIBC treated with BCG.

Table 3. Subgroup results of RFS and heterogeneity test

Variables	Study number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total RFS	7	1.331 (0.980–1.809)	9.48	0.148	36.7
Region					
Asian	2	0.892 (0.454–1.752)	0.32	0.570	0.0
Caucasian	5	1.441 (1.014–2.047)	7.52	0.111	46.8
Sample size					
>100	4	1.466 (0.986–2.181)	7.44	0.059	59.7
≤100	3	0.959 (0.534–1.725)	0.51	0.777	0.0
Follow-up (month)					
≥60	3	1.036 (0.758–1.415)	0.79	0.674	0.0
<60	4	1.853 (1.316–2.607)	2.62	0.453	0.0
Stage					
All NMIBC	3	1.575 (0.915–2.711)	4.44	0.109	54.9
Others	4	1.153 (0.821–1.620)	3.21	0.360	6.6
Cut off					
15%	2	1.625 (0.963–2.743)	0.31	0.575	0.0
Others	5	1.252 (0.839–1.869)	8.56	0.073	53.3
Patient age (year)					
≥70	3	1.352 (0.955–1.913)	1.16	0.559	0.0
<70	4	1.256 (0.717–2.198)	8.32	0.040	63.9

NMIBC, non-muscle-invasive bladder cancer; RFS, recurrence-free survival.

Influence of the expression of ki67 on PFS

A total of nine published studies reported ki67 expression and PFS results of patients with NMIBC receiving BCG. The meta-analysis indicated that ki67 had no statistical significance with RFS (HR = 2.567, 95% CI: 1.562–4.219), and the overexpression of ki67 was the risk factor for PFS. Statistical heterogeneity was found among all the included studies ($I^2 = 55.6\%$, $P = 0.021$) (**Figure 2B**). The subgroup analysis was performed based on the regions, sample size, follow-up period, stage, cutoff value, publication time, and age. The results indicated that the overexpression of ki67 was the risk factor for PFS in the Caucasian subgroup (HR = 2.883, 95% CI: 1.830–4.544), the subgroup with sample size > 100 (HR = 2.559, 95% CI: 1.372–4.774), the subgroup with the follow-up period < 6 months (HR = 3.158, 95% CI: 1.774–5.623), the subgroup with other cutoffs (HR = 2.515, 95% CI: 1.382–4.576), and the two subgroups based on age (HR = 2.800, 95% CI: 1.447–5.418 and HR = 2.654, 95% CI: 1.381–5.100, respectively) (**Table 4**).

Table 4. Subgroup results of PFS and heterogeneity test

Variables	Study number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total PFS	9	2.567 (1.562–4.219)	18.1	0.021	55.6
Region					
Asian	1	0.421 (0.084–2.114)	0.00		
Caucasian	8	2.883 (1.830–4.544)	12.99	0.072	46.1
Sample size					
>100	5	2.559 (1.372–4.774)	9.26	0.055	56.8
≤100	4	2.536 (0.943–6.818)	8.75	0.033	65.7
Follow-up (month)					
≥60	6	2.153 (0.984–4.710)	13.08	0.023	61.8
<60	3	3.158 (1.774–5.623)	3.56	0.169	43.8
Stage					
All NMIBC	3	4.673 (1.938–11.264)	3.29	0.193	39.2
Others	6	2.044 (1.213–15.040)	9.58	0.088	47.8
Cut off					
15%	1	2.800 (1.447–5.418)	0.00		
Others	8	2.515 (1.382–4.576)	17.92	0.012	60.9
Patient age (year)					
≥70	2	2.519 (1.377–4.606)	0.60	0.438	0.0
<70	7	2.654 (1.381–5.100)	17.40	0.008	65.5

NMIBC, non-muscle-invasive bladder cancer; PFS, progression-free survival.

Galbraith plot

Using Galbraith plot (**Figure 3A**), it was found that Santos³³ was the main reason for the heterogeneity of RFS. After the aforementioned study was excluded, the rest RFS studies had no significant heterogeneity according to the new meta-analysis ($I^2 = 0.0\%$, $P = 0.667$). However, the expression of ki67 still had no statistical significance with RFS (HR = 1.161, 95% CI: 0.896–1.504) (**Figure S1**). Using the Galbraith plot (**Fig. 3B**), it was found that Santos,³³ Park,¹⁴ and van Rhijn²⁹ were the main reason for the heterogeneity of PFS. After these studies were excluded, the remaining RFS studies had no significant heterogeneity according to the new meta-analysis ($I^2 = 0.0\%$, $P = 0.497$). The expression of ki67 still had statistical significance with PFS (HR = 2.922, 95% CI: 2.002–4.266) (**Figure S2**).

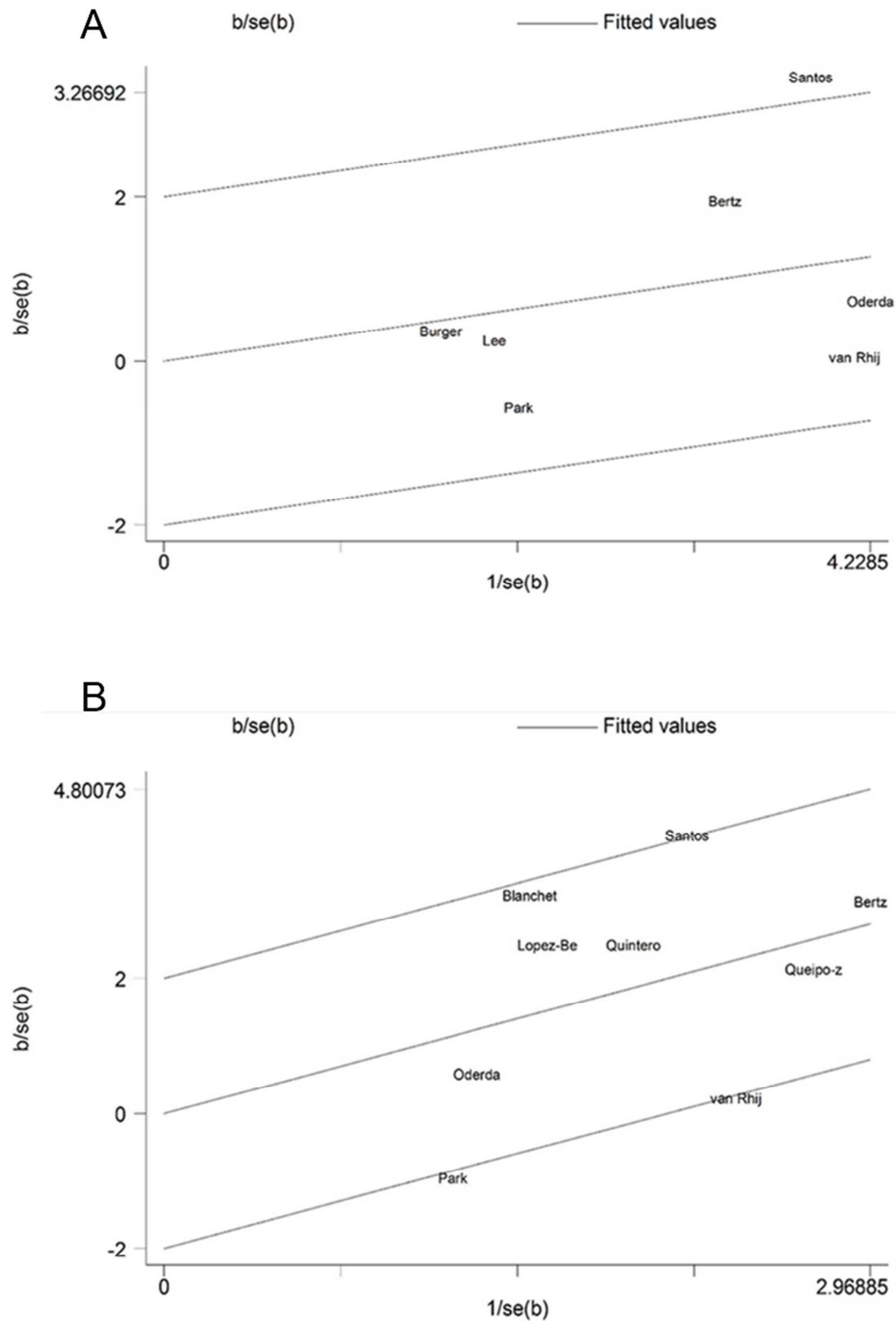


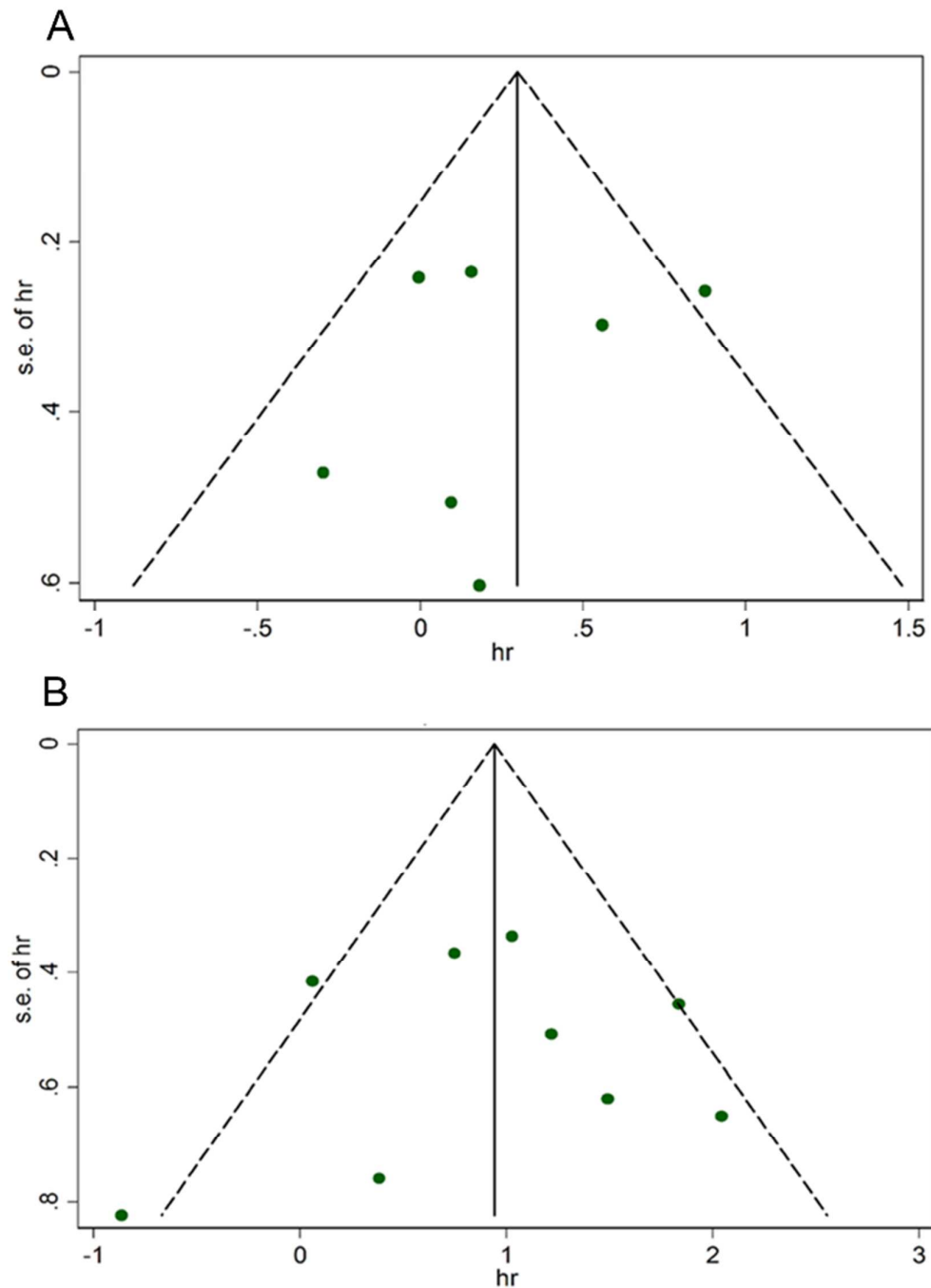
Figure 3. Galbraith plot analysis was used to evaluate heterogeneity. It suggested that two studies were the potential source of heterogeneity for RFS (A), while one was the source of heterogeneity for PFS (B).

Meta-regression analysis

The meta-regression analysis indicated that the factors influencing heterogeneity (publication time, research region, sample size, stage, cutoff value, age, and follow-up period) might not be the reason for RFS heterogeneity. Publication time was the reason for heterogeneity of PFS ($P = 0.036$), but other factors were not (**Table S2**).

Publication bias

The funnel plot (**Figure 4A**) was basically symmetrical for RFS. The results of Begg's test and Egger's test showed P (Begg's) = 0.761, P (Egger's) = 0.601. The funnel plot (**Figure 4B**) was also basically symmetrical for PFS. The results of Begg's test and Egger's test showed P (Begg's) = 0.917, P (Egger's) = 0.964.



47 **Figure 4.** Funnel plots of the expression of ki67 and RFS (A) or PFS (B).
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51 **Discussion**
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53 A total of 11 published studies with 1321 cases complying with the inclusion criteria
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3 were enrolled in this meta-analysis. The results of the meta-analysis indicated that the
4
5 expression of ki67 had no statistical significance with RFS, but it had significance
6
7 with PFS. The overexpression of ki67 was the risk factor for PFS. It suggested that
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9 ki67 was the prognostic predictive marker for patients with NMIBC after BCG
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11 therapy. Besides, the aforementioned conditions did not change after excluding the
12
13 published studies possibly causing heterogeneity and reperforming the meta-analysis.
14
15 It further proved that the result of the aforementioned meta-analysis was stable, that
16
17 is, the overexpression of ki67 was the risk factor for PFS. In the Caucasian subgroup,
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19 the overexpression of ki67 was the risk factor for PFS and RFS, suggesting the racial
20
21 classification and regional factor might play important roles in the prognosis of
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23 patients with NMIBC after BCG therapy. In the two subgroups based on age, the
24
25 overexpression of ki67 was the risk factor for PFS, suggesting that age was the
26
27 important factor influencing the prognosis of bladder cancer. The elder the patient, the
28
29 worse the prognosis would be. Besides, the meta-regression analysis indicated
30
31 publication time as the reason for PFS heterogeneity. The cumulative meta-analysis
32
33 indicated that the expression of ki67 had statistical significance with RFS and PFS. It
34
35 suggested that the correlation of the expression of ki67 with RFS and PFS needed
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37 further exploration to observe the following changes. Besides, according to the funnel
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39 plot, Begg's test, and Egger's test, the enrolled studies had no significant publication
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41 bias. Thus, the reliability of the present meta-analysis was high.

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46 In 2016, the European Association of Urology (EUA)³⁶ recommended a scoring
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48 system for the prognostic evaluation of NMIBC based on six clinical and pathological
49
50 factors built by European Organization for the Research and Treatment of
51
52 Cancer-Genito-Urinary Cancer Group (EORTC), including number of tumors, tumor
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54 size, prior recurrence rate, T category, presence of concurrent carcinoma in situ, and
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3 tumor grade (**Table S3**). The patients were categorized into low-risk tumors,
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5 intermediate-risk tumors, and high-risk tumors using this assessment system to
6
7 evaluate the prognosis. For the patients after BCG therapy, the EUA recommended
8
9 another risk calculator developed by the Club Urologico Espanol de Tratamiento
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11 Oncologico (CUETO) and the EORTC. This calculator based on gender, age,
12
13 recurrent tumor, number of tumors, T category, associated Tis, and grade. The
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15 CUETO risk calculator can be achieved at <http://www.aeu.es/Cueto.html>. For the two
16
17 scales for patients with NMIBC, no matter used alone or combined, the recommended
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19 level was B grade. The two scales could be used together in the clinic. When using the
20
21 CUETO scale, the calculated recurrent risk was lower than that from the EORTC
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23 scale,³⁷ which might be related to the special design in the CUETO scale for the
24
25 patients receiving BCG intravesical immunotherapy. However, the scoring system
26
27 only depending on clinical and pathological factors could not accurately evaluate the
28
29 prognosis of bladder cancer patients in T1 stage due to the independence of disease
30
31 condition in each patient.³⁸ The markers regulated at the genetic level may judge the
32
33 prognosis of bladder cancer patients with the development of precision medicine. A
34
35 reliable marker helps in recognizing the patients who failed in BCG intravesical
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37 immunotherapy with high risk in time. Hence, these patients can undergo radical
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39 cystectomy or other treatments in time. Unfortunately, no prognostic marker has been
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41 applied in clinic currently.

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46 Ki67 is a nucleoprotein that can be detected in the cell cycles except G0 phase.³⁹ The
47
48 expression of human ki67 protein is closely related to proliferation. Therefore, it is an
49
50 ideal marker to confirm the growth fraction of specific cell colony.⁴⁰ Ki67 is a widely
51
52 known amplified biomarker. The ki67 monoclonal antibody can be detected by the
53
54 immunohistochemical method.⁴¹ Ki67 has been proved to be a good proliferation

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3 marker in different cancers, including bladder cancer.⁴²

4
5 So far, some meta-analyses have studied the effect of ki67 on the prognostic quality of
6
7 life of esophageal cancer, breast cancer, epithelial ovarian cancer, and so on.⁴³⁻⁴⁵ Some
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9 studies have also focused on the other aspects of bladder cancer. Using meta-analysis,
10
11 Luo⁴⁶ believed that a high reactivity of ki67 could predict the poor prognosis in
12
13 patients with bladder cancer. The univariate analysis showed that cancer-specific
14
15 survival, disease-free survival, overall survival, PFS, and RFS had a significant
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17 correlation with poor prognosis in patients with a high reactivity of ki67. However,
18
19 this study enrolled all types of bladder tumors and all the therapies for NMIBC.
20
21 Currently, the treated bladder cancer in the clinic is mainly NMIBC. Thus, most of the
22
23 applied therapy is TUR combined with installations of chemotherapy or BCG
24
25 intravesical immunotherapy based on the patients' conditions. Therefore, this analysis
26
27 had a certain limitation in the prognosis judgment on the patients with NMIBC after
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29 BCG intravesical immunotherapy.
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35 Currently, few evidence-based studies focused on the prognosis of patients with
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37 NMIBC after BCG intravesical immunotherapy. Using meta-analysis, Zhou⁴⁷
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39 analyzed the correlation between the expression of p53 and quality of life of patients
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41 with NMIBC after BCG intravesical immunotherapy. They believed that the
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43 overexpression of p53 in patients with NMIBC treated with BCG might be associated
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45 with RFS, especially in Asian population. Similarly, Du⁴⁸ also performed the
46
47 meta-analysis on the relationship between p53 status and NMIBC in T1 stage and
48
49 believed that the overexpression of p53 might be related to the development of
50
51 NMIBC. The present study indicated that the overexpression of ki67 was the risk
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53 factor for PFS, but the expression of ki67 had no statistical significance with RFS. For
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3 Caucasians, the overexpression of ki67 was the risk factor for PFS and RFS. P53 is
4 the most common inactivated tumor suppressor gene in tumor cells.⁴⁹ The inactivation
5 of p53 may cause cell abnormal hyperplasia and cancerization. The variation in p53
6 results in enhanced proliferation, invasion, and metabolism.⁵⁰ The increase in the
7 expression of ki67 ,as cell proliferation marker ,suggests enhanced proliferation.⁴⁰ As
8
9 a tumor suppressor gene with complicated function, the range of effects of p53 is
10 wider. The accuracy in the prediction of quality of life may not be more appropriate
11 compared with ki67. The genetic difference between Asians and Caucasians suggests
12 that different prediction systems should be built for different races. Besides, p27,
13 E2F1, ezrin, and CK20 were also studied in other investigations for predicting
14 NMIBC prognosis, which could be further explored comparing their advantages used
15 alone or combined.

16
17 However, this study still had some limitations. First, some of the enrolled published
18 studies were retrospective studies, involving different populations, using different
19 techniques, and with different cutoff values. All these reasons might have caused the
20 heterogeneity. Second, the meta-analysis included English published studies.
21 Although Begg's test and Egger's test did not suggest publication bias, this study
22 could still be influenced by the bias. Finally, the surgical skills were different in
23 different published studies, which might affect effectiveness judgment of BCG.

24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 **Conclusions**

48
49 For the patients with NMIBC treated with BCG intravesical immunotherapy, the
50 overexpression of ki67 was the risk factor for PFS, the overexpression of ki67 was the
51 risk factor for PFS, but the relationship between ki67 expression and PFS had no

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2
3 statistical significance. In the Caucasian subgroup, the overexpression of ki67 was the
4 risk factor for PFS and RFS. Owing to the aforementioned limitations of the present
5 study, RCTs with large sample size are still required to validate the results.
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8 9 **Contributors**

10
11 Conceived and designed the experiments: YHH NW XFZ. Extracted the data: XC
12 YSD ZSD JFW YHH NW XFZ. Analyzed the data: YHH NW XFZ. Contributed
13 reagents/materials/analysis tools: ZSD JFW XC. Wrote the paper: YHH NW.
14 Critically revised the report: XFZ.
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34 study design, data collection and analysis, decision to publish, or preparation of the
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41 **Competing interests**

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43 None declared.
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48 **Data sharing statement**

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50 No additional data available
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10 **Supplementary File**

11 **Table S1.** PRISMA

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13 **Table S2.** Meta-regression analysis of RFS and PFS

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16 **Table S3.** Risk group stratification in NMIBC

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18 **Figure S1.** Forest plots of HRs estimated for the relationship between the expression
19 of ki67 and RFS after the aforementioned study was excluded.

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22 **Figure S2.** Forest plots of HRs estimated for the relationship between the expression
23 of ki67 and PFS after the aforementioned study was excluded
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Table 1. Main characteristics of all studies included in this meta-analysis

Study	Year	Country	Male/Female	No. of patients	Age (year)	Follow-up (month)	Stage	Cutoff	Survival analysis
Oderda ¹⁶	2013	Italy	166/26	192	73.2 (SD 11.9)	100 (2–229)	All NMIBC	20%	RFS
Park ¹⁴	2013	Korea	53/8	61	66 (31–85)	60 (6–217)	T1G3	10.4%	RFS/PFS
Quintero ²⁸	2013	Spain	143/21	164	61 (29–93)	75 (60–144)	Ta	13%	PFS
Bertz ¹²	2012	Germany	237/72	309	71.7 (38–87)	49 (5–172)	pT1	15%	RFS/PFS
van Rhijn ²⁹	2012	Netherlands, Canada	105/24	129	68.8 (SD 9.9)	78 (39.6–110.4)	T1	25%	RFS/PFS
Burger ³⁰	2007	Germany	45/21	71	71 (52–94)	39 (1–133)	T1/Ta	15%	RFS
Queipo-zaragoza ³¹	2007	Spain	71/12	83	68.1 (SD 8.5)	All >36	T1G3	40%	PFS
Lopez-Beltran ³²	2004	Spain	49/2	51	69.96 (49–89)	63.82 (60–144)	T1G3	13%	PFS
Santos ³³	2003	Portugal	115/44	159	66 (21–88)	46.5 (4–123)	pTa/pT1	18%	RFS/PFS
Blanchet ³⁴	2001	France	-	70	62.6 (21–84)	64 (12–111)	pT1/pTa	13%	PFS
Lee ³⁵	1997	Korea	28/4	32	57.1 (30–81)	All >24	T1G2-3	20%	RFS

No., number; NMIBC, non-muscle-invasive bladder cancer; RFS, recurrence-free survival; PFS, progression-free survival

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For peer review only

Table 2. Quality of the included studies assessed by NOS

Study	Selection		Comparability			Exposure		Non-response rate	Scores
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Ascertainment of exposure	Same method to ascertain for cases and controls		
Oderda ¹⁶	—	☆	☆	☆	☆☆	☆	☆	☆	8
Park ¹⁴	—	☆	☆	—	☆☆	☆	☆	☆	7
Quintero ²⁸	—	☆	☆	—	☆☆	☆	☆	—	6
Bertz ¹²	—	☆	☆	—	☆☆	☆	☆	☆	7
van Rhijn ²⁹	—	☆	☆	—	☆☆	☆	☆	☆	7
Burger ³⁰	☆	☆	☆	—	☆☆	☆	☆	—	7
Queipo-zaragoza ³¹	—	☆	☆	—	☆☆	☆	☆	☆	7
Lopez-Beltran ³²	—	☆	☆	☆	☆☆	☆	☆	☆	8
Santos ³³	—	☆	☆	—	☆☆	☆	☆	☆	7
Blanchet ³⁴	—	☆	☆	☆	☆☆	☆	☆	☆	8
Lee ³⁵	—	☆	☆	—	☆☆	☆	☆	☆	7

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Table 3. Subgroup results of RFS and heterogeneity test

Variables	Study number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total RFS	7	1.331 (0.980–1.809)	9.48	0.148	36.7
Region					
Asian	2	0.892 (0.454–1.752)	0.32	0.570	0.0
Caucasian	5	1.441 (1.014–2.047)	7.52	0.111	46.8
Sample size					
>100	4	1.466 (0.986–2.181)	7.44	0.059	59.7
≤100	3	0.959 (0.534–1.725)	0.51	0.777	0.0
Follow-up (month)					
≥60	3	1.036 (0.758–1.415)	0.79	0.674	0.0
<60	4	1.853 (1.316–2.607)	2.62	0.453	0.0
Stage					
All NMIBC	3	1.575 (0.915–2.711)	4.44	0.109	54.9
Others	4	1.153 (0.821–1.620)	3.21	0.360	6.6
Cut off					
15%	2	1.625 (0.963–2.743)	0.31	0.575	0.0
Others	5	1.252 (0.839–1.869)	8.56	0.073	53.3
Patient age (year)					
≥70	3	1.352 (0.955–1.913)	1.16	0.559	0.0
<70	4	1.256 (0.717–2.198)	8.32	0.040	63.9

NMIBC, non-muscle-invasive bladder cancer; RFS, recurrence-free survival.

Table 4. Subgroup results of PFS and heterogeneity test

Variables	Study number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total PFS	9	2.567 (1.562–4.219)	18.1	0.021	55.6
Region					
Asian	1	0.421 (0.084–2.114)	0.00		
Caucasian	8	2.883 (1.830–4.544)	12.99	0.072	46.1
Sample size					
>100	5	2.559 (1.372–4.774)	9.26	0.055	56.8
≤100	4	2.536 (0.943–6.818)	8.75	0.033	65.7
Follow-up (month)					
≥60	6	2.153 (0.984–4.710)	13.08	0.023	61.8
<60	3	3.158 (1.774–5.623)	3.56	0.169	43.8
Stage					
All NMIBC	3	4.673 (1.938–11.264)	3.29	0.193	39.2
Others	6	2.044 (1.213–15.040)	9.58	0.088	47.8
Cut off					
15%	1	2.800 (1.447–5.418)	0.00		
Others	8	2.515 (1.382–4.576)	17.92	0.012	60.9
Patient age (year)					
≥70	2	2.519 (1.377–4.606)	0.60	0.438	0.0
<70	7	2.654 (1.381–5.100)	17.40	0.008	65.5

NMIBC, non-muscle-invasive bladder cancer; PFS, progression-free survival.

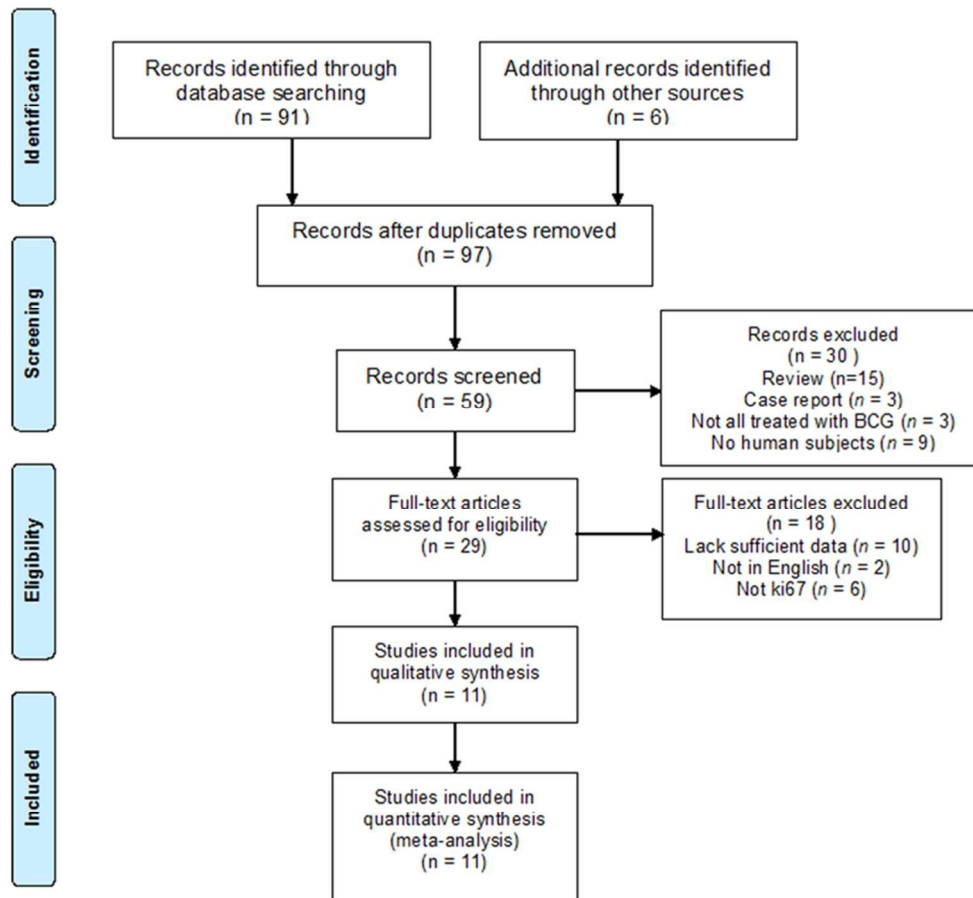
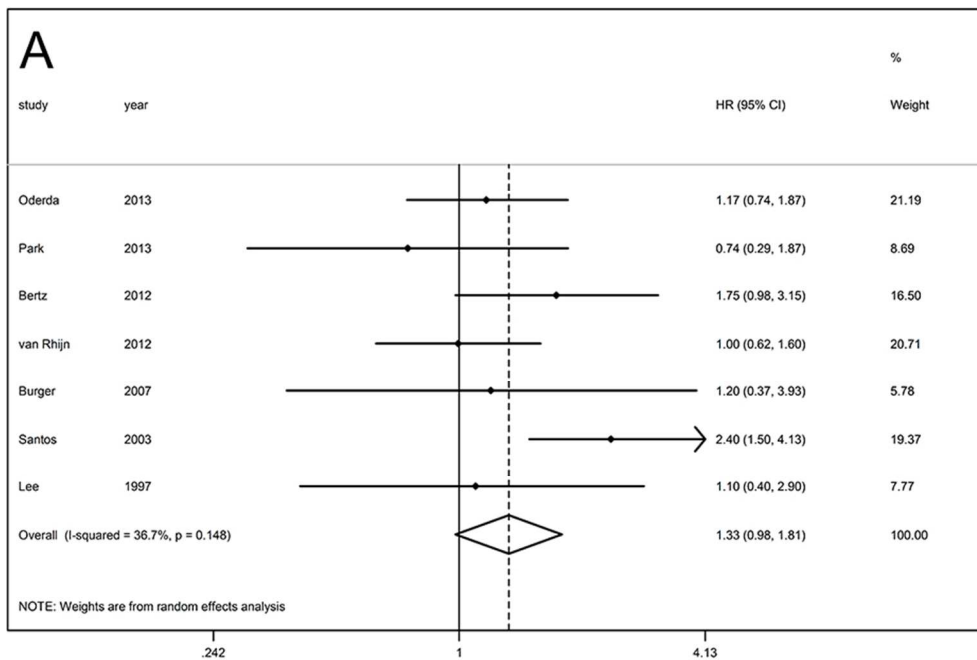


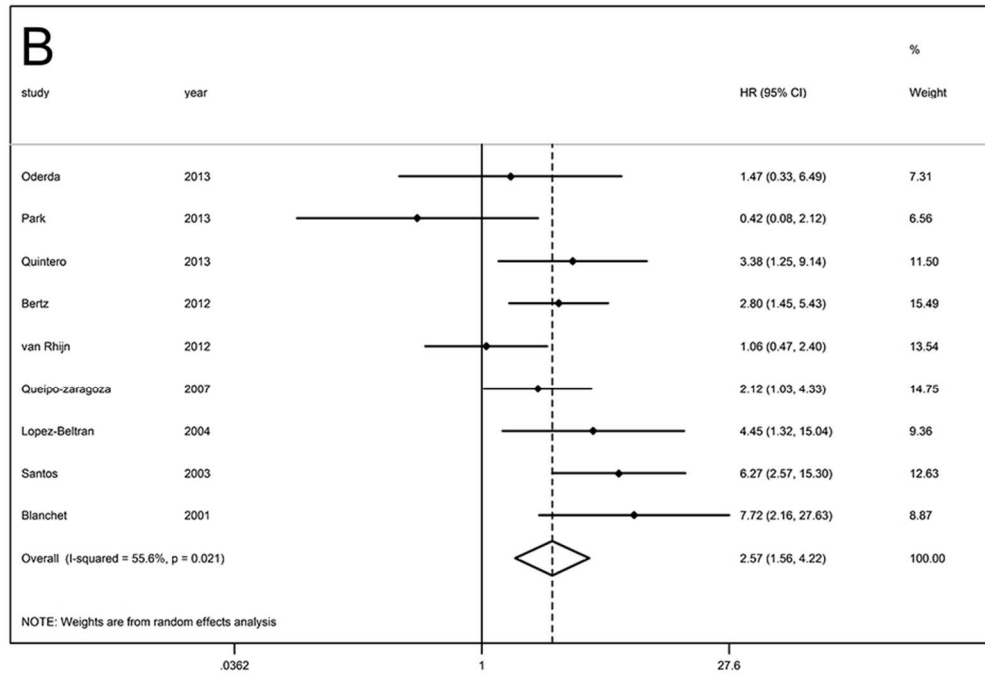
Figure 1. Flow diagram of study selection.

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Forest plots of HRs estimated for the relationship between Ki-67 expression and RFS (A) or PFS (B) among NMIBC patients treated with BCG.

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Forest plots of HRs estimated for the relationship between Ki-67 expression and RFS (A) or PFS (B) among NMIBC patients treated with BCG.

80x54mm (300 x 300 DPI)

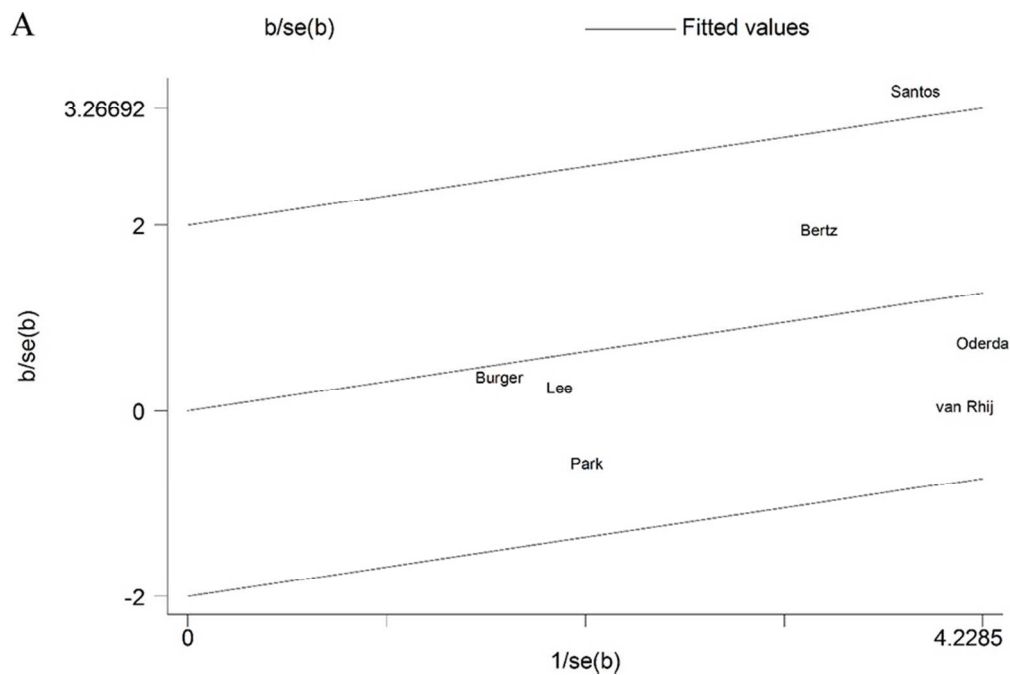


Figure 3. Galbraith plot analysis was used to evaluate heterogeneity. It suggested that two studies were the potential source of heterogeneity for RFS (A), while one was the source of heterogeneity for PFS (B)

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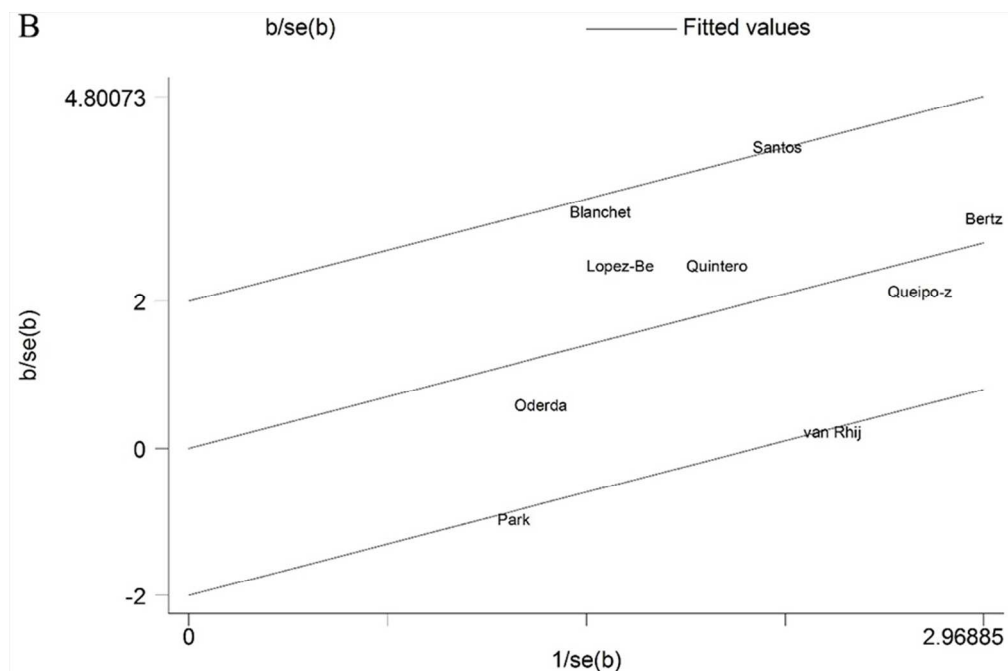
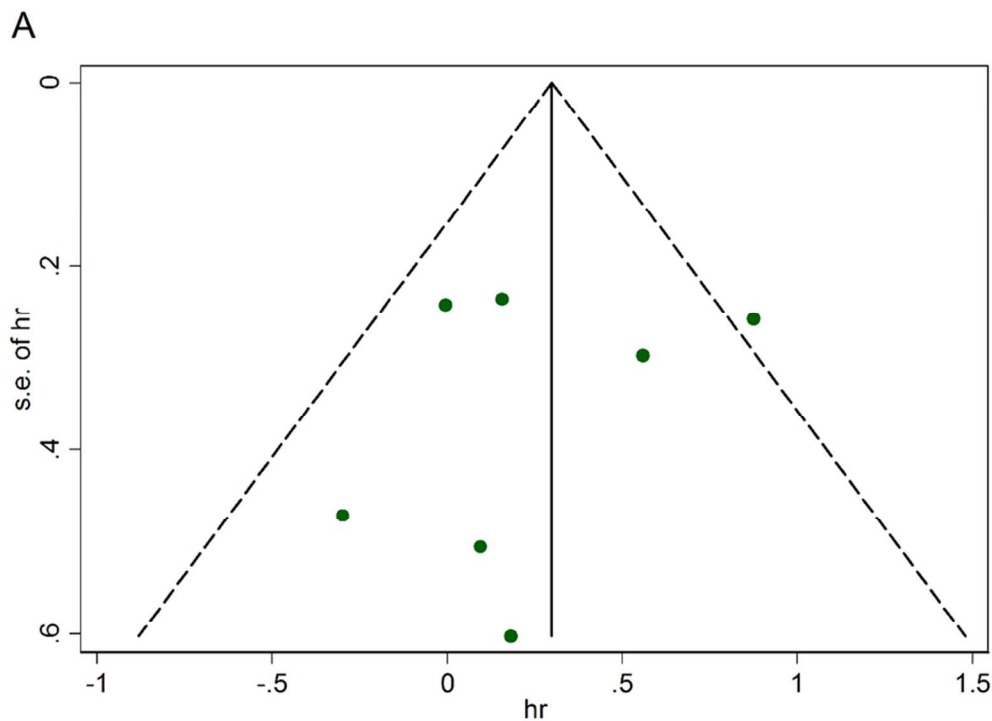


Figure 3. Galbraith plot analysis was used to evaluate heterogeneity. It suggested that two studies were the potential source of heterogeneity for RFS (A), while one was the source of heterogeneity for PFS (B)

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30 Figure 4. Funnel plots of the expression of ki67 and RFS (A) or PFS (B).

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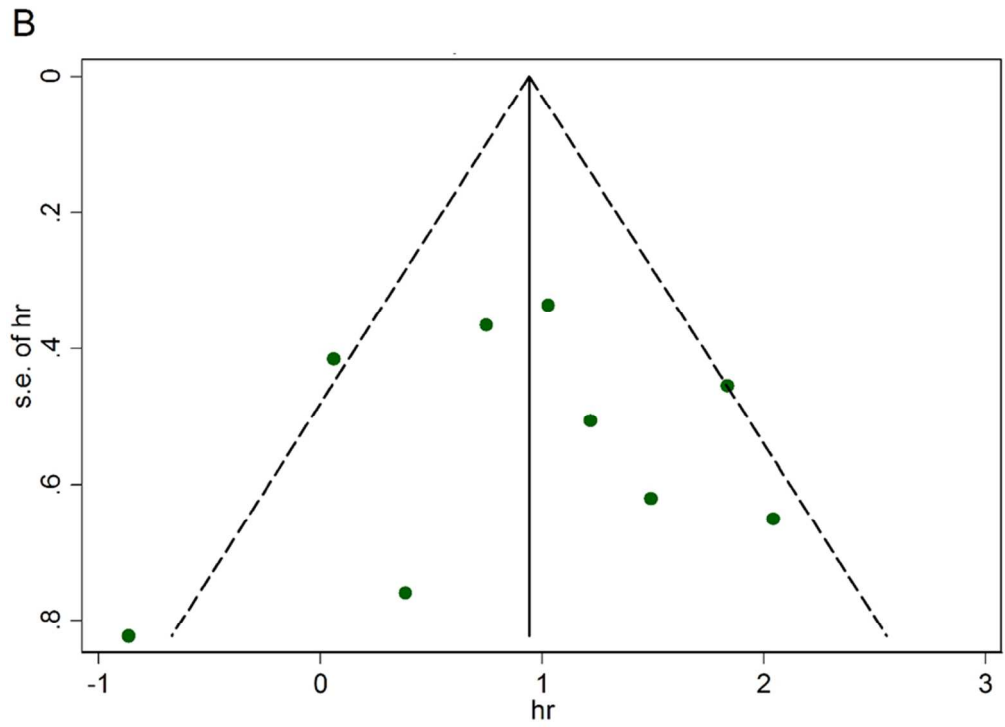


Figure 4. Funnel plots of the expression of ki67 and RFS (A) or PFS (B).

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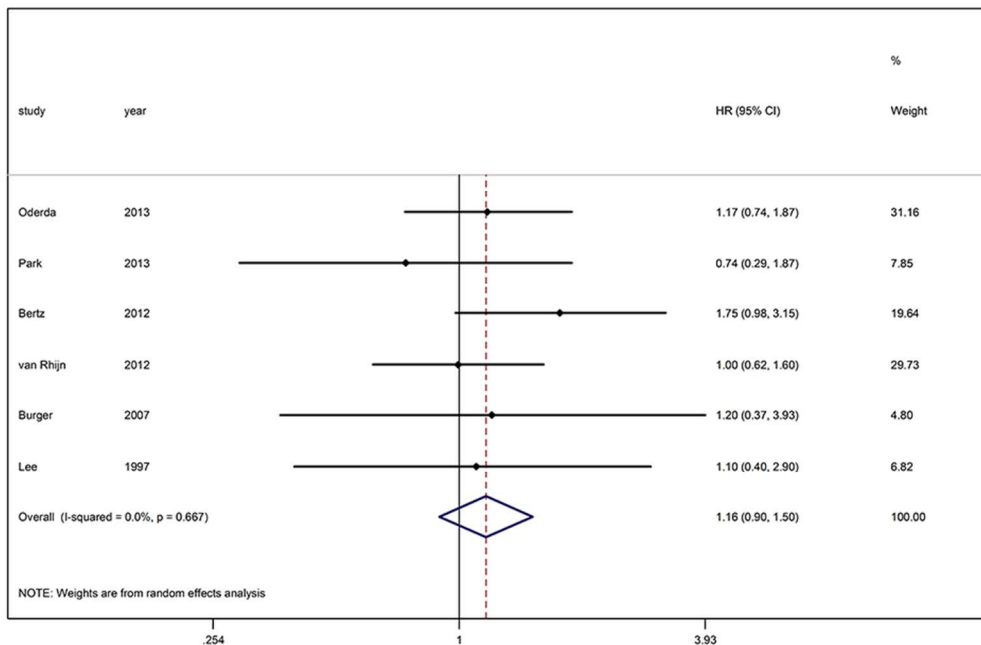


Figure S1. Forest plots of HRs estimated for the relationship between Ki-67 expression and RFS after the aforementioned study was excluded

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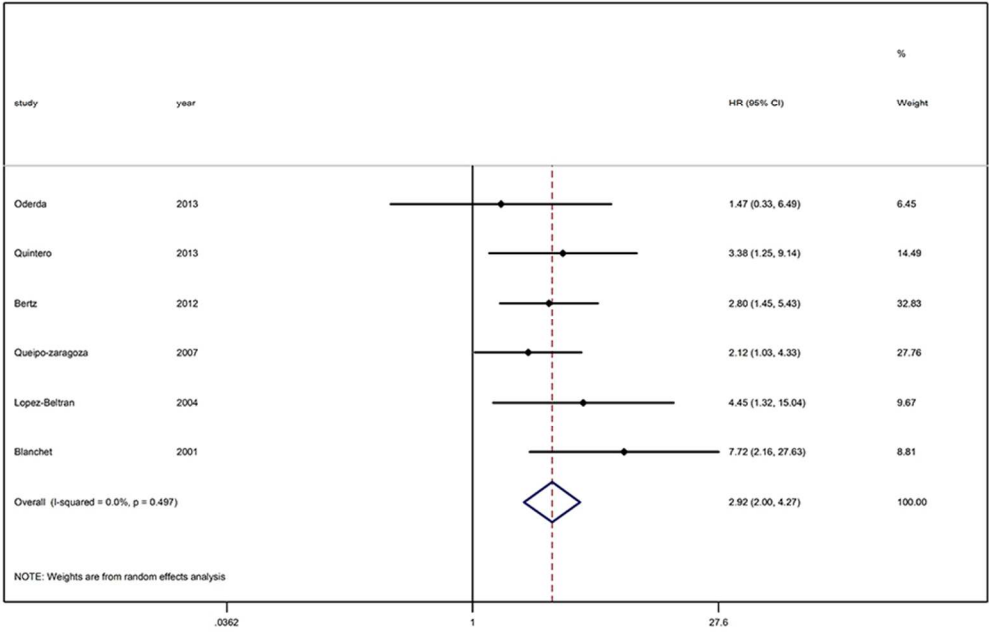


Figure S2. Forest plots of HRs estimated for the relationship between Ki-67 expression and PFS after the aforementioned study was excluded

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Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	6-8
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6-8
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	8
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	8
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	8
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	8
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8-9
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8-9
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9-10
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9-10



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Page 1 of 2

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Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	10
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10-11
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	11-13
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Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	15-21
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	15-21
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	22
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	22
DISCUSSION			
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Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	28
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	28

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Page 2 of 2

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Table S2. Meta-regression analysis of RFS and PFS

PFS				
Heterogeneity	Coefficient	SE	<i>t</i>	<i>P</i>
factor				
Years	-0.0343	0.0283	-1.21	0.280
Country				
1	-0.3899	0.3716	-1.05	0.404
2	-0.7185	0.3503	-2.05	0.177
3	-0.9900	0.4307	-2.30	0.148
4	-0.8805	0.3541	-2.49	0.131
Numbers of patients	0.0022	0.0020	1.16	0.299
Stage	None			
Cutoff	-0.0098	0.0407	-0.24	0.819
Age	0.0021	0.0395	0.05	0.959
Follow-up	-0.0062	0.0065	-0.96	0.379
PFS				
Heterogeneity	Coefficient	SE	<i>t</i>	<i>P</i>
factor				
Years	-0.1195	0.0461	-2.59	0.036
Country				

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4	1	0.2080	0.7936	0.26	0.818
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6	2	-0.8062	0.5662	-1.42	0.290
7					
8	3	-1.4505	0.8858	-1.64	0.243
9					
10					
11	4	-2.7009	0.9407	-2.87	0.103
12					
13	5	-1.7766	0.6167	-2.88	0.102
14					
15	6	-0.8158	0.5281	-1.54	0.262
16					
17					
18	Numbers	of	0.0006	0.0036	0.16
19					
20	patients				
21					
22					
23	Stage				
24					
25	1	-1.4505	1.5909	-0.91	0.458
26					
27	2	-0.8062	1.4108	-0.57	0.625
28					
29					
30	3	0.2080	1.5332	0.14	0.904
31					
32					
33	4	-1.7766	1.4353	-1.24	0.341
34					
35	5	-1.2560	1.2069	-1.04	0.407
36					
37					
38	6	-0.6170	1.4689	-0.42	0.715
39					
40	Cutoff	-0.0177	0.0309	-0.57	0.585
41					
42	Age	-0.0672	0.0757	-0.89	0.404
43					
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45	Follow-up	-0.0118	0.0159	-0.74	0.483
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Table S3. Risk group stratification in NMIBC

Low-risk tumors	Primary, solitary, Ta, LG/G1, <3 cm, no CIS
Intermediate-risk tumors	All tumors not defined in the two adjacent categories (between the category of low and high risk)
High-risk tumors	Any of the following: T1 tumor HG/G3 tumor CIS Multiple, recurrent, and large (>3 cm) Ta G1G2 tumors (all conditions must be present at this point)

NMIBC, non-muscle-invasive bladder cancer; CIS, carcinoma in situ; HG, high grade; LG, low grade.



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Page 2 of 2

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BMJ Open

Prognostic Value of ki67 in Bacillus Calmette–Guérin-Treated Non-muscle-Invasive Bladder Cancer: a Meta-analysis and Systematic Review

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019635.R1
Article Type:	Research
Date Submitted by the Author:	13-Jan-2018
Complete List of Authors:	He, Yuhui; Peking University China-Japan Friendship School of Clinical Medicine; China-Japan Friendship Hospital, Department of Urology Wang, Ning; North China University of Science and Technology Zhou, Xiaofeng; Peking University China-Japan Friendship School of Clinical Medicine; China-Japan Friendship Hospital, Department of Urology Wang, Jianfeng; China-Japan Friendship Hospital, Department of Urology Ding, Zhenshan; China-Japan Friendship Hospital, Department of Urology Chen, Xing ; China-Japan Friendship Hospital, Department of Urology Deng, Yisen; Peking University China-Japan Friendship School of Clinical Medicine; China-Japan Friendship Hospital, Department of Urology
Primary Subject Heading:	Urology
Secondary Subject Heading:	Urology
Keywords:	ki67, meta-analysis, non-muscular-invasive bladder cancer, prognosis

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3 **Prognostic Value of ki67 in Bacillus Calmette–Guérin-Treated**
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7
8 **Systematic Review**
9

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11 Yuhui He^{1,2}, Ning Wang³, Xiaofeng Zhou^{1,2*}, Jianfeng Wang², Zhenshan Ding², Xing
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49 Title page: 98
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3 Number of tables, figures, supplementary files: 4, 4, 5
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Abstract

Objectives: The aim of this study was to explore the prognostic value of ki67 as a marker in non-muscle-invasive bladder cancer (NMIBC) patients treated with Bacillus Calmette–Guérin (BCG).

Methods: Studies were systematically retrieved from the relevant databases (Web of Science, PubMed, Cochrane Library, and Embase), and the expiry date was May 2017. The research steps referred to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement.

Results: A total of 11 studies that complied with the inclusion criteria were enrolled. The expression of ki67 was not statistically significantly correlated with recurrence-free survival (RFS) [hazard ratio (HR): 1.331; 95% CI: 0.980–1.809]. No significant heterogeneity was found among all included studies ($I^2 = 36.7\%$, $P = 0.148$). The expression of ki67 was statistically significantly correlated with progression-free survival (PFS) (HR: 2.567; 95% CI: 1.562–4.219), and the overexpression of ki67 was the risk factor for PFS. Significant heterogeneity was noted among all the included studies ($I^2 = 55.6\%$, $P = 0.021$). The studies that might cause heterogeneity were excluded using the Galbraith plot, and then the meta-analysis was performed again. The results showed that the expression of ki67 was still correlated with PFS (HR: 2.922; 95% CI: 2.002–4.266).

Conclusions: The overexpression of ki67 was the risk factor for PFS, and the relationship between the expression of ki67 and RFS was not statistically significant in patients with NMIBC treated with BCG intravesical immunotherapy. Well-designed, prospective, randomized controlled trials with a large sample size are still needed to validate these findings.

Key words: ki67; meta-analysis; non-muscular-invasive bladder cancer; prognosis

Strengths and limitations of this study

This meta-analysis and systematic review was performed via a strict literature search.

It was the first meta-analysis to evaluate the prognostic value of ki67 in patients with NMIBC after transurethral resection and BCG intravesical immunotherapy.

The number of studies considered in the final meta-analysis was 11. This small sample size limited the potential analyses. The research did not consider the surgical skills mentioned in published studies.

Despite a systematic search strategy, the inclusion criteria excluded non-English documents and had language bias. The meta-regression analysis suggested no bias, but a selection bias was likely.

These limitations notwithstanding, the research can guide the follow-up research on immunohistochemical markers and clinical practice in non-muscular-invasive bladder cancer.

Introduction

Bladder cancer is one of the most common clinical urological tumors. It is a direct threat to the survival of patients with the disease. The incidence of bladder cancer varies across the world, with the highest rate in the developed communities.¹ A total of 429,800 new cases of bladder cancer and 165,100 deaths occurred in 2012 worldwide. Bladder cancer occurs mostly in men, and about a tenfold variation in incidence rates has in been reported internationally.² About 70% of these patients have non-muscle-invasive bladder cancer (NMIBC).³ Four major organizational guidelines on NMIBC, including the American Urological Association/Society of Urologic Oncology, the European Association of Urology, the National Comprehensive Cancer

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3 Network, and the National Institute for Health and Care Excellence guidelines,
4 recommend that the proper initial transurethral resection (TUR) of bladder tumor is a
5 critical step in the initial management and staging of the disease.⁴ However, TUR
6 surgery alone cannot solve the postoperative problems for NMIBC because of high
7 recurrence rate and disease development.⁵ Postoperative TUR associated with
8 Bacillus Calmette–Guérin (BCG) intravesical immunotherapy can prevent the
9 postoperative recurrence of NMIBC and significantly reduce the moderate and high
10 development risk of NMIBC.^{6, 7} However, the postoperative BCG intravesical
11 immunotherapy still has some problems. The failure rate of BCG intravesical therapy
12 in NMIBC is about 40%–50%.⁸ Furthermore, BCG also has toxic side effects, such as
13 hepatitis, pneumonitis, epididymitis/orchitis, abscess formation, bladder contracture,
14 ureteral obstruction, BCG sepsis, leukopenia, and hematuria.⁹ Therefore, BCG
15 therapy should be individually performed, and the patients in whom BCG therapy is
16 ineffective should be timely recognized. These patients or those with poor prognosis
17 should receive radical cystectomy or any other therapy in time to avoid futile
18 treatment and alleviate pain. However, the recognition of patients with no effect of
19 TUR postoperative BCG intravesical immunotherapy is still hard due to the
20 heterogeneity of bladder cancer and individuality of patients.¹⁰ Therefore, finding the
21 prognostic factors for patients with NMIBC receiving TUR and BCG therapy is
22 extremely necessary.

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46 The recurrence rate of bladder cancer treated with different therapies is between 50%
47 and 80%, and about 15% of the low-grade tumor recurrence involves high-grade
48 tumors.¹¹ The patients need periodical cystoscopy to find the recurrent focus in time.
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52 A reliable prognostic molecular marker can reduce the pain caused by cystoscopy.
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55 Because NMIBC does not have reliable prognostic markers, it is hard to decide

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3 postoperative therapy in the clinic,¹² which depends mainly on the clinical guidelines
4 and physician's experience. Currently, some of the published studies about
5 immunohistochemical markers have evaluated the prognostic value of BCG
6 intravesical immunotherapy on the patients first receiving TUR. The main
7 immunohistochemical markers include ki67, p53, p27, pRb, CD9, CD20, E2F1, and
8 so forth.^{13, 14} However, no immunohistochemical marker has been confirmed so far.
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10 The prognostic value of ki67 antigen on the survival in patients with NMIBC
11 receiving BCG intravesical immunotherapy has been controversial. For example,
12 Kruger.¹⁵ reported that ki67 antigen was an independent predictive factor for the
13 recurrence of pT1 stage tumor, but Oderde¹⁶ believed that ki67 was an independent
14 predictive factor for the recurrence of all NMIBCs. Zlotta¹⁷ reported that ki67 antigen
15 had no independent prognostic value in patients receiving BCG therapy. Saint¹⁸
16 retrospectively reviewed the recent 25-year published studies and believed that the independent
17 prognostic factor for bladder cancer in patients receiving BCG therapy was not clear.
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19 An international consensus group listed various bladder cancer prognostic indexes by
20 reviewing PubMed and considered that although some markers (such as ki67 and p53)
21 could predict the recurrence and development of bladder cancer, the data still had
22 heterogeneity. Thus, strict test criteria and clear statistical methods should be
23 established for further evaluation.¹⁹

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44 A meta-analysis can enlarge the sample size by integrating independent studies with
45 small sample size, further increase the statistical efficacy, and reduce the wrong
46 conclusion caused by the small sample size.²⁰ The aim of this study was to explore the
47 prognostic value of ki67 as a marker in patients with NMIBC treated with BCG.
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49 Based on the literature search, this study was the first meta-analysis to evaluate the
50 prognostic value of ki67 in patients with NMIBC treated with BCG.
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Methods

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (**Table S1**).²¹ The present meta-analysis did not need the approval because all the enrolled published studies were approved by the ethics committee in their research institute.

Literature retrieval strategy

The comprehensive literature search was performed on Web of Science, PubMed, Cochrane Library, and Embase databases for relevant studies. The last quest was updated on May 24, 2017, with hand-searching to identify any potentially eligible studies that might have been missed. The following search strategy was adopted for each database: ("Urinary Bladder Neoplasms"[Mesh] OR "bladder cancer" OR "bladder carcinoma" OR " bladder tumor ") AND ("BCG Vaccine"[Mesh] OR "BCG" OR "Bacillus Calmette–Guérin") AND ("ki67 antigen "[Mesh] OR " ki-67" OR " ki67" OR " MBI-1"). Filters were as follows: retrospective, array research, clinical trial, controlled clinical trial, and randomized controlled trial. Free word retrieval strategy was used. The enrolled contents included the reference lists and relevant suggestive references while searching.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) prospective or retrospective published studies evaluating the prognostic relationship between the expression of ki67 and NMIBC treated with BCG; (2) the expression of ki67 in tissues detected by immunohistochemistry analysis; (3) hazard ratio (HR) and 95% confidence interval (95% CI) directly obtained from the published studies; and (4) published English studies. The exclusion criteria were as follows: (1) review, systematic evaluation, case

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3 report, editorial, and specialist experience; (2) studies with no human subjects; and (3)
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5 published studies in which data could not be extracted or those having wrong data.
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7 ***Data extraction and evaluation of literature quality***

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9 Based on the aforementioned criteria, two reviewers independently screened the
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11 published studies by reading titles and abstracts and got preliminary conclusions. If
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13 the conclusions were not consistent, the literature was discussed by all the authors to
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15 decide its enrollment. The relevant information of the enrolled published studies was
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17 extracted, such as first author, publication time, research country, sex, case number,
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19 age, follow-up date, disease stage, cutoff values, recurrence-free survival (RFS), and
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21 progression-free survival (PFS). The Newcastle–Ottawa Scale (NOS) was used to
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23 evaluate the quality of all the published studies.²² Scores 0–3, 4–5, and 6–8 were
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25 accepted as low, medium, and high quality, respectively.
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28 ***Statistical methods***

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30 The measuring time and method of ki67 complied with the standard of clinical routine
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32 and pathological examination. Tumor tissue samples were taken in accordance with
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34 the standard surgical procedure and used for immunohistochemical analysis. RFS and
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36 PFS were the traditionally used statistical parameters. PFS was defined as the time
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38 from the beginning of treatment to the first progression. RFS was defined as the time
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40 from the removal of the lesion (or the randomization of the clinical trial) until the
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42 recurrence or death of the tumor. The impact of the expression of ki67 on survival was
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44 quantified using the combined HRs and 95% CIs. The HR and 95% CI of each study
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46 were directly extracted from each original published study. Besides, the Parmar and
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48 Tierney's²³ method was used to extract the data because some of the published studies
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50 did not directly provide HR and 95% CI. For example, some studies provided only the
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52 survival curve. In this meta-analysis, the DerSimonian–Laird random-effects model²⁴
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3 was used because when the heterogeneity was large, only the random-effects model
4 could be suitably used. Similar to traditional methods, HR >1 was considered as the
5 prognostic risk factor for the overexpression of ki67, and HR <1 was a protective
6 factor. 95% CI <1 indicated a statistically significant difference in the relationship
7 between the overexpression of ki67 and prognosis.
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11 The heterogeneity was calculated according to chi-square-based Q test and I^2
12 statistic.²⁵ The heterogeneity was judged using the I^2 value (low heterogeneity: I^2
13 <25%; moderate heterogeneity: $I^2 = 25\%–50\%$; large heterogeneity: $I^2 >50\%$).
14 Besides, A P value >0.05 was considered as low heterogeneity. Then, the subgroup
15 analysis based on regions, sample size, follow-up period, tumor grading, cutoff value,
16 publication time, and patient age was performed. A value of 1% was considered to be
17 a statistically significant level in the subgroup analysis. A Galbraith plot was used to
18 search published studies with heterogeneity²⁶, and the meta-analysis was performed
19 again after excluding these published studies. Meanwhile, the factors causing
20 heterogeneity were also explored using the residual maximum likelihood
21 (REML)-based random-effects meta-regression analysis.²⁷ All the statistical analyses
22 were performed using the Stata12.0 software (StataCorp, TX, USA), and the
23 two-sided test was used to evaluate the P value.
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41 ***Evaluation of publication bias***

42 Begg's plot and Egger's test method were used to find the possible publication bias. A
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46 P value <0.05 was considered to indicate publication bias.
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50 ***Results***

51 ***Literature screening***

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54 A total of 97 published studies were retrieved. Furthermore, 68 of them were excluded
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3 after duplicates were removed and records screened, and 18 were excluded after
4 reading the full text (10 published studies from which HR and 95% CI could not be
5 obtained, 2 non-English studies, and 6 that did not use ki67 detection). Finally, 11
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7 published studies were enrolled in the meta-analysis (**Figure 1**).
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10 11 *Basic characteristics and quality evaluation of enrolled published studies*

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13 The enrolled 11 published studies were published between 1997 and 2013, and the
14 countries included Italy, South Korea, Spain, Germany, New Zealand, Canada,
15 Portugal, and France. The largest sample size was 309, and the smallest one was 32. A
16 total of 1321 patients were enrolled in this study. The follow-up period was beyond 36
17 months, and the longest was 229 months. T1 was the main tumor grading, and the
18 cutoff value ranged from 10.4% to 40%. Seven published studies reported patients'
19 RFS, and nine reported PFS (**Table 1**). One literature was scored as 6 star by NOS,
20 seven as 7 star, and three as 8 star. The median of the NOS score was 7 (**Table 2**).
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Table 1. Main characteristics of all studies included in this meta-analysis

Study	Year	Country	Male/Female	No. of patients	Age (year)	Follow-up (month)	Stage	Cutoff	Survival analysis
Oderda ¹⁶	2013	Italy	166/26	192	73.2 (SD 11.9)	100 (2–229)	All NMIBC	20%	RFS
Park ¹⁴	2013	Korea	53/8	61	66 (31–85)	60 (6–217)	T1G3	10.4%	RFS/PFS
Quintero ²⁸	2013	Spain	143/21	164	61 (29–93)	75 (60–144)	Ta	13%	PFS
Bertz ¹²	2012	Germany	237/72	309	71.7 (38–87)	49 (5–172)	pT1	15%	RFS/PFS
van Rhijn ²⁹	2012	Netherlands, Canada	105/24	129	68.8 (SD 9.9)	78 (39.6–110.4)	T1	25%	RFS/PFS
Burger ³⁰	2007	Germany	45/21	71	71 (52–94)	39 (1–133)	T1/Ta	15%	RFS
Queipo-zaragoza ³¹	2007	Spain	71/12	83	68.1 (SD 8.5)	All >36	T1G3	40%	PFS
Lopez-Beltran ³²	2004	Spain	49/2	51	69.96 (49–89)	63.82 (60–144)	T1G3	13%	PFS
Santos ³³	2003	Portugal	115/44	159	66 (21–88)	46.5 (4–123)	pTa/pT1	18%	RFS/PFS
Blanchet ³⁴	2001	France	-	70	62.6 (21–84)	64 (12–111)	pT1/pTa	13%	PFS
Lee ³⁵	1997	Korea	28/4	32	57.1 (30–81)	All >24	T1G2-3	20%	RFS

NMIBC, Non-muscle-invasive bladder cancer; no., number; PFS, progression-free survival; RFS, recurrence-free survival.

Table 2. Quality of the included studies assessed by NOS

Study	Selection		Comparability		Exposure		Non-response rate	Scores	
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Ascertainment of exposure			Same method to ascertain for cases and controls
Oderda ¹⁶	—	☆	☆	☆	☆☆	☆	☆	☆	8
Park ¹⁴	—	☆	☆	—	☆☆	☆	☆	☆	7
Quintero ²⁸	—	☆	☆	—	☆☆	☆	☆	—	6
Bertz ¹²	—	☆	☆	—	☆☆	☆	☆	☆	7
van Rhijn ²⁹	—	☆	☆	—	☆☆	☆	☆	☆	7
Burger ³⁰	☆	☆	☆	—	☆☆	☆	☆	—	7
Queipo-zaragoza ³¹	—	☆	☆	—	☆☆	☆	☆	☆	7
Lopez-Beltran ³²	—	☆	☆	☆	☆☆	☆	☆	☆	8
Santos ³³	—	☆	☆	—	☆☆	☆	☆	☆	7
Blanchet ³⁴	—	☆	☆	☆	☆☆	☆	☆	☆	8
Lee ³⁵	—	☆	☆	—	☆☆	☆	☆	☆	7

Influence of the expression of ki67 on RFS

Seven published studies reported the expression of ki67 and PFS results of patients with NMIBC treated with BCG. The meta-analysis indicated that ki67 had no statistically significant association with RFS (HR: 1.331; 95% CI: 0.980–1.809), and no heterogeneity among the enrolled studies was reported ($I^2 = 36.7\%$, $P = 0.148$) (**Figure 2A**). The subgroup analysis was performed based on the regions, sample size, follow-up period, stage, cutoff value, publication time, and age. Meanwhile, all the original published studies on the correlation between ki67 expression and RFS in patients with NMIBC treated with BCG were multivariate, and the HRs were adjusted. The stratification analysis by region indicated that ki67 was also significantly associated with RFS in Caucasians and a follow-up period shorter than 6 months. (HR: 1.441, 95% CI: 1.014–2.047; HR: 1.853, 95% CI: 1.316–2.607) (**Table 3**).

Table 3. Subgroup results of RFS and heterogeneity test

Variables	Study number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total RFS	7	1.331 (0.980–1.809)	9.48	0.148	36.7
Region					
Asian	2	0.892 (0.454–1.752)	0.32	0.570	0.0
Caucasian	5	1.441 (1.014–2.047)	7.52	0.111	46.8
Sample size					
>100	4	1.466 (0.986–2.181)	7.44	0.059	59.7
≤100	3	0.959 (0.534–1.725)	0.51	0.777	0.0
Follow-up (month)					
≥60	3	1.036 (0.758–1.415)	0.79	0.674	0.0
<60	4	1.853 (1.316–2.607)	2.62	0.453	0.0
Stage					
All NMIBC	3	1.575 (0.915–2.711)	4.44	0.109	54.9
Others	4	1.153 (0.821–1.620)	3.21	0.360	6.6
Cutoff					
15%	2	1.625 (0.963–2.743)	0.31	0.575	0.0
Others	5	1.252 (0.839–1.869)	8.56	0.073	53.3
Publication year					
≥2012	4	1.164 (0.874,1.550)	3.20	0.362	6.3
<2012	3	1.774 (1.046,3.008)	2.57	0.277	22.1
Patient age (year)					
≥70	3	1.352 (0.955–1.913)	1.16	0.559	0.0
<70	4	1.256 (0.717–2.198)	8.32	0.040	63.9

NMIBC, Non-muscle-invasive bladder cancer; RFS, recurrence-free survival.

Influence of the expression of ki67 on PFS

A total of nine published studies reported the expression of ki67 and PFS results of patients in NMIBC treated with BCG. The meta-analysis indicated that ki67 had no statistically significant association with RFS (HR:2.567, 95% CI: 1.562–4.219), and the overexpression of ki67 was the risk factor for PFS. Statistically significant heterogeneity was found among all the included studies ($I^2 = 55.6\%$, $P = 0.021$) (**Figure 2B**). The subgroup analysis was performed based on the regions, sample size, follow-up period, stage, cutoff value, publication time, and age. However, the data extracted from six original published studies on the correlation between the expression of p53 and PFS in patients with NMIBC treated with BCG were multivariate with adjusted HRs, whereas three original published studies were univariate with unadjusted HRs. In the stratified analyses by the region, sample size, follow-up time, stage, cutoff, publication year, and patient age, significant associations were observed in the studies with Caucasian subgroup, sample size >100, follow-up period <6 months, other cutoffs, and two subgroups based on age (HR: 1.97, 95% CI: 1.04–3.74; HR: 2.37, 95% CI: 1.23–4.55; HR:2.49, 95% CI: 1.19–5.21; HR: 2.515, 95% CI: 1.382–4.576; HR: 2.800, 95% CI: 1.447–5.418; and HR: 2.654, 95% CI: 1.381–5.100, respectively). However, significant associations were also observed in both multivariate and univariate analyses (HR: 2.10, 95% CI: 1.07–1.12; HR: 2.80, 95%CI: 1.65–7.85, respectively), and the effect size suggested the same outcomes (HR: 2.567, 95% CI: 1.562–4.219) (**Table 4**).

Table 4. Subgroup results of PFS and heterogeneity test

Variables	Study number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total PFS	9	2.567 (1.562–4.219)	18.1	0.021	55.6
Region					
Asian	1	0.421 (0.084–2.114)	0.00		
Caucasian	8	2.883 (1.830–4.544)	12.99	0.072	46.1
Sample size					
>100	5	2.559 (1.372–4.774)	9.26	0.055	56.8
≤100	4	2.536 (0.943–6.818)	8.75	0.033	65.7
Follow-up (month)					
≥60	6	2.153 (0.984–4.710)	13.08	0.023	61.8
<60	3	3.158 (1.774–5.623)	3.56	0.169	43.8
Stage					
All NMIBC	3	4.673 (1.938–11.264)	3.29	0.193	39.2
Others	6	2.044 (1.213–15.040)	9.58	0.088	47.8
Cut off					
15%	1	2.800 (1.447–5.418)	0.00		
Others	8	2.515 (1.382–4.576)	17.92	0.012	60.9
Publication year					
≥2012	5	1.685 (0.883,3.215)	8.04	0.090	50.2
<2012	4	4.176 (2.209,7.884)	5.00	0.172	40.0
Patient age (year)					
≥70	2	2.519 (1.377–4.606)	0.60	0.438	0.0
<70	7	2.654 (1.381–5.100)	17.40	0.008	65.5
Multivariate/Univariate					
Multivariate	6	2.101 (1.070–1.121)	13.83	0.031	63.8
Univariate	3	2.803 (1.652–7.856)	3.38	0.001	40.8

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NMIBC, Non-muscle-invasive bladder cancer; PFS, progression-free survival.

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Galbraith plot

Using Galbraith plot (**Figure 3A**), it was found that Santos³³ was the main reason for the heterogeneity of RFS. After the aforementioned study was excluded, the remaining RFS studies had no significant heterogeneity according to the new meta-analysis ($I^2 = 0.0\%$, $P = 0.667$). However, the expression of ki67 still had no statistically significant association with RFS (HR: 1.161, 95% CI: 0.896–1.504) (**Figure S1**). Using the Galbraith plot (**Figure 3B**), it was found that Santos,³³ Park,¹⁴ and van Rhijn²⁹ were the main reason for the heterogeneity of PFS. After these studies were excluded, the remaining RFS studies had no significant heterogeneity according to the new meta-analysis ($I^2 = 0.0\%$, $P = 0.497$). The expression of ki67 still had statistically significant association with PFS (HR: 2.922, 95% CI: 2.002–4.266) (**Figure S2**).

Meta-regression analysis

The meta-regression analysis indicated that the factors influencing heterogeneity (publication time, research region, sample size, stage, cutoff value, age, and follow-up period) might not be the reason for RFS heterogeneity. Publication time was the reason for heterogeneity of PFS ($P = 0.036$), but other factors were not (**Table S2**).

Publication bias

The funnel plot (**Figure 4A**) was basically symmetrical for RFS. The results of Begg's test and Egger's test showed P (Begg's) = 0.761, P (Egger's) = 0.601. The funnel plot (**Figure 4B**) was also basically symmetrical for PFS. The results of Begg's test and Egger's test showed P (Begg's) = 0.917, P (Egger's) = 0.964.

Discussion

A total of 11 published studies with 1321 cases complying with the inclusion criteria

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3 were enrolled in this meta-analysis. The results of the meta-analysis indicated that the
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5 expression of ki67 had no statistically significant association with RFS, but it was
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7 significantly correlated with PFS. The overexpression of ki67 was the risk factor for
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9 PFS. It suggested that ki67 was the prognostic predictive marker in patients with
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11 NMIBC treated with BCG. Besides, the aforementioned conditions did not change
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13 after excluding the published studies possibly causing heterogeneity and reperforming
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15 the meta-analysis. It further proved that the result of the aforementioned meta-analysis
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17 was stable, that is, the overexpression of ki67 was the risk factor for PFS. In the
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19 Caucasian subgroup for PFS, racial classification and regional factors might be crucial
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21 in the prognosis of patients with NMIBC after BCG therapy. This may be related to
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23 the existence of different drug gene susceptibilities in people of different races and
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25 living areas. The two subgroups based on age in PFS, suggesting that age might be the
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27 important factor influencing the prognosis of bladder cancer. This also comply to our
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29 clinical practice. The elder the patient, the worse the prognosis. There are several
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31 sources of heterogeneity in the above-mentioned subgroup analysis: (1) Due to the
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33 influence of race and environment, the documents included in this article come from
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35 different regions and countries. There are a large number of studies that confirm the
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37 differences in disease susceptibility between ethnic groups and regions. (2) Because
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39 of different regions and different clinicians, in the TUR and BGC perfusion treatment,
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41 there are differences in the operation of health care workers. Such as surgical
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43 clearance of the tumor. The tumor with a broad base surface is often not easy to
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45 remove completely, which also depends on the surgeon's experience and surgical
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47 skills. And the quality of BCG manufacturers may vary from region to region. (3)
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49 Different researchers' literature may include the bias of research object, research
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51 design, measuring instrument and so on. However, in general, heterogeneity does not
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3 affect the conclusion. Besides, the meta-regression analysis indicated publication time
4 as the reason for PFS heterogeneity. We consider it is relate to the improvement of
5 testing technology, research level as well as the quality and quantity of published
6 articles, which will be helpful for the follow-up researches. As all the original data
7 extracted from published studies on the correlation between the expression of ki67
8 and RFS in patients with NMIBC treated with BCG were multivariate, the result was
9 considered to be precise because the HRs were adjusted, excluding the confounding
10 factors such as age and gender. However, the original data extracted from published
11 studies on the correlation between ki67 expression and PFS were both multivariate
12 and univariate. It was believed that the aforementioned adjustments did not have a
13 significant impact on meta-analyses. Besides, according to the funnel plot, Begg's test
14 and Egger's test, the enrolled studies had no statistically significant publication bias.
15 Thus, the reliability of the present meta-analysis was high.

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18 In 2016, the European Association of Urology (EUA)³⁶ recommended a scoring
19 system for the prognostic evaluation of NMIBC based on six clinical and pathological
20 factors proposed by the European Organization for the Research and Treatment of
21 Cancer-Genito-Urinary Cancer Group (EORTC), including number of tumors, tumor
22 size, prior recurrence rate, T category, presence of concurrent carcinoma in situ, and
23 tumor grade (**Table S3**). The tumors were categorized into low-risk tumors,
24 intermediate-risk tumors, and high-risk tumors using this assessment system to
25 evaluate the prognosis. For the patients after BCG therapy, the EUA recommended
26 another risk calculator developed by the Club Urologico Espanol de Tratamiento
27 Oncologico (CUETO) and the EORTC. This calculator was based on gender, age,
28 recurrent tumor, number of tumors, T category, associated Tis, and grade. The
29 CUETO risk calculator can be achieved at <http://www.aeu.es/Cueto.html>. The

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3 recommended level was B grade for the two scales for patients with NMIBC, whether
4 used alone or combined. The two scales could be used together in the clinic. When
5 using the CUETO scale, the calculated recurrent risk was lower than that from the
6 EORTC scale,³⁷ which might be related to the special design in the CUETO scale for
7 the patients receiving BCG intravesical immunotherapy. However, the scoring system
8 only depending on clinical and pathological factors could not accurately evaluate the
9 prognosis of patients with bladder cancer in T1 stage due to the independence of
10 disease condition in each patient.³⁸ The markers regulated at the genetic level may
11 judge the prognosis of patients with bladder cancer with the development of precision
12 medicine. A reliable marker helps in recognizing the patients who have failed in BCG
13 intravesical immunotherapy with high risk in time. Hence, these patients can undergo
14 radical cystectomy or other treatments in time. Unfortunately, no prognostic marker
15 has been applied in clinic currently. The results of this study potentially help to
16 remind clinicians that patients with high expression of ki67 may need to develop more
17 personalized follow-up plans, such as shorter follow-up and cystoscopy cycles. For
18 patients with high risk of clinical evaluation of the guidelines and ki67 overexpression
19 may need to promptly change the treatment strategy.

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39 Ki67 is a nucleoprotein that can be detected in the cell cycles except G0 phase.³⁹ The
40 expression of human ki67 protein is closely related to proliferation. Therefore, it is an
41 ideal marker to confirm the growth fraction of specific cell colonies.⁴⁰ Ki67 is a
42 widely known amplified biomarker. The ki67 monoclonal antibody can be detected by
43 the immunohistochemical method.⁴¹ Ki67 has been proved to be a good proliferation
44 marker in different cancers, including bladder cancer.⁴²

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52 So far, some meta-analyses have studied the effect of ki67 on the prognostic quality of
53 life of patients with esophageal cancer, breast cancer, epithelial ovarian cancer, and so
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3 on.⁴³⁻⁴⁵ Some studies have also focused on the other aspects of bladder cancer. Using
4 meta-analysis, Luo⁴⁶ believed that a high reactivity of ki67 could predict the poor
5 prognosis in patients with bladder cancer. The univariate analysis showed that
6 cancer-specific survival, disease-free survival, overall survival, PFS, and RFS had a
7 significant correlation with poor prognosis in patients with a high reactivity of ki67.
8
9 However, this study enrolled all types of bladder tumors and all the therapies for
10 NMIBC. Currently, the bladder cancer treated in the clinic is mainly NMIBC. Thus,
11 most of the applied therapy is TUR combined with installations of chemotherapy or
12 BCG intravesical immunotherapy based on the patients' conditions. Therefore, this
13 analysis had a certain limitation in the prognosis of patients with NMIBC after BCG
14 intravesical immunotherapy.

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Currently, few evidence-based studies focused on the prognosis of patients with
NMIBC after BCG intravesical immunotherapy. Using meta-analysis, Zhou⁴⁷
analyzed the correlation between the expression of p53 and quality of life of patients
with NMIBC after BCG intravesical immunotherapy. They believed that the
overexpression of p53 in patients with NMIBC treated with BCG might be associated
with RFS, especially in Asian population. Similarly, Du⁴⁸ also performed the
meta-analysis on the relationship between p53 status and NMIBC in T1 stage and
believed that the overexpression of p53 might be related to the development of
NMIBC. The present study indicated that the overexpression of ki67 was the risk
factor for PFS, but the expression of ki67 had no statistically significant association
with RFS. P53 is the most common inactivated tumor suppressor gene in tumor
cells.⁴⁹ The inactivation of p53 may cause cell abnormal hyperplasia and
cancerization. The variation in p53 results in enhanced proliferation, invasion, and

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3 metabolism.⁵⁰ The increase in the expression of ki67 , as cell proliferation marker
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6 suggests enhanced proliferation.⁴⁰ As a tumor suppressor gene with complicated
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8 function, p53 has a wider range of effects. The accuracy in the prediction of quality of
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10 life may not be more appropriate compared with ki67. The genetic difference between
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12 Asians and Caucasians suggests that different prediction systems should be built for
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14 different races. Besides, p27, E2F1, ezrin, and CK20 were also studied in other
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16 investigations for predicting NMIBC prognosis, which could be explored further
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18 comparing the advantages of using them alone or combined.
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21 However, this study still had some limitations. First, the enrolled published studies
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23 involved different populations, used similar detection equipment, and had different
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25 cutoff values. All these reasons might have caused the heterogeneity. Further, the
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27 sample size of the meta-analysis also limited its significance. Second, the
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29 meta-analysis included English published studies. Although Begg's test and Egger's
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31 test did not suggest publication bias, this study was still influenced by some bias.
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33 Finally, the surgical skills were different in different published studies, affecting the
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35 judgment regarding the effectiveness of BCG.
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38 ***Conclusions***

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40 The overexpression of ki67 was the risk factor for PFS in patients with NMIBC after
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42 TUR and BCG intravesical immunotherapy, but the relationship between the
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44 expression of ki67 and RFS was not statistically significant. Owing to the
45
46 aforementioned limitations of the present study, RCTs with large sample size are still
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48 required to validate the results.
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51 ***Authors' contributions***

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3 YHH, NW, and XFZ conceived and designed the experiments. XC and YSD extracted
4 the data. YHH, NW, and XFZ analyzed the data. ZSD, JFW, and XC contributed
5 reagents/materials/analysis tools. YHH and NW wrote the paper. XFZ critically
6 revised the report.
7
8
9

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12
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14 of China (ISTCP) (Grant No.2014DFE30010).
15

16 17 **Disclaimer**

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19 The contents of the present study are solely the responsibility of the author. The
20 funders had no role in study design, data collection and analysis, decision to publish,
21 or preparation of the manuscript.
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26 27 **Competing interests**

28
29 None declared.
30

31 32 **Data sharing statement**

33
34 Datasets used and/or analyzed in the present study are available from the
35 corresponding author on reasonable request.
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39 40 **References**

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Figure File

Figure 1. Flow diagram of study selection.

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3 **Figure 2.** Forest plots of HRs estimated for the relationship between the expression of
4 ki67 and RFS (A) or PFS (B) among patients in NMIBC treated with BCG.

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7 **Figure 3.** Galbraith plot analysis was used to evaluate heterogeneity and RFS (A) or
8 PFS (B).

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11 **Figure 4.** Funnel plots of the expression of ki67 and RFS (A) or PFS (B).

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16 **Table File**

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18 **Table 1.** Main characteristics of all studies included in this meta-analysis

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20 **Table 2.** Quality of the included studies assessed by NOS

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22 **Table 3.** Subgroup results of RFS and heterogeneity test

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24 **Table 4.** Subgroup results of PFS and heterogeneity test

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29 **Supplementary File**

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31 **Table S1.** PRISMA

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33 **Table S2.** Meta-regression analysis of RFS and PFS

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35 **Table S3.** Risk group stratification in NMIBC

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38 **Figure S1.** Forest plots of HRs estimated for the relationship between the expression
39 of ki67 and RFS after the aforementioned study was excluded

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42 **Figure S2.** Forest plots of HRs estimated for the relationship between the expression
43 of ki67 and PFS after the aforementioned study was excluded

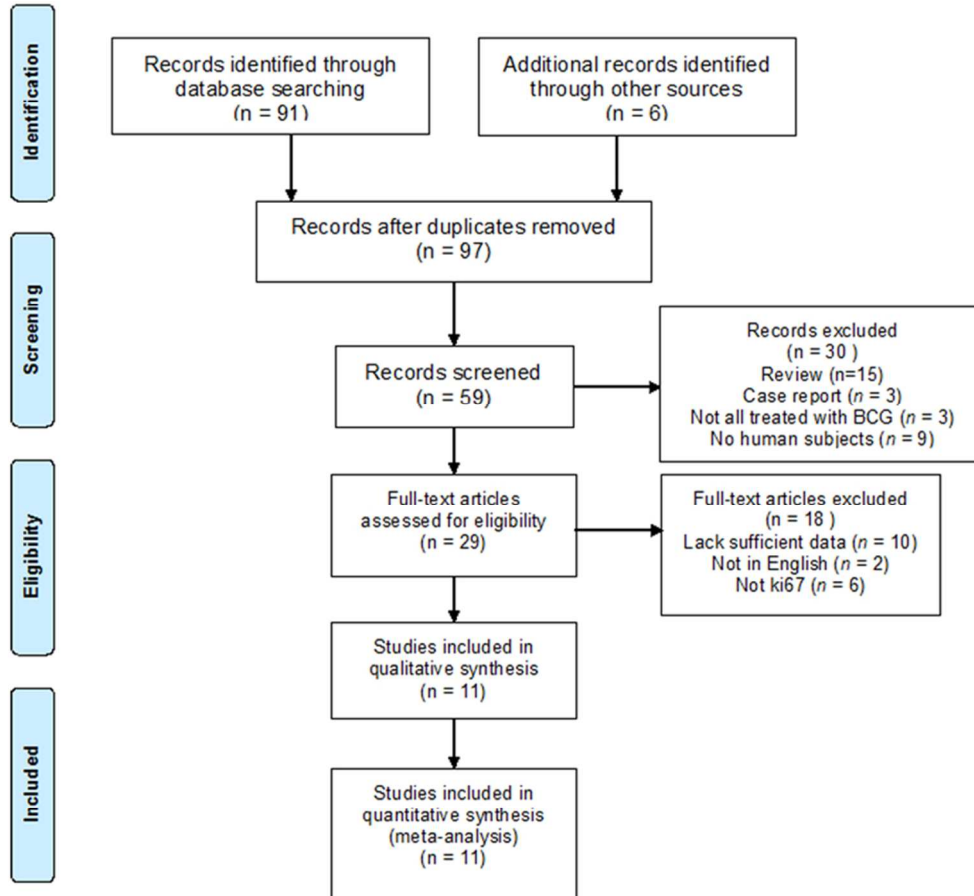
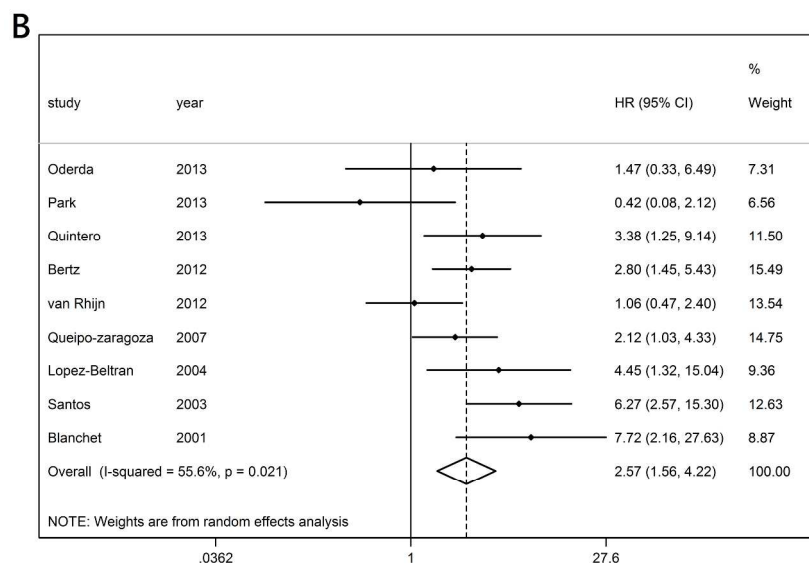
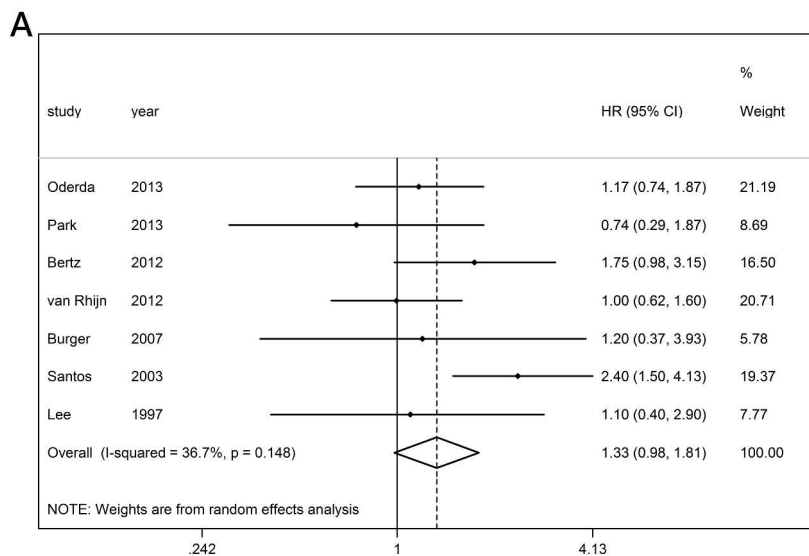


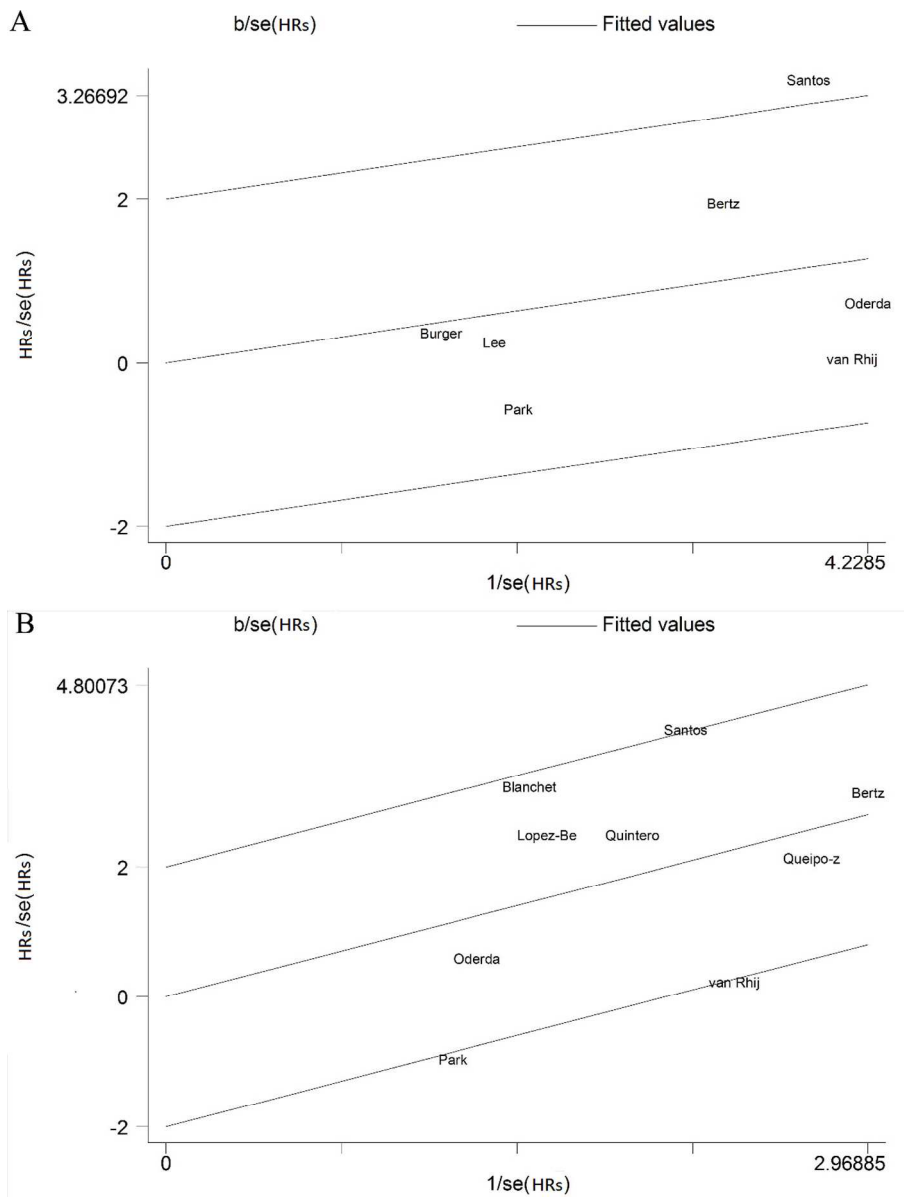
Figure 1. Flow diagram of study selection.

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Forest plots of HRs estimated for the relationship between the expression of ki67 and RFS (A) or PFS (B) among patients in NMIBC treated with BCG.

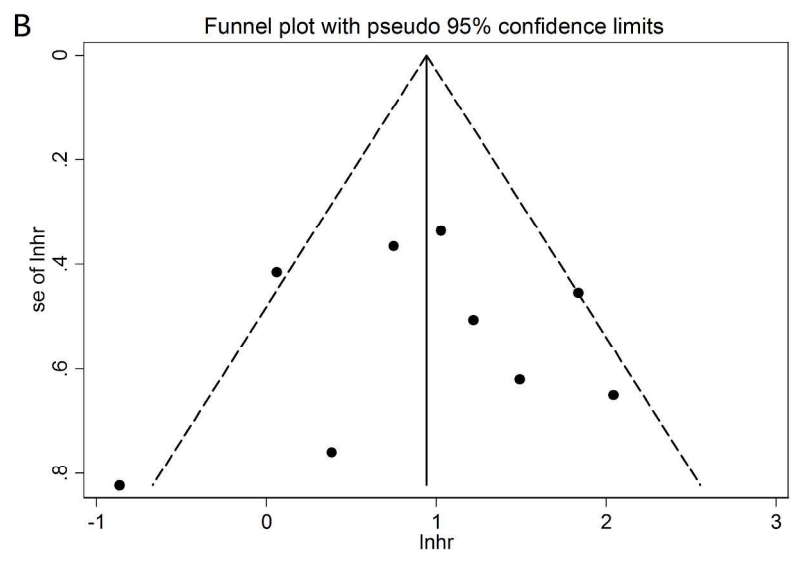
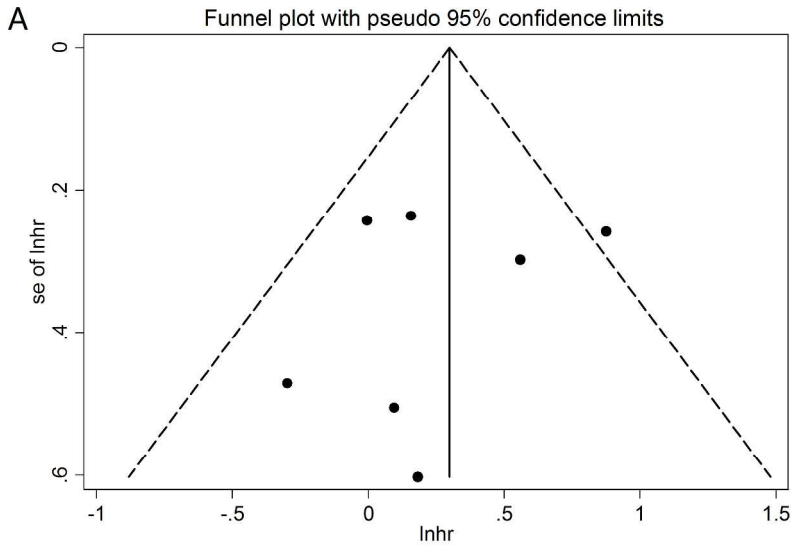
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Galbraith plot analysis was used to evaluate heterogeneity and RFS (A) or PFS (B).

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Funnel plots of the expression of ki67 and RFS (A) or PFS (B).

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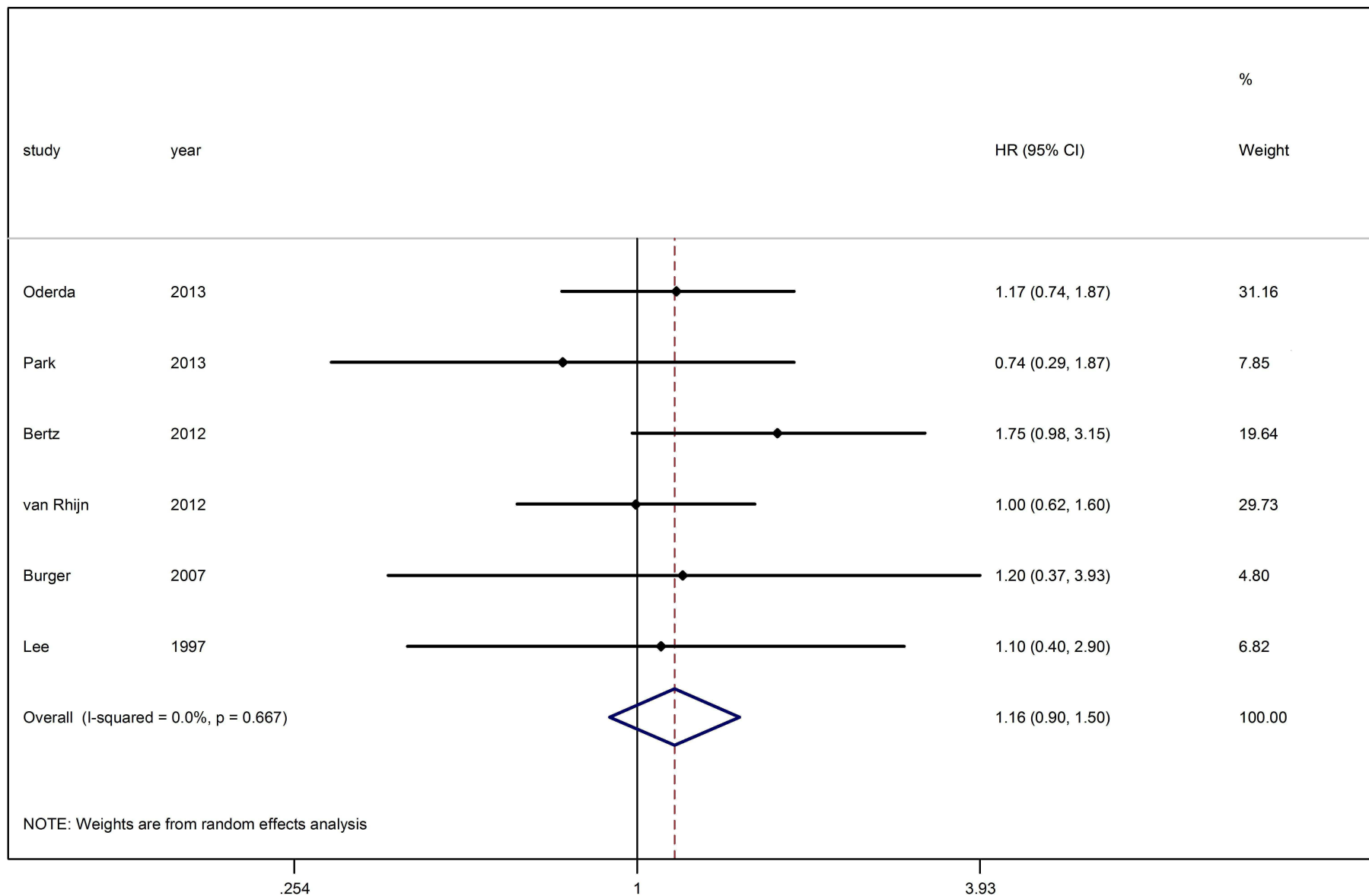


Figure S1. Forest plots of HRs estimated for the relationship between the expression of ki67 and RFS after the aforementioned study was excluded

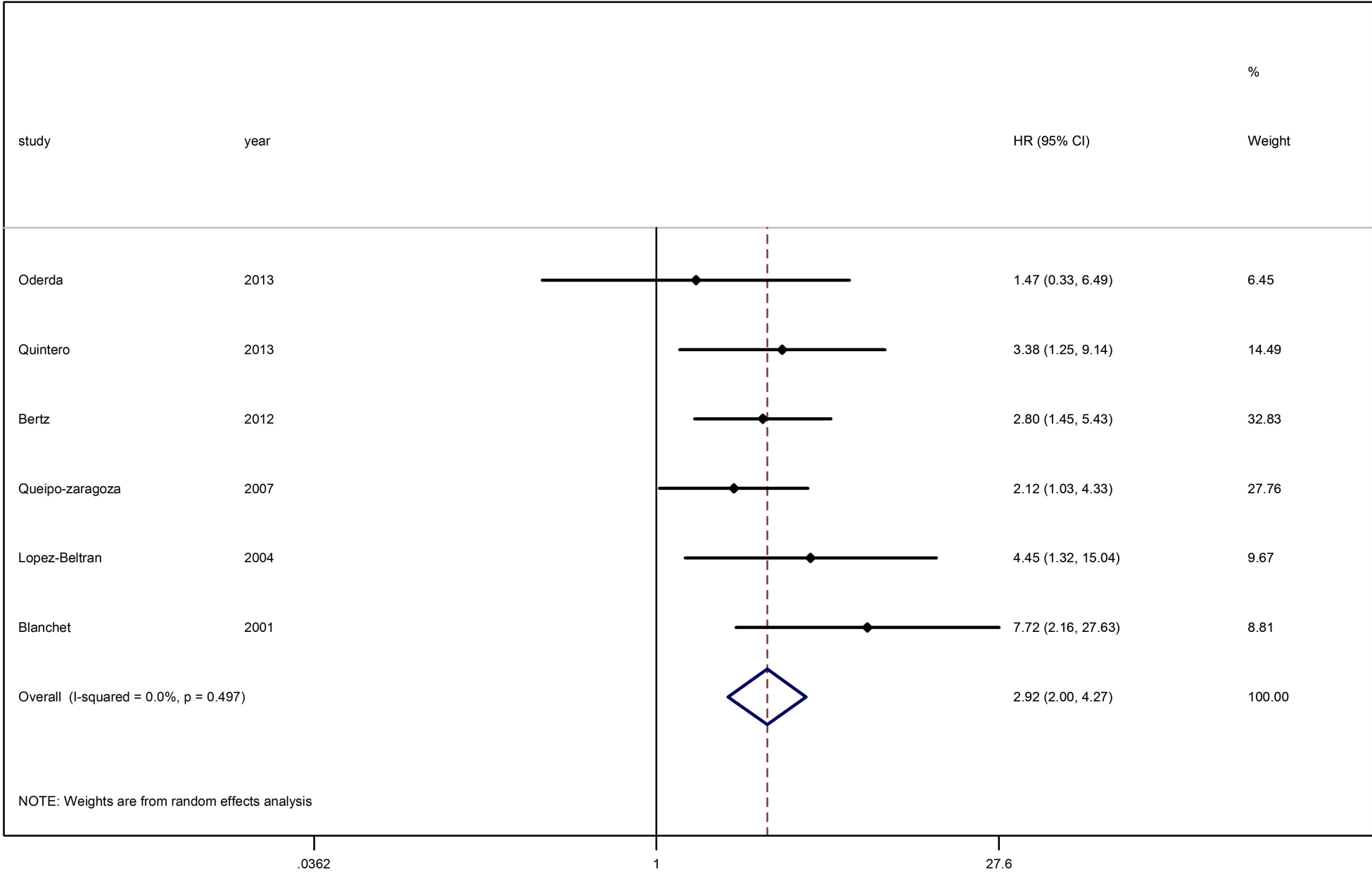


Figure S2. Forest plots of HRs estimated for the relationship between the expression of ki67 and PFS after the aforementioned study was excluded



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7-8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7-8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8-9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8-9



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9-10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10-12
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	17
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	13-16
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-17
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	17
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	17
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	17-22
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	23

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Table S2. Meta-regression analysis of RFS and PFS

PFS

Heterogeneity factor	Coefficient	SE	<i>t</i>	<i>P</i>
Years	-0.0343	0.0283	-1.21	0.280
Country				
1	-0.3899	0.3716	-1.05	0.404
2	-0.7185	0.3503	-2.05	0.177
3	-0.9900	0.4307	-2.30	0.148
4	-0.8805	0.3541	-2.49	0.131
Numbers of patients	0.0022	0.0020	1.16	0.299
Stage	None			
Cutoff	-0.0098	0.0407	-0.24	0.819
Age	0.0021	0.0395	0.05	0.959
Follow-up	-0.0062	0.0065	-0.96	0.379

PFS

Heterogeneity factor	Coefficient	SE	<i>t</i>	<i>P</i>
Years	-0.1195	0.0461	-2.59	0.036
Country				

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2					
3					
4	1	0.2080	0.7936	0.26	0.818
5					
6	2	-0.8062	0.5662	-1.42	0.290
7					
8					
9	3	-1.4505	0.8858	-1.64	0.243
10					
11	4	-2.7009	0.9407	-2.87	0.103
12					
13					
14	5	-1.7766	0.6167	-2.88	0.102
15					
16	6	-0.8158	0.5281	-1.54	0.262
17					
18					
19	Numbers	of	0.0006	0.0036	0.16
20					
21	patients				
22					
23					
24					
25	Stage				
26					
27	1	-1.4505	1.5909	-0.91	0.458
28					
29	2	-0.8062	1.4108	-0.57	0.625
30					
31	3	0.2080	1.5332	0.14	0.904
32					
33	4	-1.7766	1.4353	-1.24	0.341
34					
35	5	-1.2560	1.2069	-1.04	0.407
36					
37	6	-0.6170	1.4689	-0.42	0.715
38					
39					
40					
41					
42	Cutoff	-0.0177	0.0309	-0.57	0.585
43					
44	Age	-0.0672	0.0757	-0.89	0.404
45					
46	Follow-up	-0.0118	0.0159	-0.74	0.483
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Table S3. Risk group stratification in NMIBC

Low-risk tumors	Primary, solitary, Ta, LG/G1, <3 cm, no CIS
Intermediate-risk tumors	All tumors not defined in the two adjacent categories (between the category of low and high risk)
High-risk tumors	Any of the following: T1 tumor HG/G3 tumor CIS Multiple, recurrent, and large (>3 cm) Ta G1G2 tumors (all conditions must be present at this point)

NMIBC, non-muscle-invasive bladder cancer; CIS, carcinoma in situ; HG, high grade; LG, low grade.



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7-8
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Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-17
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	17
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Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	23

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BMJ Open

Prognostic Value of ki67 in Bacillus Calmette–Guérin-Treated Non-muscle-Invasive Bladder Cancer: a Meta-analysis and Systematic Review

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Manuscript ID	bmjopen-2017-019635.R2
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Date Submitted by the Author:	14-Feb-2018
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Primary Subject Heading:	Urology
Secondary Subject Heading:	Urology
Keywords:	ki67, meta-analysis, non-muscular-invasive bladder cancer, prognosis

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3 **Prognostic Value of ki67 in Bacillus Calmette–Guérin-Treated**
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6 **Non-muscle-Invasive Bladder Cancer: a Meta-analysis and**
7
8 **Systematic Review**
9

10
11 Yuhui He^{1,2}, Ning Wang³, Xiaofeng Zhou^{1,2*}, Jianfeng Wang², Zhenshan Ding², Xing
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For peer review only

Abstract

Objectives: The aim of this study was to explore the prognostic value of ki67 as a marker in non-muscle-invasive bladder cancer (NMIBC) patients treated with Bacillus Calmette–Guérin (BCG).

Methods: Studies were systematically retrieved from the relevant databases (Web of Science, PubMed, Cochrane Library, and Embase), and the expiry date was May 2017. The research steps referred to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement.

Results: A total of 11 studies that complied with the inclusion criteria were enrolled. The expression of ki67 was not statistically significantly associated with recurrence-free survival (RFS) [hazard ratio (HR): 1.331; 95% CI: 0.980–1.809]. No significant heterogeneity was found among all included studies ($I^2 = 36.7%$, $P = 0.148$). The expression of ki67 was statistically significantly associated with progression-free survival (PFS) (HR: 2.567; 95% CI: 1.562–4.219), and the overexpression of ki67 was the risk factor for PFS. Significant heterogeneity was noted among all the included studies ($I^2 = 55.6%$, $P = 0.021$). The studies that might cause heterogeneity were excluded using the Galbraith plot, and then the meta-analysis was performed again. The results showed that the expression of ki67 was still associated with PFS (HR: 2.922; 95% CI: 2.002–4.266).

Conclusions: The overexpression of ki67 was the risk factor for PFS, and the relationship between the expression of ki67 and RFS was not statistically significant in patients with NMIBC treated with BCG intravesical immunotherapy. Well-designed, prospective, randomized controlled trials with a large sample size are still needed to validate the findings.

Key words: ki67; meta-analysis; non-muscular-invasive bladder cancer; prognosis

Strengths and limitations of this study

This meta-analysis and systematic review was performed via a strict literature search.

It was the first meta-analysis to evaluate the prognostic value of ki67 in patients with NMIBC after transurethral resection and BCG intravesical immunotherapy.

The number of studies considered in the final meta-analysis was 11. This small sample size limited the potential analyses. The research did not consider the surgical skills mentioned in published studies.

Despite a systematic search strategy, the inclusion criteria excluded non-English documents and had language bias. The meta-regression analysis suggested no bias, but a selection bias was likely.

These limitations notwithstanding, the research can guide the follow-up research on immunohistochemical markers and clinical practice in non-muscular-invasive bladder cancer.

Introduction

Bladder cancer is one of the most common clinical urological tumors. It is a direct threat to the survival of patients with the disease. The incidence of bladder cancer varies across the world, with the highest rate in the developed communities.¹ A total of 429,800 new cases of bladder cancer and 165,100 deaths occurred in 2012 worldwide. Bladder cancer occurs mostly in men, and about a tenfold variation in incidence rates has been reported internationally.² About 70% of these patients have non-muscle-invasive bladder cancer (NMIBC).³ Four major organizational guidelines on NMIBC, including the American Urological Association/Society of Urologic Oncology, the European Association of Urology, the National Comprehensive Cancer

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3 Network, and the National Institute for Health and Care Excellence guidelines,
4 recommend that the proper initial transurethral resection (TUR) of bladder tumor is a
5 critical step in the initial management and staging of the disease.⁴ However, TUR
6 surgery alone cannot solve the postoperative problems for NMIBC because of high
7 recurrence rate and disease development.⁵ Postoperative TUR associated with
8 Bacillus Calmette–Guérin (BCG) intravesical immunotherapy can prevent the
9 postoperative recurrence of NMIBC and significantly reduce the moderate and high
10 development risk of NMIBC.^{6, 7} However, the postoperative BCG intravesical
11 immunotherapy still has some problems. The failure rate of BCG intravesical therapy
12 in NMIBC is about 40%–50%.⁸ Furthermore, BCG has toxic side effects, such as
13 hepatitis, pneumonitis, epididymitis/orchitis, abscess formation, bladder contracture,
14 ureteral obstruction, BCG sepsis, leukopenia, and hematuria.⁹ Therefore, BCG
15 therapy should be individually performed, and the patients in whom BCG therapy is
16 ineffective should be timely recognized. These patients or those with poor prognosis
17 should receive radical cystectomy or any other therapy in time to avoid futile
18 treatment and alleviate pain. However, the recognition of patients with no effect of
19 TUR postoperative BCG intravesical immunotherapy is still hard due to the
20 heterogeneity of bladder cancer and individuality of patients.¹⁰ Therefore, finding the
21 prognostic factors for patients with NMIBC receiving TUR and BCG therapy is
22 extremely necessary.

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46 The recurrence rate of bladder cancer treated with different therapies is between 50%
47 and 80%, and about 15% of the low-grade tumor recurrence involves high-grade
48 tumors.¹¹ The patients need periodical cystoscopy to find the recurrent focus in time.
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52 A reliable prognostic molecular marker can reduce the pain caused by cystoscopy.
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55 Because NMIBC does not have reliable prognostic markers, it is hard to decide

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3 postoperative therapy in the clinic,¹² which depends mainly on the clinical guidelines
4 and physician's experience. Currently, some of the published studies about
5 immunohistochemical markers have evaluated the prognostic value of BCG
6 intravesical immunotherapy on the patients first receiving TUR. The main
7 immunohistochemical markers include ki67, p53, p27, pRb, CD9, CD20, E2F1, and
8 so forth.^{13, 14} However, no immunohistochemical marker has been confirmed so far.
9
10 The prognostic value of ki67 antigen on the survival in patients with NMIBC
11 receiving BCG intravesical immunotherapy has been controversial. For example,
12 Kruger¹⁵ reported that ki67 antigen was an independent predictive factor for the
13 recurrence of pT1 stage tumor, but Oderde¹⁶ believed that ki67 was an independent
14 predictive factor for the recurrence of all NMIBCs. Zlotta¹⁷ reported that ki67 antigen
15 had no independent prognostic value in patients receiving BCG therapy. Saint¹⁸
16 retrospectively reviewed the recent 25-year published studies and believed that the independent
17 prognostic factor for bladder cancer in patients receiving BCG therapy was not clear.
18
19 An international consensus group listed various bladder cancer prognostic indexes by
20 reviewing PubMed and considered that although some markers (such as ki67 and p53)
21 could predict the recurrence and development of bladder cancer, the data still had
22 heterogeneity. Thus, strict test criteria and clear statistical methods should be
23 established for further evaluation.¹⁹

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44 A meta-analysis can enlarge the sample size by integrating independent studies with
45 small sample size, further increase the statistical efficacy, and reduce the wrong
46 conclusion caused by the small sample size.²⁰ The aim of this study was to explore the
47 prognostic value of ki67 as a marker in patients with NMIBC treated with BCG.
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49 Based on the literature search, this study was the first meta-analysis to evaluate the
50 prognostic value of ki67 in patients with NMIBC treated with BCG.
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Methods

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (**Table S1**).²¹ The present meta-analysis did not need the approval because all the enrolled published studies were approved by the ethics committee in their research institute.

Literature retrieval strategy

The comprehensive literature search was performed on Web of Science, PubMed, Cochrane Library, and Embase databases for relevant studies. The last quest was updated on May 24, 2017, with hand-searching to identify any potentially eligible studies that might have been missed. The following search strategy was adopted for each database: ("Urinary Bladder Neoplasms"[Mesh] OR "bladder cancer" OR "bladder carcinoma" OR " bladder tumor ") AND ("BCG Vaccine"[Mesh] OR "BCG" OR "Bacillus Calmette–Guérin") AND ("ki67 antigen "[Mesh] OR " ki-67" OR " ki67" OR " MBI-1"). Filters were as follows: retrospective, array research, clinical trial, controlled clinical trial, and randomized controlled trial. Free word retrieval strategy was used. The enrolled contents included the reference lists and relevant suggestive references while searching (File S1).

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) prospective or retrospective published studies evaluating the prognostic relationship between the expression of ki67 and NMIBC treated with BCG; (2) the expression of ki67 in tissues detected by immunohistochemistry analysis; (3) hazard ratio (HR) and 95% confidence interval (95% CI) directly obtained from the published studies; and (4) published English studies. The exclusion criteria were as follows: (1) review, systematic evaluation, case

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3 report, editorial, and specialist experience; (2) studies with no human subjects; and (3)
4 published studies in which data could not be extracted or those having wrong data.
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6 7 ***Data extraction and evaluation of literature quality***

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9 Based on the aforementioned criteria, two reviewers independently screened the
10 published studies by reading titles and abstracts and got preliminary conclusions. If
11 the conclusions were not consistent, the literature was discussed by all the authors to
12 decide its enrollment. The relevant information of the enrolled published studies was
13 extracted, such as first author, publication time, research country, sex, case number,
14 age, follow-up date, disease stage, cutoff values, recurrence-free survival (RFS), and
15 progression-free survival (PFS). The Newcastle–Ottawa Scale (NOS) was used to
16 evaluate the quality of all the published studies.²² Scores 0–3, 4–5, and 6–8 were
17 accepted as low, medium, and high quality, respectively.
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28 29 ***Statistical methods***

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31 The measuring time and method of ki67 complied with the standard of clinical routine
32 and pathological examination. Tumor tissue samples were taken in accordance with
33 the standard surgical procedure and used for immunohistochemical analysis. RFS and
34 PFS were the traditionally used statistical parameters. PFS was defined as the time
35 from the beginning of treatment to the first progression. RFS was defined as the time
36 from the removal of the lesion (or the randomization of the clinical trial) until the
37 recurrence or death of the tumor. The impact of the expression of ki67 on survival was
38 quantified using the combined HRs and 95% CIs. The HR and 95% CI of each study
39 were directly extracted from each original published study. Besides, the Parmar and
40 Tierney's²³ method was used to extract the data because some of the published studies
41 did not directly provide HR and 95% CI. For example, some studies provided only the
42 survival curve. In this meta-analysis, the DerSimonian–Laird random-effects model²⁴
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3 was used because when the heterogeneity was large, only the random-effects model
4 could be suitably used. Similar to traditional methods, HR >1 was considered as the
5 prognostic risk factor for the overexpression of ki67, and HR <1 was a protective
6 factor. 95% CI <1 indicated a statistically significant difference in the relationship
7 between the overexpression of ki67 and prognosis.
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11 The heterogeneity was calculated according to chi-square-based Q test and I^2
12 statistic.²⁵ The heterogeneity was judged using the I^2 value (low heterogeneity: I^2
13 <25%; moderate heterogeneity: $I^2 = 25\%–50\%$; large heterogeneity: $I^2 >50\%$).
14 Besides, A P value >0.05 was considered as low heterogeneity. Then, the subgroup
15 analysis based on regions, sample size, follow-up period, tumor grading, cutoff value,
16 publication time, and patient age was performed. A value of 1% was considered to be
17 a statistically significant level in the subgroup analysis. A Galbraith plot was used to
18 search published studies with heterogeneity²⁶, and the meta-analysis was performed
19 again after excluding these published studies. Meanwhile, the factors causing
20 heterogeneity were also explored using the residual maximum likelihood
21 (REML)-based random-effects meta-regression analysis.²⁷ All the statistical analyses
22 were performed using the Stata12.0 software (StataCorp, TX, USA), and the
23 two-sided test was used to evaluate the P value.
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41 ***Evaluation of publication bias***

42 Begg's plot and Egger's test method were used to find the possible publication bias. A
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46 P value <0.05 was considered to indicate publication bias.
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50 ***Results***

51 ***Literature screening***

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54 A total of 97 published studies were retrieved. Furthermore, 68 of them were excluded
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3 after duplicates were removed and records screened, and 18 were excluded after
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5 reading the full text (10 published studies from which HR and 95% CI could not be
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7 obtained, 2 non-English studies, and 6 that did not use ki67 detection). Finally, 11
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9 published studies were enrolled in the meta-analysis (**Figure 1**).

11 ***Basic characteristics and quality evaluation of enrolled published studies***

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13 The enrolled 11 published studies were published between 1997 and 2013, and the
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15 countries included Italy, South Korea, Spain, Germany, New Zealand, Canada,
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17 Portugal, and France. The largest sample size was 309, and the smallest one was 32. A
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19 total of 1321 patients were enrolled in this study. The follow-up period was more than
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21 36 months, and the longest was 229 months. T1 was the main tumor grading, and the
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23 cutoff value ranged from 10.4% to 40%. Seven published studies reported patients'
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25 RFS, and nine reported PFS (**Table 1**). One literature was scored as 6 star by NOS,
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27 seven as 7 star, and three as 8 star. The median of the NOS score was 7 (**Table 2**).

Table 1. Main characteristics of all studies included in this meta-analysis

Study	Year	Country	Male/ Female	No. of patients	Age (year)	Follow-up (month)	Stage	Cutoff	Survival analysis
Oderda ¹⁶	2013	Italy	166/26	192	73.2 (SD 11.9)	100 (2–229)	All NMIBC	20%	RFS
Park ¹⁴	2013	Korea	53/8	61	66 (31–85)	60 (6–217)	T1G3	10.4%	RFS/PFS
Quintero ²⁸	2013	Spain	143/21	164	61 (29–93)	75 (60–144)	Ta	13%	PFS
Bertz ¹²	2012	Germany	237/72	309	71.7 (38–87)	49 (5–172)	pT1	15%	RFS/PFS
van Rhijn ²⁹	2012	Netherlands, Canada	105/24	129	68.8 (SD 9.9)	78 (39.6–110.4)	T1	25%	RFS/PFS
Burger ³⁰	2007	Germany	45/21	71	71 (52–94)	39 (1–133)	T1/Ta	15%	RFS
Queipo-zaragoza ³¹	2007	Spain	71/12	83	68.1 (SD 8.5)	All >36	T1G3	40%	PFS
Lopez-Beltran ³²	2004	Spain	49/2	51	69.96 (49–89)	63.82 (60–144)	T1G3	13%	PFS
Santos ³³	2003	Portugal	115/44	159	66 (21–88)	46.5 (4–123)	pTa/pT1	18%	RFS/PFS
Blanchet ³⁴	2001	France	-	70	62.6 (21–84)	64 (12–111)	pT1/pTa	13%	PFS
Lee ³⁵	1997	Korea	28/4	32	57.1 (30–81)	All >24	T1G2-3	20%	RFS

NMIBC, Non-muscle-invasive bladder cancer; no., number; PFS, progression-free survival; RFS, recurrence-free survival.

Table 2. Quality of the included studies assessed by NOS

Study	Selection		Comparability		Exposure		Non-response rate	Scores	
	Adequate definition of cases	Representativeness of cases	Selection of controls	Definition of controls	Control for important factor	Ascertainment of exposure			Same method to ascertain for cases and controls
Oderda ¹⁶	—	☆	☆	☆	☆☆	☆	☆	☆	8
Park ¹⁴	—	☆	☆	—	☆☆	☆	☆	☆	7
Quintero ²⁸	—	☆	☆	—	☆☆	☆	☆	—	6
Bertz ¹²	—	☆	☆	—	☆☆	☆	☆	☆	7
van Rhijn ²⁹	—	☆	☆	—	☆☆	☆	☆	☆	7
Burger ³⁰	☆	☆	☆	—	☆☆	☆	☆	—	7
Queipo-zaragoza ³¹	—	☆	☆	—	☆☆	☆	☆	☆	7
Lopez-Beltran ³²	—	☆	☆	☆	☆☆	☆	☆	☆	8
Santos ³³	—	☆	☆	—	☆☆	☆	☆	☆	7
Blanchet ³⁴	—	☆	☆	☆	☆☆	☆	☆	☆	8
Lee ³⁵	—	☆	☆	—	☆☆	☆	☆	☆	7

Influence of the expression of ki67 on RFS

Seven published studies reported the expression of ki67 and PFS results of patients with NMIBC treated with BCG. The meta-analysis indicated that ki67 had no statistically significant association with RFS (HR: 1.331; 95% CI: 0.980–1.809), and no heterogeneity among the enrolled studies was reported ($I^2 = 36.7%$, $P = 0.148$) (**Figure 2A**). The subgroup analysis was performed based on the regions, sample size, follow-up period, stage, cutoff value, publication time, and age. Meanwhile, all the original published analyses on the association between the expression of ki67 and RFS in patients with NMIBC treated with BCG were multivariate, and the HRs were adjusted. The stratification analysis by region indicated that ki67 was also significantly associated with RFS in Caucasians and a follow-up period shorter than 6 months. (HR: 1.441, 95% CI: 1.014–2.047; HR: 1.853, 95% CI: 1.316–2.607) (**Table 3**).

Table 3. Subgroup results of RFS and heterogeneity test

Variables	Analysis number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total RFS	7	1.331 (0.980–1.809)	9.48	0.148	36.7
Region					
Asian	2	0.892 (0.454–1.752)	0.32	0.570	0.0
Caucasian	5	1.441 (1.014–2.047)	7.52	0.111	46.8
Sample size					
>100	4	1.466 (0.986–2.181)	7.44	0.059	59.7
≤100	3	0.959 (0.534–1.725)	0.51	0.777	0.0
Follow-up (month)					
≥60	3	1.036 (0.758–1.415)	0.79	0.674	0.0
<60	4	1.853 (1.316–2.607)	2.62	0.453	0.0
Stage					
All NMIBC	3	1.575 (0.915–2.711)	4.44	0.109	54.9
Others	4	1.153 (0.821–1.620)	3.21	0.360	6.6
Cutoff					
15%	2	1.625 (0.963–2.743)	0.31	0.575	0.0
Others	5	1.252 (0.839–1.869)	8.56	0.073	53.3
Publication year					
≥2012	4	1.164 (0.874,1.550)	3.20	0.362	6.3
<2012	3	1.774 (1.046,3.008)	2.57	0.277	22.1
Patient age (year)					
≥70	3	1.352 (0.955–1.913)	1.16	0.559	0.0
<70	4	1.256 (0.717–2198)	8.32	0.040	63.9

NMIBC, Non-muscle-invasive bladder cancer; RFS, recurrence-free survival.

Influence of the expression of ki67 on PFS

A total of nine published studies reported the expression of ki67 and PFS results of patients in NMIBC treated with BCG. The meta-analysis indicated that ki67 had no statistically significant association with RFS (HR:2.567, 95% CI: 1.562–4.219), and the overexpression of ki67 was the risk factor for PFS. Statistically significant heterogeneity was found among all the included studies ($I^2 = 55.6\%$, $P = 0.021$) (**Figure 2B**). The subgroup analysis was performed based on the regions, sample size, follow-up period, stage, cutoff value, publication time, and age. However, the data extracted from six original published analyses on the association between the expression of ki67 and PFS in patients with NMIBC treated with BCG were multivariate with adjusted HRs, whereas the data from three original published analyses were univariate with unadjusted HRs. In the stratified analyses by the region, sample size, follow-up time, stage, cutoff, publication year, and patient age, significant associations were observed in the studies with Caucasian subgroup, sample size >100, follow-up period <6 months, other cutoffs, and two subgroups based on age (HR: 1.97, 95% CI: 1.04–3.74; HR: 2.37, 95% CI: 1.23–4.55; HR:2.49, 95% CI: 1.19–5.21; HR: 2.515, 95% CI: 1.382–4.576; HR: 2.800, 95% CI: 1.447–5.418; and HR: 2.654, 95% CI: 1.381–5.100, respectively). However, significant associations were also observed in both multivariate and univariate analyses (HR: 2.10, 95% CI: 1.07–1.12; HR: 2.80, 95%CI: 1.65–7.85, respectively), and the effect size suggested the same outcomes (HR: 2.567, 95% CI: 1.562–4.219) (**Table 4**).

Table 4. Subgroup results of PFS and heterogeneity test

Variables	Analysis number	HR (95% CI)	Heterogeneity test		
			<i>Q</i>	<i>P</i>	<i>I</i> ² (%)
Total PFS	9	2.567 (1.562–4.219)	18.1	0.021	55.6
Region					
Asian	1	0.421 (0.084–2.114)	0.00		
Caucasian	8	2.883 (1.830–4.544)	12.99	0.072	46.1
Sample size					
>100	5	2.559 (1.372–4.774)	9.26	0.055	56.8
≤100	4	2.536 (0.943–6.818)	8.75	0.033	65.7
Follow-up (month)					
≥60	6	2.153 (0.984–4.710)	13.08	0.023	61.8
<60	3	3.158 (1.774–5.623)	3.56	0.169	43.8
Stage					
All NMIBC	3	4.673 (1.938–11.264)	3.29	0.193	39.2
Others	6	2.044 (1.213–15.040)	9.58	0.088	47.8
Cut off					
15%	1	2.800 (1.447–5.418)	0.00		
Others	8	2.515 (1.382–4.576)	17.92	0.012	60.9
Publication year					
≥2012	5	1.685 (0.883,3.215)	8.04	0.090	50.2
<2012	4	4.176 (2.209,7.884)	5.00	0.172	40.0
Patient age (year)					
≥70	2	2.519 (1.377–4.606)	0.60	0.438	0.0
<70	7	2.654 (1.381–5.100)	17.40	0.008	65.5
Multivariate/Univariate					
Multivariate	6	2.101 (1.070–1.121)	13.83	0.031	63.8
Univariate	3	2.803 (1.652–7.856)	3.38	0.001	40.8

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NMIBC, Non-muscle-invasive bladder cancer; PFS, progression-free survival.

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Galbraith plot

Using Galbraith plot (**Figure 3A**), it was found that the study by Santos³³ was the main reason for the heterogeneity of RFS. After the aforementioned study was excluded, the remaining RFS studies had no significant heterogeneity according to the new meta-analysis ($I^2 = 0.0\%$, $P = 0.667$). However, the expression of ki67 still had no statistically significant association with RFS (HR: 1.161, 95% CI: 0.896–1.504) (**Figure S1**). Using the Galbraith plot (**Figure 3B**), it was found that the study by Santos,³³ Park,¹⁴ and van Rhijn²⁹ were the main reason for the heterogeneity of PFS. After these studies were excluded, the remaining RFS studies had no significant heterogeneity according to the new meta-analysis ($I^2 = 0.0\%$, $P = 0.497$). The expression of ki67 still had a statistically significant association with PFS (HR: 2.922, 95% CI: 2.002–4.266) (**Figure S2**).

Meta-regression analysis

The meta-regression analysis indicated that the factors influencing heterogeneity (publication time, research region, sample size, stage, cutoff value, age, and follow-up period) might not be the reason for RFS heterogeneity. Publication time was the reason for heterogeneity of PFS ($P = 0.036$), but other factors were not (**Table S2**).

Publication bias

The funnel plot (**Figure 4A**) was basically symmetrical for RFS. The results of Begg's test and Egger's test showed P (Begg's) = 0.761, P (Egger's) = 0.601. The funnel plot (**Figure 4B**) was also basically symmetrical for PFS. The results of Begg's test and Egger's test showed P (Begg's) = 0.917, P (Egger's) = 0.964.

Discussion

A total of 11 published studies with 1321 cases complying with the inclusion criteria

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3 were enrolled in this meta-analysis. The results of the meta-analysis indicated that the
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5 expression of ki67 had no statistically significant association with RFS, but it was
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7 significantly associated with PFS. The overexpression of ki67 was the risk factor for
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9 PFS. It suggested that ki67 was the prognostic predictive marker in patients with
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11 NMIBC treated with BCG. Besides, the aforementioned conditions did not change
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13 after excluding the published studies, possibly leading to heterogeneity and
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15 reperforming of the meta-analysis. It further proved that the result of the
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17 aforementioned meta-analysis was stable, that is, the overexpression of ki67 was the
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19 risk factor for PFS. In the Caucasian subgroup for PFS, racial classification and
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21 regional factors might be crucial in the prognosis of patients with NMIBC after BCG
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23 therapy. This might be related to the existence of different drug gene susceptibilities in
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25 people belonging to different races and living areas. The two subgroups were based on
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27 age in PFS, suggesting that age might be the important factor influencing the
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29 prognosis of bladder cancer. This also complies with our clinical practice. The elder
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31 the patient, the worse the prognosis. Several factors led to heterogeneity in the
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33 aforementioned subgroup analysis: (1) Due to the influence of race and environment,
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35 the documents included in this study came from different regions and countries. A
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37 large number of studies confirmed the differences in disease susceptibility between
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39 ethnic groups and regions. (2) Differences existed in the operation of health care
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41 workers in TUR and BGC intravesical immunotherapy because of different regions
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43 and different clinicians, such as surgical clearance of the tumor. The tumor with a
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45 broad base surface is often not easy to remove completely, which also depends on the
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47 surgeon's experience and surgical skills. In addition, the quality of BCG
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49 manufacturers may vary from region to region. (3) Different literature might include
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51 the bias of research object, research design, measuring instrument, and so on.
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3 However, in general, heterogeneity did not affect the conclusion. Besides, the
4 meta-regression analysis indicated publication time as the reason for PFS
5 heterogeneity. It might be related to the improvement in testing technology, research
6 level, and the quality and number of published studies, facilitating follow-up studies.
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8 As all the original data extracted from published studies on the association between
9 the expression of ki67 and RFS in patients with NMIBC treated with BCG were
10 multivariate, the result was considered to be precise because the HRs were adjusted,
11 excluding the confounding factors such as age and gender. However, the original data
12 extracted from published analyses on the association between the expression of ki67
13 and PFS were both multivariate and univariate. It was believed that the
14 aforementioned adjustments did not have a significant impact on meta-analyses.
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16 Besides, according to the funnel plot, Begg's test and Egger's test, the enrolled studies
17 had no statistically significant publication bias. Thus, the reliability of the present
18 meta-analysis was high.
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33 In 2016, the European Association of Urology (EUA)³⁶ recommended a scoring
34 system for the prognostic evaluation of NMIBC based on six clinical and pathological
35 factors proposed by the European Organization for the Research and Treatment of
36 Cancer-Genito-Urinary Cancer Group (EORTC), including number of tumors, tumor
37 size, prior recurrence rate, T category, presence of concurrent carcinoma in situ, and
38 tumor grade (**Table S3**). The tumors were categorized into low-risk tumors,
39 intermediate-risk tumors, and high-risk tumors using this assessment system to
40 evaluate the prognosis. For the patients after BCG therapy, the EUA recommended
41 another risk calculator developed by the Club Urologico Espanol de Tratamiento
42 Oncologico (CUETO) and the EORTC. This calculator was based on gender, age,
43 recurrent tumor, number of tumors, T category, associated Tis, and grade. The
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3 CUETO risk calculator can be achieved at <http://www.aeu.es/Cueto.html>. The
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5 recommended level was B grade for the two scales for patients with NMIBC, whether
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7 used alone or combined. The two scales could be used together in the clinic. When
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9 using the CUETO scale, the calculated recurrent risk was lower than that from the
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11 EORTC scale,³⁷ which might be related to the special design in the CUETO scale for
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13 the patients receiving BCG intravesical immunotherapy. However, the scoring system
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15 only depending on clinical and pathological factors could not accurately evaluate the
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17 prognosis of patients with bladder cancer in T1 stage due to the independence of
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19 disease condition in each patient.³⁸ The markers regulated at the genetic level may
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21 judge the prognosis of patients with bladder cancer with the development of precision
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23 medicine. A reliable marker helps in recognizing the patients who have failed in BCG
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25 intravesical immunotherapy with high risk in time. Hence, these patients can undergo
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27 radical cystectomy or other treatments in time. Unfortunately, no prognostic marker
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29 has been applied in clinic currently. The results of this study potentially help to
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31 remind clinicians that patients with high expression of ki67 may need to develop more
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33 personalized follow-up plans, such as shorter follow-up and cystoscopy cycles.
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35 Patients with high risk of clinical evaluation of the guidelines and overexpression of
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37 ki67 may need to promptly change the treatment strategy.

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41 Ki67 is a nucleoprotein that can be detected in the cell cycles except G0 phase.³⁹ The
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43 expression of human ki67 protein is closely related to proliferation. Therefore, it is an
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45 ideal marker to confirm the growth fraction of specific cell colonies.⁴⁰ Ki67 is a
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47 widely known amplified biomarker. The ki67 monoclonal antibody can be detected by
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49 the immunohistochemical method.⁴¹ Ki67 has been proved to be a good proliferation
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51 marker in different cancers, including bladder cancer.⁴²

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54 So far, some meta-analyses have studied the effect of ki67 on the prognostic quality of

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3 life of patients with esophageal cancer, breast cancer, epithelial ovarian cancer, and so
4 on.⁴³⁻⁴⁵ Some studies have also focused on the other aspects of bladder cancer. Using
5 meta-analysis, Luo⁴⁶ believed that a high reactivity of ki67 could predict the poor
6 prognosis in patients with bladder cancer. The univariate analysis showed that
7 cancer-specific survival, disease-free survival, overall survival , PFS, and RFS had a
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9 significant association with poor prognosis in patients with a high reactivity of ki67.
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11 However, this study enrolled all types of bladder tumors and all the therapies for
12 NMIBC. Currently, the bladder cancer treated in the clinic is mainly NMIBC. Thus,
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14 most of the applied therapy is TUR combined with installations of chemotherapy or
15 BCG intravesical immunotherapy based on the patients' conditions. Therefore, this
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17 analysis had a certain limitation in the prognosis of patients with NMIBC after BCG
18 intravesical immunotherapy.

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21 Currently, few evidence-based studies focused on the prognosis of patients with
22 NMIBC after BCG intravesical immunotherapy. Using meta-analysis, Zhou⁴⁷
23 analyzed the association between the expression of p53 and quality of life of patients
24 with NMIBC after BCG intravesical immunotherapy. They believed that the
25 overexpression of p53 in patients with NMIBC treated with BCG might be associated
26 with RFS, especially in Asian population. Similarly, Du⁴⁸ also performed the
27 meta-analysis on the relationship between p53 status and NMIBC in T1 stage and
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29 believed that the overexpression of p53 might be related to the development of
30 NMIBC. The present study indicated that the overexpression of ki67 was the risk
31 factor for PFS, but the expression of ki67 had no statistically significant association
32 with RFS. P53 is the most common inactivated tumor suppressor gene in tumor
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34 cells.⁴⁹ The inactivation of p53 may cause cell abnormal hyperplasia and
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3 cancerization. The variation in p53 results in enhanced proliferation, invasion, and
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5 metabolism.⁵⁰ The increase in the expression of ki67 , as cell proliferation marker
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8 suggests enhanced proliferation.⁴⁰ As a tumor suppressor gene with complicated
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10 function, p53 has a wider range of effects. The accuracy in the prediction of quality of
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12 life may not be more appropriate compared with ki67. The genetic difference between
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14 Asians and Caucasians suggests that different prediction systems should be built for
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16 different races. Besides, p27, E2F1, ezrin, and CK20 were also studied in other
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18 investigations for predicting NMIBC prognosis, which could be explored further
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20 comparing the advantages of using them alone or combined.
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23 However, this study still had some limitations. First, the enrolled published studies
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25 involved different populations, used similar detection equipment, and had different
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27 cutoff values. All these reasons might have led to heterogeneity. Further, the sample
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29 size of the meta-analysis also limited its significance. Second, the meta-analysis
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31 included English published studies. Although Begg's test and Egger's test did not
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33 suggest publication bias, this study was still influenced by some bias. Finally, the
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35 surgical skills were different in different published studies, affecting the judgment
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37 regarding the effectiveness of BCG.
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40 **Conclusions**

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42 The overexpression of ki67 was the risk factor for PFS in patients with NMIBC after
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44 TUR and BCG intravesical immunotherapy, but the relationship between the
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46 expression of ki67 and RFS was not statistically significant. Owing to the
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48 aforementioned limitations of the present study, RCTs with large sample size are still
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50 required to validate the results.
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Authors' contributions

YHH, NW, and XFZ conceived and designed the experiments. XC and YSD extracted the data. YHH, NW, and XFZ analyzed the data. ZSD, JFW, and XC contributed reagents/materials/analysis tools. YHH and NW wrote the paper. XFZ critically revised the report.

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Competing interests

None declared.

Data sharing statement

Extra data can be accessed via the Dryad data repository at <http://datadryad.org/> with the <https://doi.org/10.5061/dryad.hf06q72>

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55 **Figure File**

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3 **Figure 1.** Flow diagram of study selection.

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5 **Figure 2.** Forest plots of HRs estimated for the relationship between the expression of
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7 ki67 and RFS (A) or PFS (B) among patients in NMIBC treated with BCG.

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9 **Figure 3.** Galbraith plot analysis was used to evaluate heterogeneity and RFS (A) or
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11 PFS (B).

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13 **Figure 4.** Funnel plots of the expression of ki67 and RFS (A) or PFS (B).

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18 **Table File**

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20 **Table 1.** Main characteristics of all studies included in this meta-analysis.

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22 **Table 2.** Quality of the included studies assessed by NOS.

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24 **Table 3.** Subgroup results of RFS and heterogeneity test.

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26 **Table 4.** Subgroup results of PFS and heterogeneity test.

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31 **Supplementary File**

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33 **File S1.** Electronic search strategy in PubMed.

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35 **Table S1.** PRISMA.

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37 **Table S2.** Meta-regression analysis of RFS and PFS.

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39 **Table S3.** Risk group stratification in NMIBC.

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42 **Figure S1.** Forest plots of HRs estimated for the relationship between the expression
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44 of ki67 and RFS after the aforementioned study was excluded.

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46 **Figure S2.** Forest plots of HRs estimated for the relationship between the expression
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48 of ki67 and PFS after the aforementioned study was excluded.

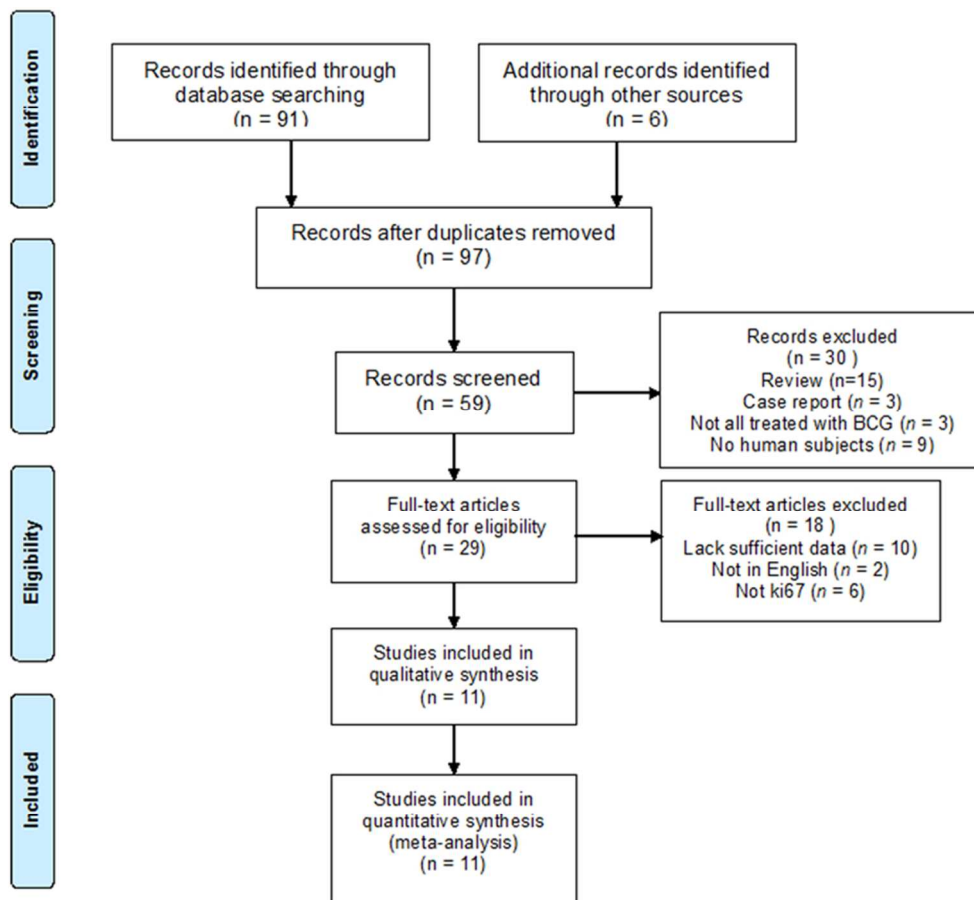
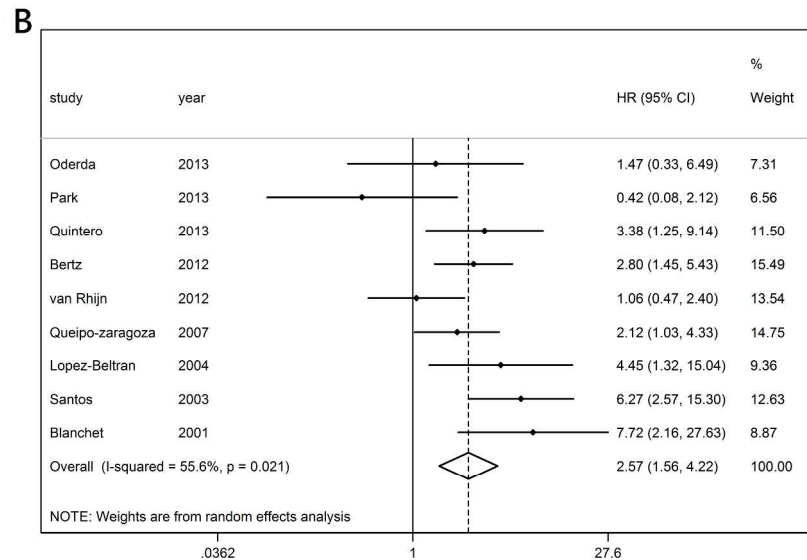
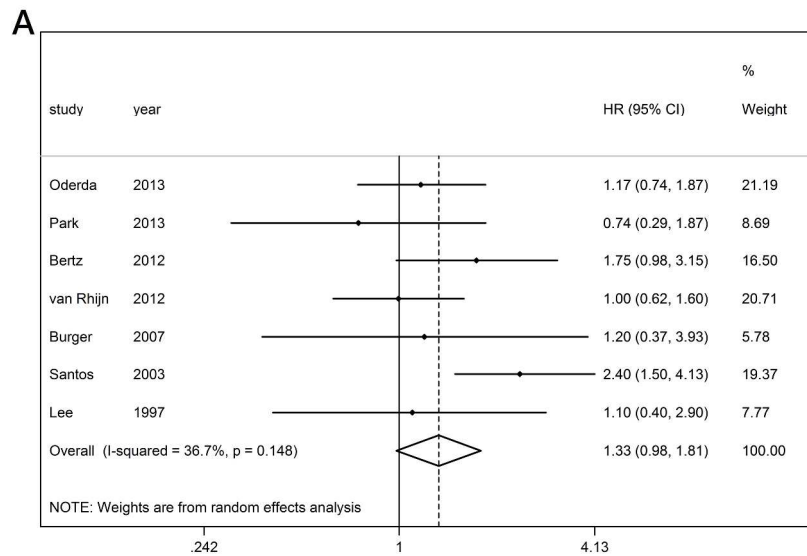


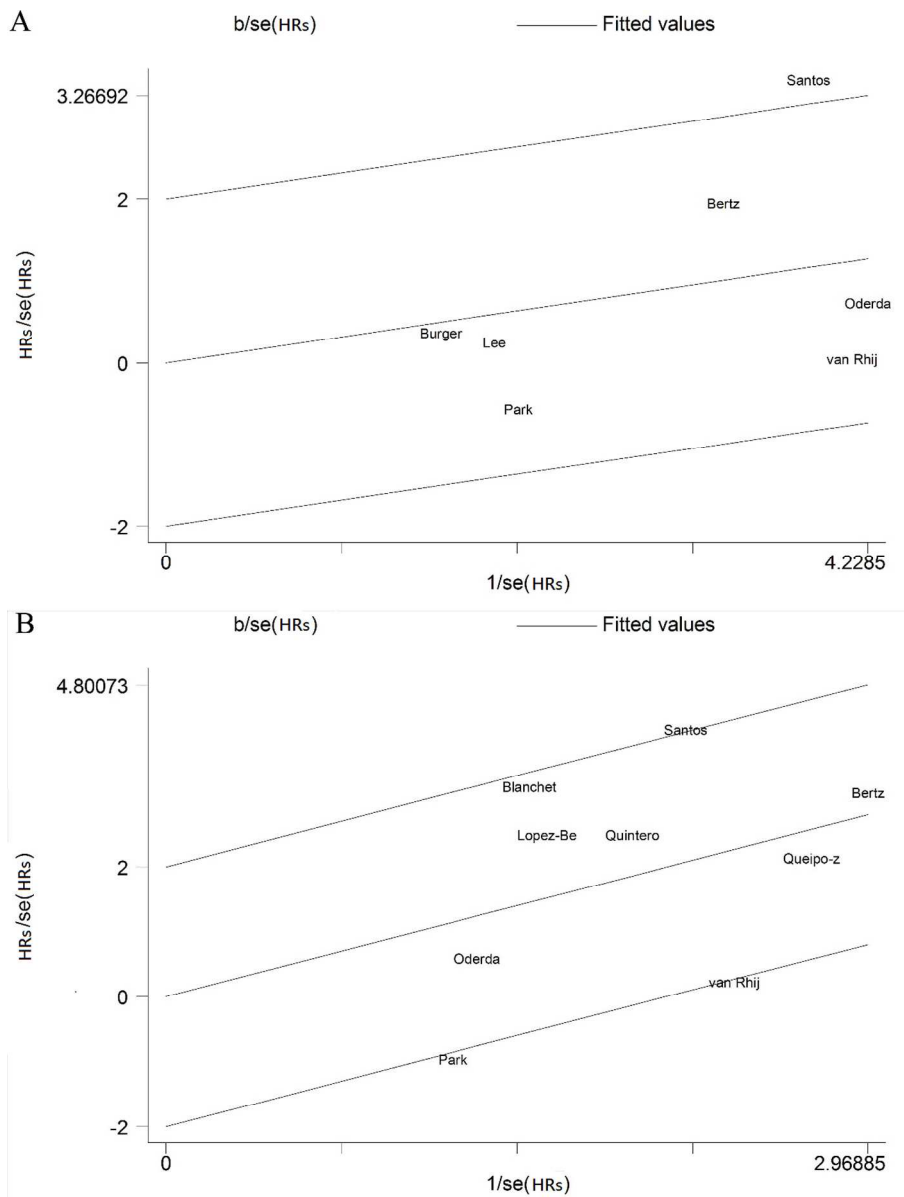
Figure 1. Flow diagram of study selection.

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Forest plots of HRs estimated for the relationship between the expression of ki67 and RFS (A) or PFS (B) among patients in NMIBC treated with BCG.

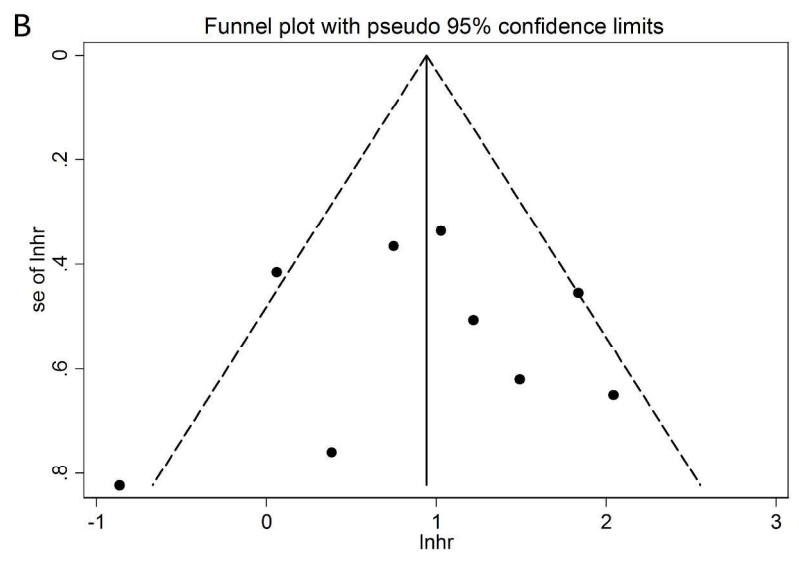
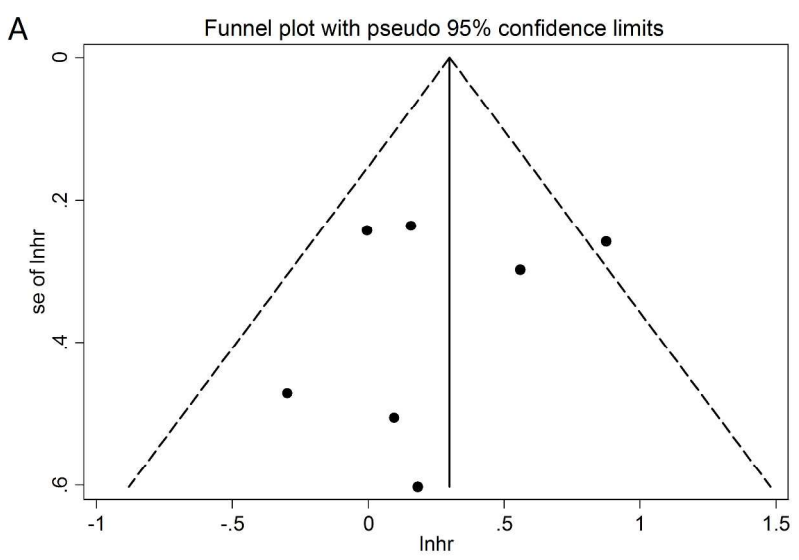
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Galbraith plot analysis was used to evaluate heterogeneity and RFS (A) or PFS (B).

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Funnel plots of the expression of ki67 and RFS (A) or PFS (B).

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1 2 3 4 **Prognostic Value of ki67 in Bacillus Calmette–Guérin-Treated** 5 6 **Non-muscle-Invasive Bladder Cancer: a Meta-analysis and** 7 8 **Systematic Review** 9

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13 Search strategy in PubMed.

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20 #1 Search "Urinary Bladder Neoplasms" [Mesh]
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34 #8 Search Bacillus Calmette–Guérin
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38 #10 Search "ki-67 antigen" [Mesh]
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42 #12 Search ki67
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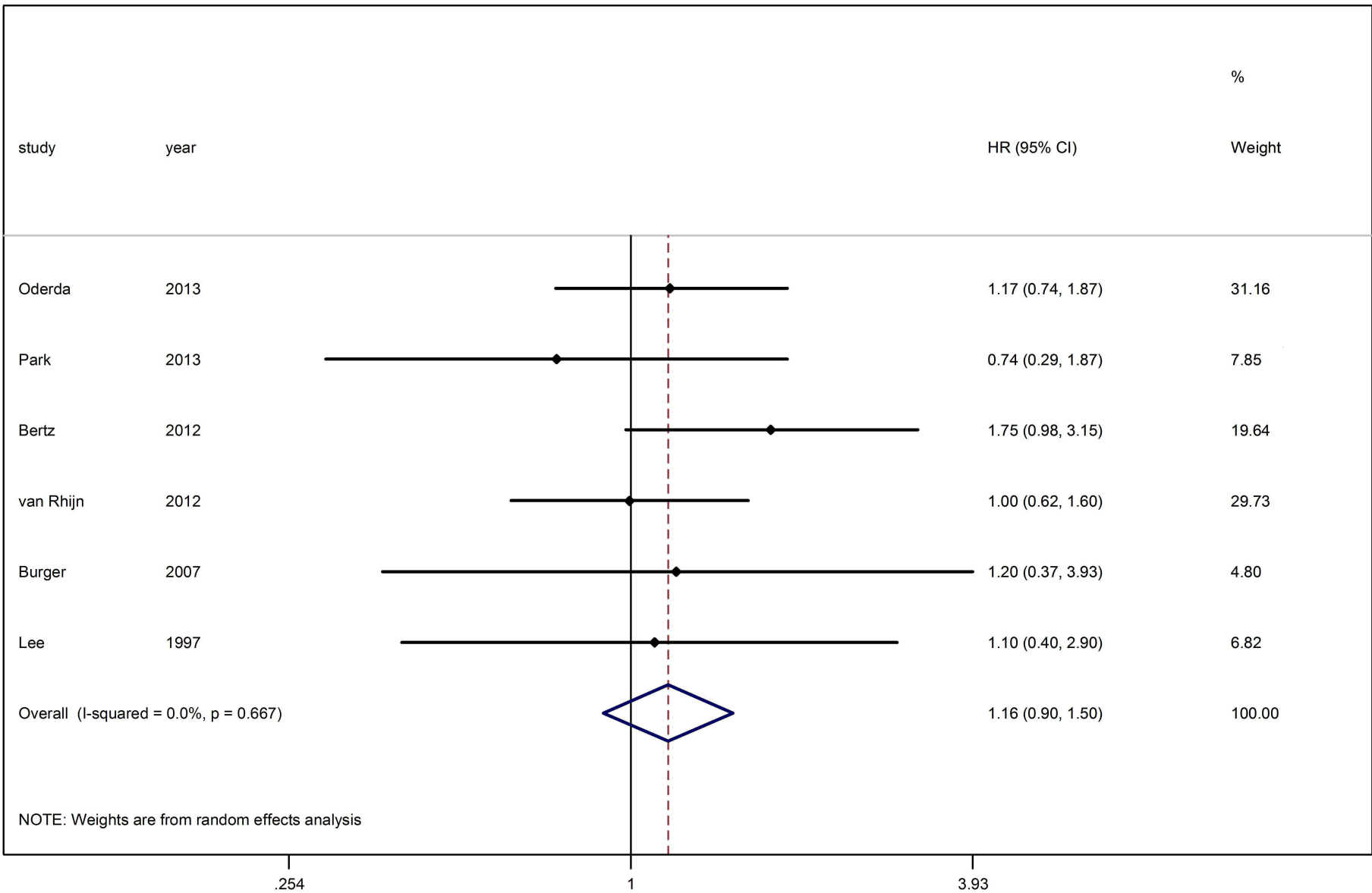


Figure S1. Forest plots of HRs estimated for the relationship between the expression of ki67 and RFS after the aforementioned study was excluded

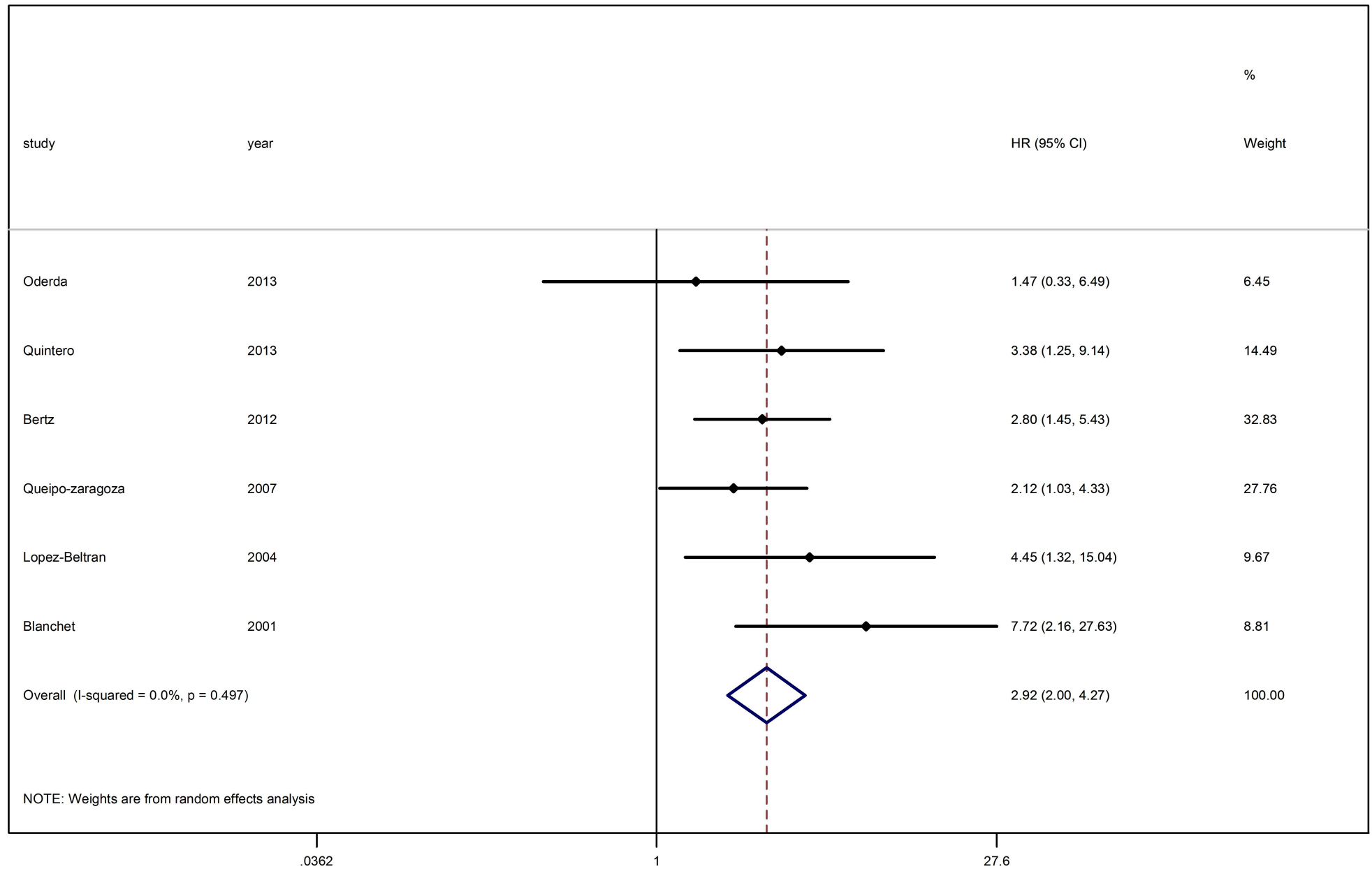


Figure S2. Forest plots of HRs estimated for the relationship between the expression of ki67 and PFS after the aforementioned study was excluded



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7-8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7-8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8-9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8-9



PRISMA 2009 Checklist

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Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9-10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10-12
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Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	13-16
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-17
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	17
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	17
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	17-22
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	23

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Table S2. Meta-regression analysis of RFS and PFS

PFS

Heterogeneity factor	Coefficient	SE	<i>t</i>	<i>P</i>
Years	-0.0343	0.0283	-1.21	0.280
Country				
1	-0.3899	0.3716	-1.05	0.404
2	-0.7185	0.3503	-2.05	0.177
3	-0.9900	0.4307	-2.30	0.148
4	-0.8805	0.3541	-2.49	0.131
Numbers of patients	0.0022	0.0020	1.16	0.299
Stage	None			
Cutoff	-0.0098	0.0407	-0.24	0.819
Age	0.0021	0.0395	0.05	0.959
Follow-up	-0.0062	0.0065	-0.96	0.379

PFS

Heterogeneity factor	Coefficient	SE	<i>t</i>	<i>P</i>
Years	-0.1195	0.0461	-2.59	0.036
Country				

1					
2					
3					
4	1	0.2080	0.7936	0.26	0.818
5					
6	2	-0.8062	0.5662	-1.42	0.290
7					
8					
9	3	-1.4505	0.8858	-1.64	0.243
10					
11	4	-2.7009	0.9407	-2.87	0.103
12					
13					
14	5	-1.7766	0.6167	-2.88	0.102
15					
16					
17	6	-0.8158	0.5281	-1.54	0.262
18					
19	Numbers	of	0.0006	0.0036	0.16
20					
21	patients				
22					
23					
24	Stage				
25					
26					
27	1	-1.4505	1.5909	-0.91	0.458
28					
29					
30	2	-0.8062	1.4108	-0.57	0.625
31					
32					
33	3	0.2080	1.5332	0.14	0.904
34					
35	4	-1.7766	1.4353	-1.24	0.341
36					
37					
38	5	-1.2560	1.2069	-1.04	0.407
39					
40					
41	6	-0.6170	1.4689	-0.42	0.715
42					
43	Cutoff	-0.0177	0.0309	-0.57	0.585
44					
45	Age	-0.0672	0.0757	-0.89	0.404
46					
47					
48	Follow-up	-0.0118	0.0159	-0.74	0.483
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Table S3. Risk group stratification in NMIBC

Low-risk tumors	Primary, solitary, Ta, LG/G1, <3 cm, no CIS
Intermediate-risk tumors	All tumors not defined in the two adjacent categories (between the category of low and high risk)
High-risk tumors	Any of the following: T1 tumor HG/G3 tumor CIS Multiple, recurrent, and large (>3 cm) Ta G1G2 tumors (all conditions must be present at this point)

NMIBC, non-muscle-invasive bladder cancer; CIS, carcinoma in situ; HG, high grade; LG, low grade.



PRISMA 2009 Checklist

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Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	13-16
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-17
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	17
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	17
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	17-22
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	23

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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