The predictive value of PRDM2 in solid tumor: a systematic review and meta-analysis

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Background. Many studies have reported the presence of Positive Regulatory/Su(var)3-9,Enhancer-of-zeste and Trithorax Domain 2 (PRDM2) downregulation in cancer. However, its potential as a diagnostic biomarker is still unclear. Hence, a systematic review and meta-analysis were conducted to address this issue.

Introduction. As of 2018, cancer has become the second leading cause of death worldwide. Thus, cancer control is exceptionally vital in reducing mortality. One such example is through early diagnosis of cancer using tumor biomarkers. Having a function as a tumor suppressor gene (TSG), *PRDM2* has been linked with carcinogenesis in several solid tumor. This study aims to assess the relationship between *PRDM2* downregulation and solid tumor, its relationship with clinicopathological data, and its potential as a diagnostic biomarker. This study also aims to evaluate the quality of the studies, data reliability and confidence in cumulative evidence.

Materials & Methods. A protocol of this study is registered at the International Prospective Register of Systematic Reviews (PROSPERO) with the following registration number: CRD42019132156. PRISMA was used as a guideline to conduct this review. A comprehensive electronic search was performed from inception to June 2019 in Pubmed, Cochrane Library, ProQuest, EBSCO and ScienceDirect. Studies were screened and included studies were identified based on the criteria made. Finally, data synthesis and quality assessment were conducted.

Results. There is a significant relationship between *PRDM2* downregulation with solid tumor (RR 4.29, 95% CI 2.58 – 7.13, P < 0.00001). The overall sensitivity and specificity of *PRDM2* downregulation in solid tumors is 84% (95% CI 39-98%) and 86% (95% CI 71-94%), respectively. There is a low risk of bias for the studies used. TSA results suggested the presence of marked imprecision. The overall quality of evidence for this study is very low.

Discussion. We present the first meta-analysis that investigated the potential of *PRDM2* downregulation as a diagnostic biomarker in solid tumor. In line with previous studies, our results demonstrated that *PRDM2* downregulation occurs in solid tumor. A major source of limitation in this study is the small number of studies.

Conclusions. Our review proved that *PRDM2* is downregulated in solid tumor. The relationship between *PRDM2* downregulation and clinicopathological data is still inconclusive. Although the sensitivity and

specificity of *PRDM2* downregulation are imprecise, its high values, in addition to the evidence presented that confirmed *PRDM2* downregulation in solid tumor suggested that it might still have a potential to be used as a diagnostic biomarker. In order to further strengthen these findings, more research regarding *PRDM2* in solid tumors are encouraged.

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Abstract

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Introduction

Cancer has long been considered a catastrophic public health problem due to its high mortality rates. As of 2018, cancer has become the second leading cause of death worldwide, with an estimate of 9.6 million death. Moreover, the incidence of cancer is also growing at an alarming rate due to the exponential increase of aging population, as well as changes in the prevalence and distribution of cancer risk factors. It was estimated that the incidence of cancer would rise to 18.1 million new cases in 2018. To summarise, 1 in 6 women and 1 in 5 men will develop cancer, while 1 in 10 women and 1 in 8 men are dying as a result of cancer (Bray et al. 2018; World Health Organization 2018).

Thus, cancer control is extremely vital in reducing mortality. One example of cancer control is an early diagnosis of cancer through the use of tumor biomarkers. However, despite the potential of biomarkers for early detection of cancer, its implementation in the clinical setting is still lacking (Goossens et al. 2015; Schiffman et al. 2015; World Health Organization 2017). Hence, further research to identify novel biomarkers should be performed.

Positive Regulatory/Su(var)3-9, Enhancer-of-zeste and Trithorax Domain 2 (PRDM2) is a tumor suppressor gene (TSG) that belongs to the nuclear histone/protein methyltransferase superfamily. *PRDM2* gene products are involved in DNA-binding and transcription factor binding-activities, implicating its role in carcinogenesis (Zhang et al. 2015). Furthermore, studies have also reported *PRDM2* downregulation in cancers that exhibit high incidence and mortality (Cheng et al. 2010; Cui et al. 2016; Johansson et al. 2018; Lal et al. 2006; Michalak & Visvader 2016; Oshimo et al. 2004; Pandzic et al. 2017; Rossi et al. 2009; Sakurada et al. 2001; Tan et al. 2014; Wu et al. 2016; Yang et al. 2017; Zhang et al. 2016). However, the potential of *PRDM2* as a diagnostic biomarker is still unclear.

Therefore, we performed a systematic review and meta-analysis that investigated the relationship between *PRDM2* expression level and solid tumor, as well as its potential as a diagnostic

biomarker. If there is sufficient data, we will also investigate if there is any correlation between *PRDM2* expression level with clinicopathological data.

Materials and Methods

Study Registration and Methodology

A protocol of this study is registered at the International Prospective Register of Systematic Reviews (PROSPERO) with the following registration number: CRD42019132156 (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=132156) (National Institute for Health Research). Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram was used as a guideline to conduct our systematic review and meta-analysis (Moher et al. 2009).

Search Strategy and Study Selection

A comprehensive electronic search was done in PubMed, Cochrane Library, ProQuest, EBSCO and ScienceDirect from inception to July 2019 using the following search terms: (PRDM2 OR RIZ OR RIZ1 OR RIZ2 OR KMT8 OR KMT8A OR MTB-ZF OR HUMHOXY1) AND (Cancer OR Cancers OR Malignant OR Malignancy OR Malignancies OR Neoplasm OR Neoplasms OR Neoplasia OR Neoplasias OR Tumor OR Tumors OR Tumour OR Tumours).

All of the search outputs were exported into the EndNote software. Duplicates were removed, and screening was performed based on the title and abstract of the study. Probable or included studies were identified and assessed for eligibility according to the criteria above. Finally, included studies were identified, and data extraction was performed. Each step of the process was performed by two independent reviewers (AB and IPW), and any differences were solved through a discussion.

A study is included if it meets the following criteria: (1) The study used human subjects; (2) The study investigated the relationship between *PRDM2* expression level and solid tumor through the use of gene expression analysis; (3) The study used histopathological examination as a comparator; (4) The study is a clinical trial or cross-sectional study. A study is excluded if: (1) The study does not have a control group (people without cancer or non-cancer specimens); (2) The study did not use an appropriate or did not state the gene expression analysis method used; (3) The expression level of *PRDM2* in the study is not clearly stated or unquantifiable; (4) The study is a review, case series, conference abstracts, in vitro or in vivo study. (5) The study is not written in English.

Data Extraction

The included studies were then analyzed further and the following informations are extracted: First author, publication year, country of origin, age, gender, race, type of cancer, cancer differentiation state, stage of cancer, type of control, number of cases and controls, gene

expression analysis method, *PRDM2* expression level and conclusion of the study. In the case of missing data, the authors will be contacted via email to request access to those missing data. This phase was independently performed by two reviewers (AB and AS), and any differences were solved by a consensus.

Data Synthesis and Statistical Analysis

Sensitivity and specificity of PDRM2 were assessed in order to elucidate the potential of *PRDM2* expression level as a diagnostic biomarker in solid tumor. Sensitivity and specificity are said to be significant if >50%. Risk ratio (RR) with a 95% confidence interval (CI) was used to determine the relationship between *PRDM2* expression level and risk of cancer, as well as the relationship between *PRDM2* expression level and clinicopathological data. If heterogeneity is present, Random Effects Model (REM) will be used. However, if heterogeneity is absent, Fixed Effects Model (FEM) will be used instead.

Cochrane's Q test (chi-squared test) and Higgins I^2 statistics were used to assess for the presence of heterogeneity statistically. Heterogeneity is said to be present if $P<0.10$ or $I^2>75\%$ (Higgins & Green 2011; Higgins et al. 2003). To assess for the presence of heterogeneity visually, a forest plot will be generated. Meta-regression and subgroup analysis will be conducted when there are at least 10 studies used in the meta-analysis (Baker et al. 2009). The possible causes of heterogeneity are: Age, gender, ethnicity, country of origin, type of cancer, cancer differentiation state, stage of cancer and genotyping method.

Funnel plot and Deek's test will be used to assess publication bias when the number of included studies is at least 10. If the funnel plot is asymmetric, publication bias is present. If the P-value for Deek's test is <0.10, there is funnel plot asymmetry (Deeks et al. 2005). If publication bias is found, the trim and fill method will be used to correct this bias (Duval & Tweedie 2000).

Furthermore, sensitivity analysis was performed to elucidate the effect and stability of a single study on the pooled estimates by deleting one study at a time. Additionally, sensitivity analysis was also conducted to compare the pooled estimates using odds ratio (OR) and RR, as well as using REM and FEM. All statistical analyses were generated using RevMan 5.3 and STATA 12.0. All data synthesis and statistical analysis were conducted independently by two reviewers (CT and IPW), and any differences were solved by a discussion.

Quality Assessment and Data Reliability

In order to claim that the meta-analysis conducted has been conclusive, the required information size has to be achieved. Thus, a trial sequential analysis (TSA) was performed using TSA software in order to determine the required information size (Wetterslev et al. 2017). Quality of evidence will be assessed using Quality Assessment of Diagnostic Accuracy Studies - 2

(QUADAS-2) which consists of the following key domains: patient selection, index test, reference standard, as well as flow and timing (Whiting et al. 2011).

Confidence in Cumulative Evidence

Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) was used to evaluate the confidence in cumulative evidence. Overall certainty of evidence can be written as high, moderate, low or very low (Schünemann et al. 2013).

Results

Search Results

Using the search method previously described above, a total of 58 potential studies were identified. Out of these 58 studies, 52 were excluded due to the studies being irrelevant, in vitro and/or in vivo, used unsuitable methods, written in non-English, or is a review. The remaining six studies (Akahira et al. 2007; Dong et al. 2012; Ge et al. 2015; Geli et al. 2005; Jiang et al. 1999; Tan et al. 2018) were included in the systematic review while only five studies (Akahira et al. 2007; Dong et al. 2012; Geli et al. 2005; Jiang et al. 1999; Tan et al. 2018) were included in the meta-analysis due to lack of suitable data. The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram for this study is shown in Fig. 1.

The studies that were eligible for systematic review were published from 1999 to 2015. There was a total of 314 samples of solid tumors and 225 controls obtained from patients in China (Dong et al. 2012; Ge et al. 2015; Tan et al. 2018), Japan (Akahira et al. 2007), Sweden (Geli et al. 2005) and United States of America (Jiang et al. 1999). All of these six studies are cross-sectional studies. The solid tumors included in this study are ovarian cancer (Akahira et al. 2007), esophageal squamous cell carcinoma (Dong et al. 2012), renal cell carcinoma (RCC) (Ge et al. 2015), pheochromocytoma (Geli et al. 2005), abdominal paraganglioma (Geli et al. 2005), hepatoma (Jiang et al. 1999), lung squamous cell carcinoma (LSCC) (Tan et al. 2018) and lung adenocarcinoma (LAC) (Tan et al. 2018). Out of these six studies, one used immunohistochemistry (IHC) only (Akahira et al. 2007), three used reverse transcription-polymerase chain reaction (RT-PCR) only (Ge et al. 2015; Geli et al. 2005; Jiang et al. 1999) and two used both IHC and RT-PCR (Dong et al. 2012; Tan et al. 2018). One study, Geli et al. (2005), did not mention the number of samples and controls that expressed *PRDM2* downregulation. Thus, only five studies were included in the meta-analysis. A summary of the main characteristics of the included studies for systematic review and meta-analysis could be viewed in Table 1 and Table 2, respectively.

Systematic Review Results

All six studies concluded that *PRDM2* gene expression is significantly decreased in solid tumor compared to control, with the P-value ranging from <0.05 to <0.001 using CI 95%. Akahira et al. (2007) stated that there was a significant correlation between *PRDM2* downregulation with

cancer grade (P<0.0345) and stage (P<0.0153) in ovarian cancer. On the other hand, Ge et al. (2015) stated otherwise, concluding that there was no significant relationship between RCC with tumor progression (P=0.19). A study by Geli et al. (2005) reported that decreased *PRDM2* gene expression was not correlated significantly with gender and tumor size, but was found to be weakly correlated with younger age (Spearman rank-order correlations; R=0.4). Other clinicopathological data were either absent or not investigated in the studies. Hence, the role of *PRDM2* downregulation in cancer grade, stage, gender, age and other clinicopathological data is still unclear. Due to the lack of sufficient clinicopathological data, only *PRDM2* gene expression and its sensitivity and specificity were further analysed in the meta-analysis.

Meta-Analysis Results

Five studies were included in this meta-analysis to further investigate the relationship between *PRDM2* downregulation with solid tumor (Akahira et al. 2007; Dong et al. 2012; Geli et al. 2005; Jiang et al. 1999; Tan et al. 2018). The pooled analysis suggested that *PRDM2* gene expression is decreased in solid tumor (RR 4.29, 95% CI 2.58 – 7.13, P < 0.00001; Fig. 2). Based on this pooled analysis, three sensitivity analyses were conducted to evaluate the stability of our findings: with and without the deletion of Jiang et al. (1999) (Fig. 3), RR or OR (Fig. 4), and FEM or REM (Fig. 5). All three sensitivity analyses did not have meaningful differences, proving that our results are stable.

The sensitivity and specificity of *PRDM2* downregulation in solid tumor were also assessed in order to investigate its potential as a diagnostic biomarker. A split forest plot displaying the sensitivity and specificity of the included studies is shown in Fig. 6. As demonstrated in the summary receiver operating characteristic (SROC) curve (Fig. 7), the summary sensitivity and specificity of decreased *PRDM2* gene expression in solid tumor is 84% (95% CI 39-98%) and 86% (95% CI 71-94%), respectively. This result is in favor of *PRDM2* downregulation as a potential diagnostic biomarker. However, the confidence interval for *PRDM2* downregulation is wide, suggesting that there is marked imprecision. This was later confirmed on TSA (Fig. 8). In Fig. 8, the line representing the cumulative Z-curve failed to cross the significance boundary and did not reach the required number of studies which is 7743. Therefore, it can be concluded that the usage of *PRDM2* downregulation as a diagnostic biomarker in solid tumor is still inconclusive.

Quality Assessment of Included Studies

The quality of the included studied was evaluated using the QUADAS-2 tool, and a summary of the results can be viewed in Table 3. As shown in Table 3, in the index test domain there are four studies (Ge et al. 2015; Geli et al. 2005; Jiang et al. 1999; Tan et al. 2018) having an unclear risk of bias. These four studies did not directly state whether the index test (gene expression analysis) was interpreted independently from the reference standard (histopathological examination). Thus, we decided that unclear was most fit as the risk of bias. One of the studies, Jiang et al.

(1999) also had missing information on how the patients were recruited, leading to an unclear risk of bias for one other domain. In general, the quality of the included studies was superior, ensuring the reliability of our systematic review and meta-analysis.

Confidence in Cumulative Evidence

By assessing five domains, including the risk of bias (by using the results from QUADAS-2 risk of bias assessment), indirectness, inconsistency, imprecision (by using the results from TSA) and risk of publication bias, a GRADE evidence profile was constructed as shown in Table 4. To be noted, all of the included studies used diagnostic accuracy test as their design, whereby all of the samples and controls will undergo both the index test and reference standard. Ideally, diagnostic studies should randomize which of the samples and controls will undergo the index test only and which will undergo the reference standard only. Hence, we put serious in the indirectness domain. In addition, most of the included studies have wide confidence interval and inconclusive TSA results. Thus, serious was placed in the imprecision domain. As for publication bias, since the number of included studies is <10, publication bias could not be evaluated. Unfortunately, this does not entirely rule out the possibility of publication bias being present in our study, and thus we decided to downgrade the quality of evidence further. Overall, we have very low confidence in the pooled estimates obtained for our meta-analysis.

Discussion

In this study, we have successfully generated the first meta-analysis that investigated the potential of *PRDM2* downregulation as a diagnostic biomarker in solid tumor. Compared to previous primary studies on *PRDM2* thus far, we investigated the significance of *PRDM2* with solid tumor on the level of a review. This includes the evaluation of quality assessment, data reliability and confidence in cumulative evidence, proving that our study was more comprehensive.

Meta-regression, funnel plot and Deek's test were not performed due to the small number of studies obtained. Due to the inability to confirm the presence of publication bias, we also could not perform trim and fill method. Since our results indicated that there was no heterogeneity in the studies used, a subgroup analysis was not required.

In line with previous studies, our results demonstrated that *PRDM2* downregulation occurs in solid tumor. However, compared to the findings of Sorrentino et al. (2018), we did not find the relationship between *PRDM2* and several solid tumors such as neuroblastoma, breast cancers, melanoma, parathyroid adenoma and Merkel cell carcinoma. Another notable difference is the inconclusive results linking *PRDM2* downregulation with cancer stage and grade even though *PRDM2* downregulation has been associated with cancer progression (Sun et al. 2011). A possible explanation for these inconsistencies might be due to the fact that our study only accepted human studies, and thus limited the possibility of encountering such studies.

Interestingly, all of the individual studies did not have a standardised baseline to define *PRDM2* downregulation. Although this could lead to possible heterogeneity, our study demonstrated otherwise.

Following these findings, an important question to address is whether *PRDM2* downregulation could be used as a diagnostic biomarker in solid tumor. As described above, the high sensitivity and specificity of *PRDM2* downregulation suggested its potential as a diagnostic biomarker. However, these values have wide confidence intervals and inconclusive TSA results, implying there was marked imprecision (Chai-Adisaksopha et al. 2016; Tan & Tan 2010). Thus, the use of *PRDM2* downregulation as a diagnostic biomarker is still inconclusive. This imprecision might be due to the small number of sample and controls used in the individual studies or low variability in the subjects used (Carlson & Morrison 2009). Although our present study could not fully prove the potential of *PRDM2* downregulation as a diagnostic biomarker due to its imprecision, it is important to highlight that these results can potentially improve with the addition of new studies. This has been proven by our TSA results whereby the line representing the cumulative Z-curve did not cross the futility boundary.

Another issue that should be addressed in the future is whether the quality of our evidence is satisfying enough. The quality of evidence is judged based on five domains: risk of bias, indirectness, inconsistency, imprecision and publication bias. It should be noted that all of the studies used in this review are diagnostic accuracy studies which are considered a proxy to randomised-controlled trials. Hence, indirectness is present, and this could lead to overestimation of sensitivity and specificity, resulting in the downgrading of the quality of evidence (Schmidt & Factor 2013). As discussed before, imprecision is present, and publication bias could not be assessed, leading to further downgrading. Together, these three domains led to the downgrading of the quality of evidence from high to very low. Although there is very low confidence for our results, it is important to highlight once again that these results can improve if new studies are added.

Limitations of our study are the lack of RCTs as part of our included studies which made it difficult to evaluate the internal validity of our results (Carlson & Morrison 2009). As mentioned before, our study also lacks clinicopathological data in order to assess the potential of *PRDM2* further. Interestingly, none of the included studies investigated *PRDM2* gene expression in the same type of solid tumor. Hence, we were unable to evaluate in which type of solid tumor is *PRDM2* downregulation most suitable to be used as a biomarker. Furthermore, there was no standardised baseline among studies. Another limitation of this study involves the issue of only using studies written in English, leading to the possibility of language bias. Most of the individual studies have a wide confidence interval and inconclusive TSA results, indicating there is insufficient knowledge about the effect and that further research should be done. Based on the

points above, it can be concluded that a major source of limitation is due to the small number of studies.

Conclusions

In conclusion, our review demonstrated that *PRDM2* gene expression is decreased or downregulated in solid tumor. Due to insufficient data, we are unable to determine the relationship between *PRDM2* downregulation and clinicopathological data. Although the sensitivity and specificity of *PRDM2* downregulation are imprecise, its high values, in addition to the evidence presented that confirmed *PRDM2* downregulation in solid tumor, suggested that it might still have a potential to be used as a diagnostic biomarker. However, more samples are required in order to solidify this conclusion. Thus, we suggest more research to be conducted, especially those with RCT as their design. More study is urgently needed to determine a standardised baseline for *PRDM2* downregulation level. We would also recommend more research regarding the relationship between *PRDM2* gene expression with clinicopathological data to further evaluate the potential of *PRDM2* gene expression in solid tumor. Finally, we suggest a new systematic review and meta-analysis to be done in order to renew the findings of our study.

Abbrevations

CI: Confidence interval
Df: Degree of freedom
F: Female
FN: False negative
FP: False positive
GRADE: Grading of recommendations, assessment, development, and evaluations
IHC: Immunohistochemistry
LAC: Lung adenocarcinoma
LSCC: Lung squamous cell carcinoma
M: Male
M-H: Mantel-Haenszel
ND: Not determined
OR: Odds ratio
PRDM2: Positive regulatory/su(var)3-9, enhancer-of-zeste and trithorax domain 2
PRISMA: Preferred reporting items for systematic reviews and meta-analyses
PROSPERO: International prospective register of systematic reviews
qRT-PCR: Quantitative reverse transcription-polymerase chain reaction
QUADAS-2: Quality assessment of diagnostic accuracy studies - 2
RCC: Renal cell carcinoma
REM: Random effects model
RR: Risk ratio

RT-PCR: Reverse transcription-polymerase chain reaction
SROC: Summary receiver operating characteristic
TN: True negative
TP: True positive
TSA: Trial sequential analysis
TSG: Tumor suppressor gene

Additional Information and Declarations

Competing Interests

The authors declare no conflicts of interest.

Authors' Contributions

All authors contributed to the drafting of this manuscript. AB and IPW searched and obtained the data. AB and AS abstracted and summarised the data. CT and IPW performed all statistical analyses and evaluated the quality of studies. CT and AB reviewed and edited the first draft of the manuscript. All authors reviewed, commented and approved the final draft of the manuscript.

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Figure 1

PRISMA flow diagram for selection of included studies.

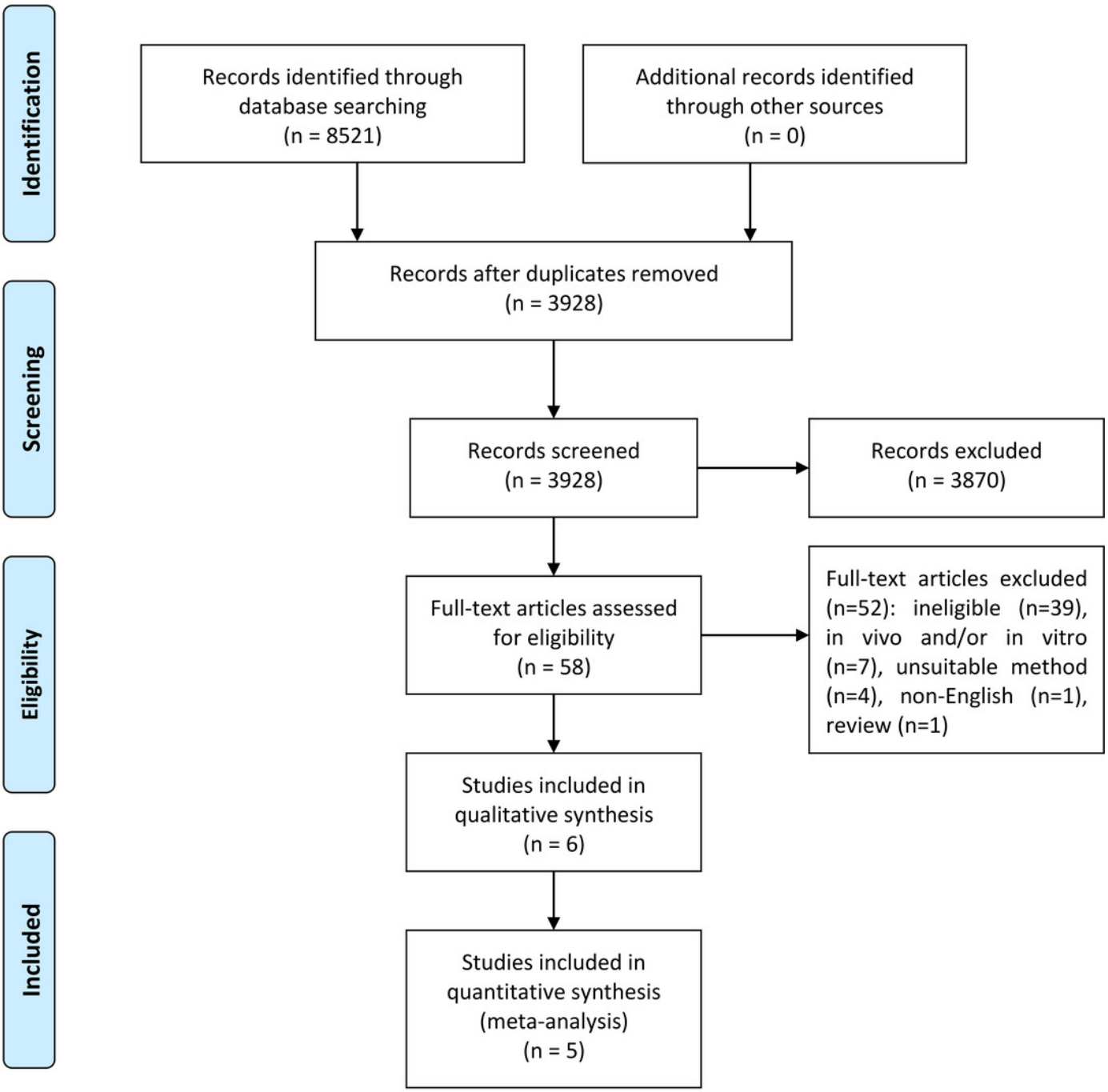


Figure 2

Forest plot of *PRDM2* downregulation in solid tumors and control.

Studies with notable weights are Tan et al. (2018) (46.2%) and Dong et al. (2012) (31.2%). The results from this forest plot demonstrated that *PRDM2* downregulation occurs more often in solid tumor when compared to control (RR 4.29, 95% CI 2.58-7.13, P<0.00001). There was no significant heterogeneity in this analysis (X^2 = 2.85, I^2 = 0%). The horizontal line represents 95% CI. The blue box is the result of each individual study. The black diamond at the bottom of the plot is the pooled analysis of all studies. CI = Confidence interval. df = Degree of freedom. I^2 = Test of heterogeneity. M-H = Mantel-Haenszel.

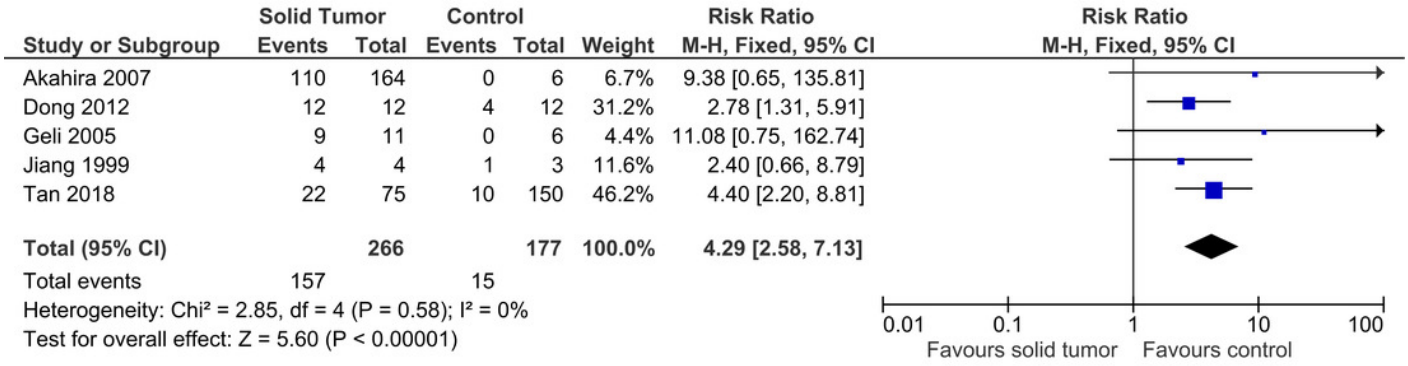


Figure 3

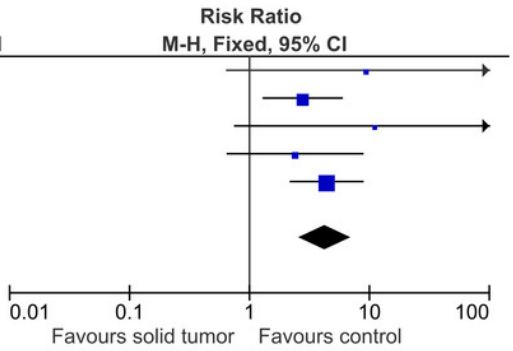
Sensitivity analysis to compare the use of all studies with deletion of a study.

The deleted study, Jiang et al. (1999), is a study that has the most questionable results based on the risk of bias assessment. There was a significant result for both analyses: (A) Without deletion of Jiang et al. (1999): RR 4.29, 95% CI 2.58-7.13, P<0.00001, X^2 = 2.85, P<0.58, I^2 = 0%; (B) With deletion of Jiang et al. (1999): RR 4.53, 95% CI 2.63-7.82, P<0.00001, X^2 = 2.33, P<0.51, I^2 = 0%. The deletion of Jiang et al. (1999) increased RR by 1.1 times higher with the 95% CI 1.2 times wider. The deletion of study also slightly lowered heterogeneity. This sensitivity analysis proved that the results were stable. The horizontal line represents 95% CI. The blue box is the result of each individual study. CI = Confidence interval. df = Degree of freedom. I^2 = Test of heterogeneity. M-H = Mantel-Haenszel.

A

Study or Subgroup	Solid Tumor Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Akahira 2007	110	164	0	6	6.7%	9.38 [0.65, 135.81]
Dong 2012	12	12	4	12	31.2%	2.78 [1.31, 5.91]
Geli 2005	9	11	0	6	4.4%	11.08 [0.75, 162.74]
Jiang 1999	4	4	1	3	11.6%	2.40 [0.66, 8.79]
Tan 2018	22	75	10	150	46.2%	4.40 [2.20, 8.81]
Total (95% CI)		**266**		**177**	**100.0%**	**4.29 [2.58, 7.13]**
Total events	157		15			

Heterogeneity: Chi² = 2.85, df = 4 (P = 0.58); I² = 0%
Test for overall effect: Z = 5.60 (P < 0.00001)



B

Study or Subgroup	Solid Tumor Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Akahira 2007	110	164	0	6	7.5%	9.38 [0.65, 135.81]
Dong 2012	12	12	4	12	35.3%	2.78 [1.31, 5.91]
Geli 2005	9	11	0	6	5.0%	11.08 [0.75, 162.74]
Tan 2018	22	75	10	150	52.3%	4.40 [2.20, 8.81]
Total (95% CI)		**262**		**174**	**100.0%**	**4.53 [2.63, 7.82]**
Total events	153		14			

Heterogeneity: Chi² = 2.33, df = 3 (P = 0.51); I² = 0%
Test for overall effect: Z = 5.43 (P < 0.00001)

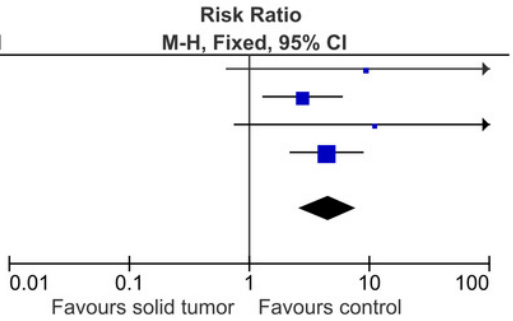


Figure 4

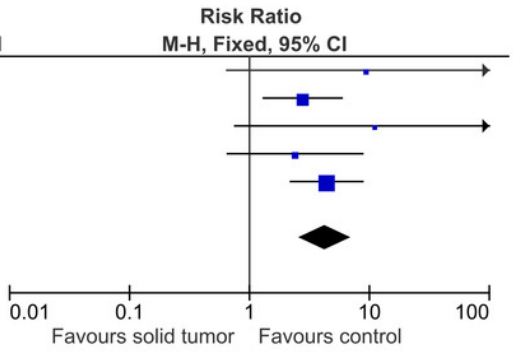
Sensitivity analysis to compare the use of Risk Ratio (RR) with Odds Ratio (OR).

There was a significant result for both analyses: (A) RR: RR 4.29, 95% CI 2.58-7.13, P<0.00001; (B) OR: OR 9.62, 95% CI 4.82-19.19, P<0.00001). The use of OR gave a result two times higher with the 95% CI three times wider when compared to RR. RR had a slightly lower heterogeneity when compared to OR (RR: X^2 = 2.58, P<0.58, I^2 = 0%; OR: X^2 = 4.06, P<0.40, I^2 = 1%). This sensitivity analysis proved that the results were stable. The horizontal line represents 95% CI. The blue box is the result of each individual study. The black diamond at the bottom of the plot is the pooled analysis of all studies. CI = Confidence interval. df = Degree of freedom. I^2 = Test of heterogeneity. M-H = Mantel-Haenszel.

A

Study or Subgroup	Solid Tumor Events	Solid Tumor Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Akahira 2007	110	164	0	6	6.7%	9.38 [0.65, 135.81]
Dong 2012	12	12	4	12	31.2%	2.78 [1.31, 5.91]
Geli 2005	9	11	0	6	4.4%	11.08 [0.75, 162.74]
Jiang 1999	4	4	1	3	11.6%	2.40 [0.66, 8.79]
Tan 2018	22	75	10	150	46.2%	4.40 [2.20, 8.81]
Total (95% CI)		**266**		**177**	**100.0%**	**4.29 [2.58, 7.13]**
Total events	157		15			

Heterogeneity: Chi² = 2.85, df = 4 (P = 0.58); I² = 0%
Test for overall effect: Z = 5.60 (P < 0.00001)



B

Study or Subgroup	Solid Tumor Events	Solid Tumor Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Akahira 2007	110	164	0	6	5.8%	26.36 [1.46, 476.49]
Dong 2012	12	12	4	12	3.1%	47.22 [2.24, 996.02]
Geli 2005	9	11	0	6	2.4%	49.40 [2.02, 1207.15]
Jiang 1999	4	4	1	3	3.0%	15.00 [0.43, 524.53]
Tan 2018	22	75	10	150	85.7%	5.81 [2.58, 13.08]
Total (95% CI)		**266**		**177**	**100.0%**	**9.62 [4.82, 19.19]**
Total events	157		15			

Heterogeneity: Chi² = 4.06, df = 4 (P = 0.40); I² = 1%
Test for overall effect: Z = 6.43 (P < 0.00001)

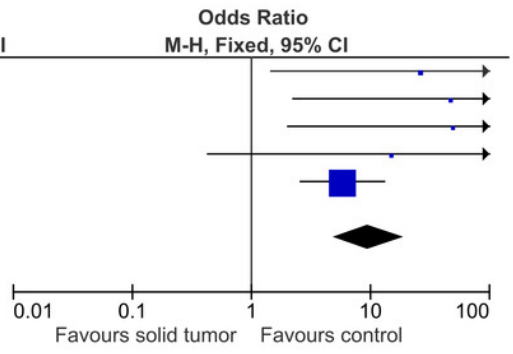


Figure 5

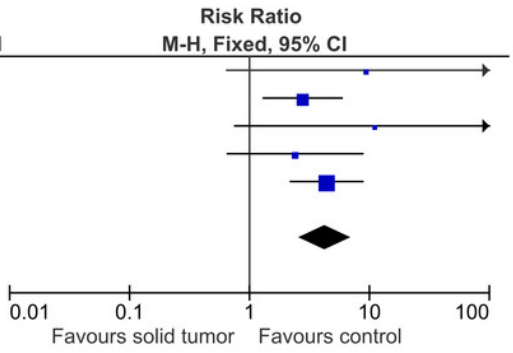
Sensitivity analysis to compare the use of Fixed Effects Model (FEM) with Random Effects Model (REM).

There was a significant result for both analyses: (A) FEM: RR 4.29, 95% CI 2.58-7.13, P<0.00001; (B) REM: RR 3.61, 95% CI 2.28-5.72, P<0.00001. FEM increased RR by 1.2 times higher with 95% CI 1.3 times wider. This sensitivity analysis proved that the results were stable. The horizontal line represents 95% CI. The blue box is the result of each individual study. The black diamond at the bottom of the plot is the pooled analysis of all studies. CI = Confidence interval. df = Degree of freedom. I^2 = Test of heterogeneity. M-H = Mantel-Haenszel.

A

Study or Subgroup	Solid Tumor Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Akahira 2007	110	164	0	6	6.7%	9.38 [0.65, 135.81]
Dong 2012	12	12	4	12	31.2%	2.78 [1.31, 5.91]
Geli 2005	9	11	0	6	4.4%	11.08 [0.75, 162.74]
Jiang 1999	4	4	1	3	11.6%	2.40 [0.66, 8.79]
Tan 2018	22	75	10	150	46.2%	4.40 [2.20, 8.81]
Total (95% CI)		**266**		**177**	**100.0%**	**4.29 [2.58, 7.13]**
Total events	157		15			

Heterogeneity: Chi² = 2.85, df = 4 (P = 0.58); I² = 0%
Test for overall effect: Z = 5.60 (P < 0.00001)



B

Study or Subgroup	Solid Tumor Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Akahira 2007	110	164	0	6	3.0%	9.38 [0.65, 135.81]
Dong 2012	12	12	4	12	37.3%	2.78 [1.31, 5.91]
Geli 2005	9	11	0	6	2.9%	11.08 [0.75, 162.74]
Jiang 1999	4	4	1	3	12.6%	2.40 [0.66, 8.79]
Tan 2018	22	75	10	150	44.1%	4.40 [2.20, 8.81]
Total (95% CI)		**266**		**177**	**100.0%**	**3.61 [2.28, 5.72]**
Total events	157		15			

Heterogeneity: Tau² = 0.00; Chi² = 2.85, df = 4 (P = 0.58); I² = 0%
Test for overall effect: Z = 5.45 (P < 0.00001)

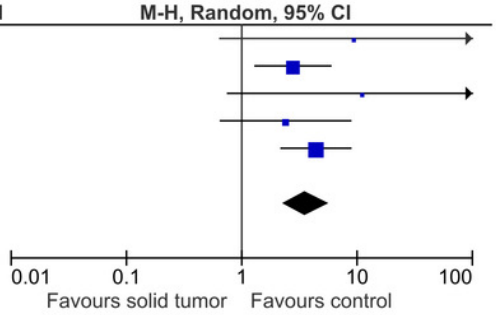


Figure 6

Forest plot for sensitivity and specificity of decreased *PRDM2* gene expression in solid tumor.

Studies that have high sensitivities include Dong et al. (2012) (Sensitivity 1.00, 95% CI 0.74-1.00) and Jiang et al. (1999) (Sensitivity 1.00, 95% CI 0.40-1.00). Studies that have high specificities are Akahira et al. (2007) (Specificity 1.00, 95% CI 0.54-1.00) and Geli et al. (2005) (Specificity 1.00, 95% CI 0.54-1.00). The horizontal line represents 95% CI. The blue box is the result of each individual study. CI = Confidence interval. FN = False negative. FP = False positive. TN = True negative. TP = True positive.

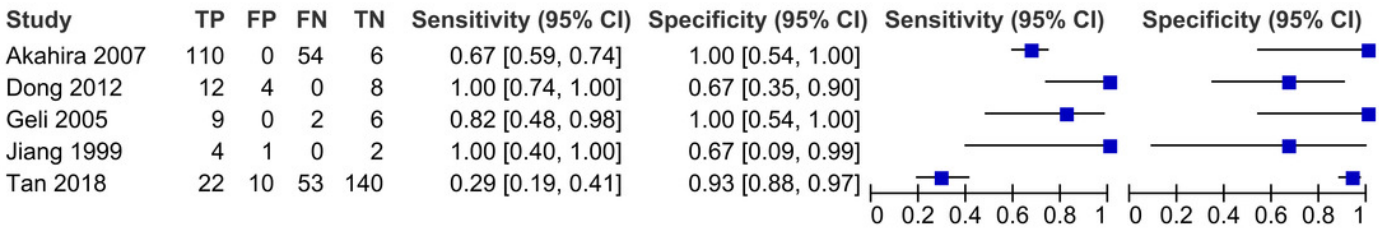


Figure 7

Summary receiver operating characteristic (SROC) curve of decreased *PRDM2* gene expression in solid tumor.

The overall sensitivity and specificity is 84% (95% CI 39-98%) and 86% (95% CI 71-94%), respectively. The calculation of these results can be viewed at Fig. S1. The black circle (summary estimate) represents the summary estimate of sensitivity and specificity. The dotted lines around the summary point represents the 95% confidence region. The dashed lines represent the 95% prediction region (the region within which we are 95% certain that the results of a new study will lie).

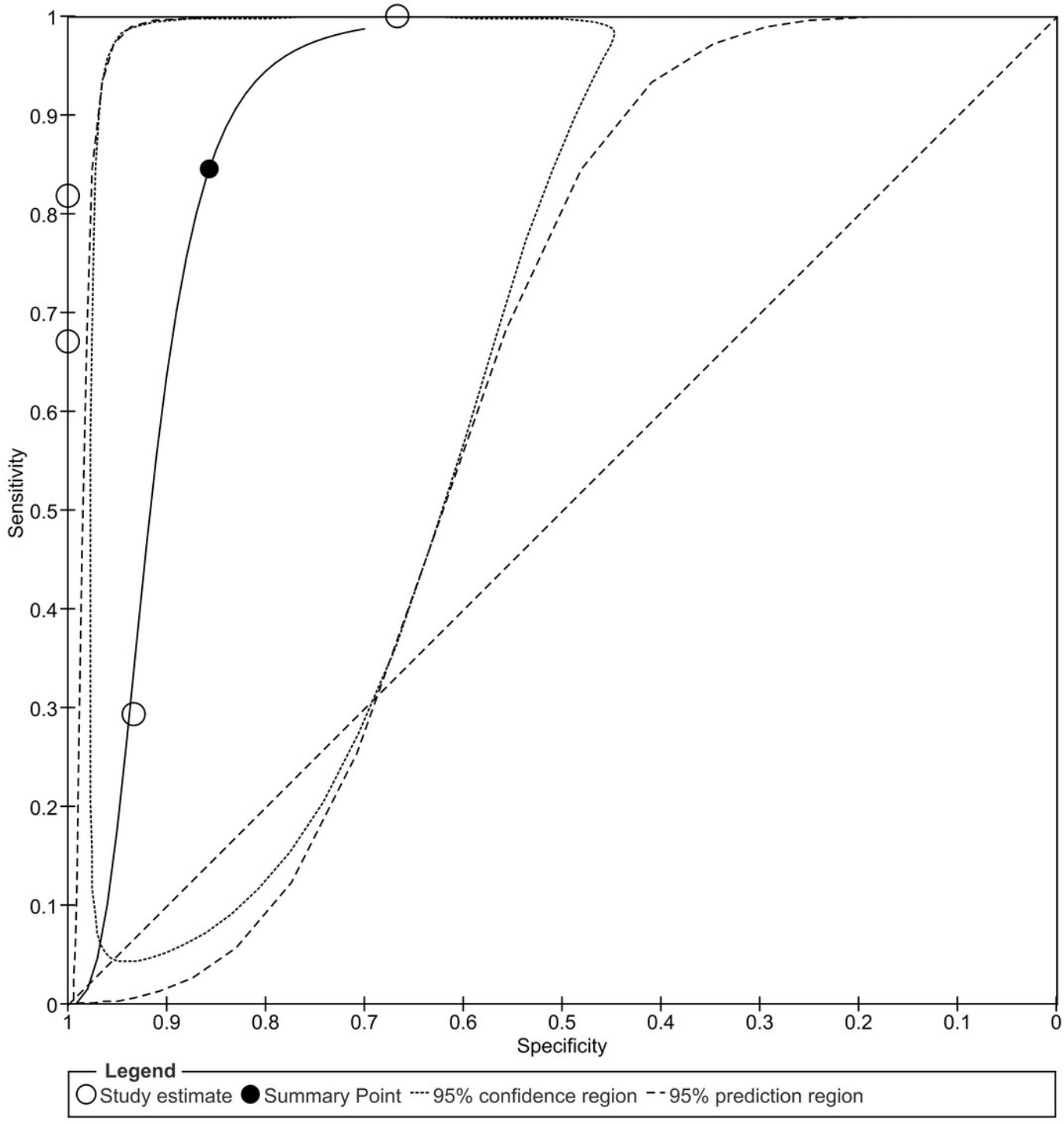

Sensitivity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Specificity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Legend
Study estimate
Summary Point
95% confidence region
95% prediction region

Figure 8

Trial Sequential Analysis (TSA) results of the meta-analysis.

The cumulative Z-curve (blue line) crossed the conventional meta-analysis significance boundary (horizontal red lines at Z = + 1,96 and Z = - 1,96), confirming that type I error was avoided. However, the cumulative Z-curve has not crossed the trial sequential significance boundary (diagonal red line at the top and bottom of the plot), suggesting that type II error might have not been avoided. Furthermore, the cumulative Z-score has also failed to reach the vertical red line on the right, indicating that this review has not reached the required sample size which is 7743. It is interesting to note that the cumulative Z-curve did not cross the trial sequential futility boundary (triangular red line on the right), implying that the addition of new samples could potentially improve the TSA results. In conclusion, this TSA analysis proved that this meta-analysis still requires more samples in order ensure that type II error was avoided. This is a magnified version of the TSA. The TSA results on a standard scale can be viewed at Fig. S2.

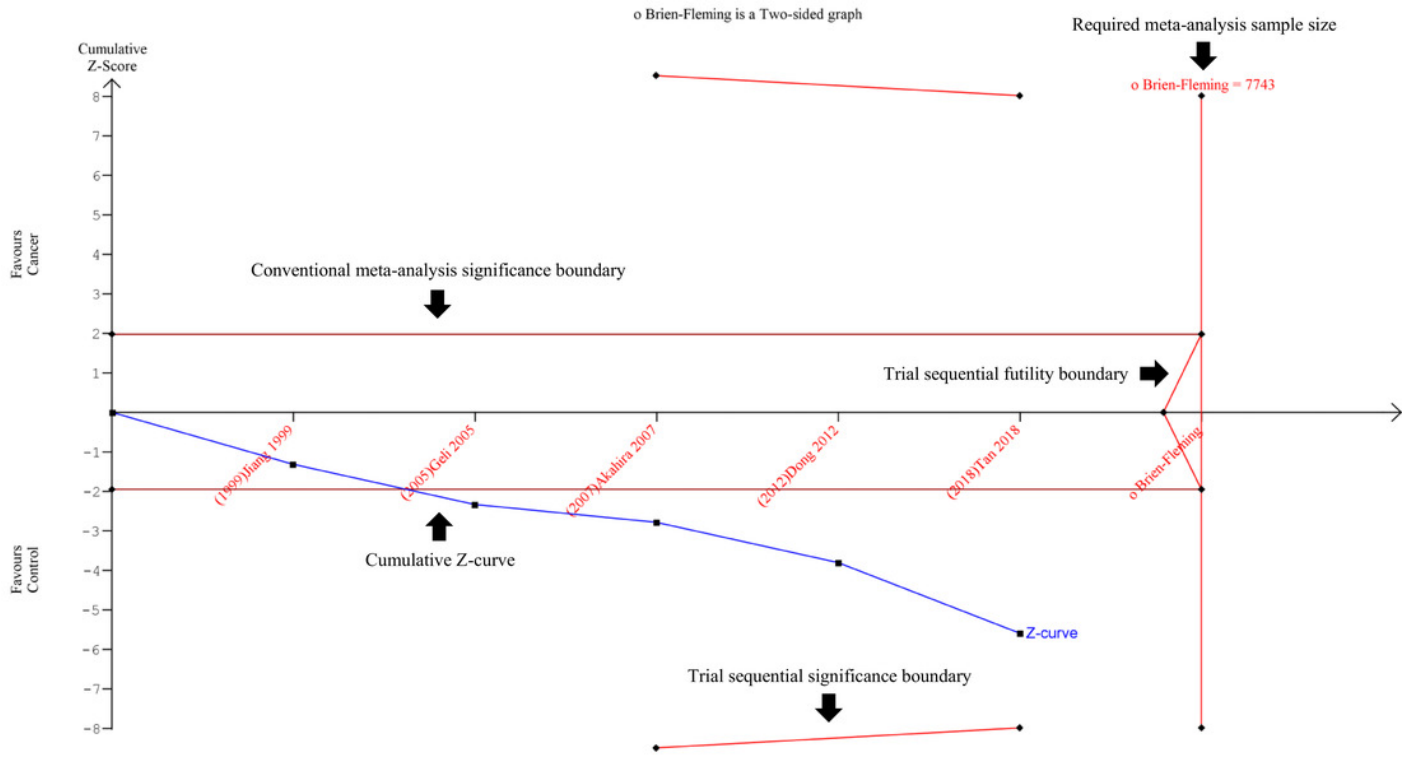

o Brien-Fleming is a Two-sided graph
Required meta-analysis sample size
o Brien-Fleming = 7743
Cumulative Z-Score
Favours Cancer
Favours Control
Conventional meta-analysis significance boundary
Trial sequential futility boundary
(1999)Jiang 1999
(2005)Geli 2005
(2007)Akahira 2007
(2012)Dong 2012
(2018)Tan 2018
o Brien-Fleming
Cumulative Z-curve
Z-curve
Trial sequential significance boundary

Table 1(on next page)

Study characteristics of studies included in systematic review.

F = Female. IHC = Immunohistochemistry. LAC = Lung adenocarcinoma. LSCC = Lung squamous cell carcinoma. M = Male. ND = Not determined. PRDM2 = Positive Regulatory/Su(var)3-9,Enhancer-of-zeste and Trithorax Domain 2. qRT-PCR = Quantitative reverse transcription-polymerase chain reaction. RT-PCR = Reverse transcription-polymerase chain reaction.

Study	Country	Age	Gender	Race	Method	No. of sample	No. of control	Cancer					*PRDM2* expression	P value
								Type	Stage		Differentiation			
									I+II	III+IV	Well/ Moderate	Poor		
Akahira et al. (2007)	Japan	<50 (n=42/67) >=50 (n=68/97)	ND	Asian	IHC	164	6 [a]	Ovarian cancer	69	95	107 [h]	36 [h]	Decreased [i]	<0.05
Dong et al. (2012)	China	ND	ND	Asian	RT-PCR IHC	40	40 [b]	Esophageal squamous cell carcinoma	ND	ND	ND	ND	Decreased [i]	<0.05 [j]
Ge et al. (2015)	China	ND	ND	Asian	qRT-PCR	20	20 [c]	Renal cell carcinoma	ND	ND	ND	ND	Decreased [i]	<0.001 [k]
Geli et al. (2005)	Sweden	ND	7 M 4 F	Caucasian	qRT-PCR	11	6 [d]	Pheochromocytoma (n=4) Abdominal paraganglioma (n=7)	ND	ND	ND	ND	Decreased [i]	<0.001 [l]
Jiang et al. (1999)	United States of America	ND	ND	Caucasian	RT-PCR	4	3 [e]	Hepatoma	ND	ND	ND	ND	Decreased [i]	ND
Tan et al. (2018)	China	<60 (n=30) >=60 (n=45)	56 M 19 F	Asian	RT-PCR IHC	75	150 [f]	LSCC (n=52) LAC (n=23)	63 [g]	12 [g]	46 [g]	29 [g]	Decreased [i]	<0.05 [m]

[a] Normal ovaries
[b] Adjacent non-cancerous tissue
[c] Adjacent non-malignant renal tissue
[d] Normal adrenal cells
[e] Normal liver tissue
[f] Tumor adjacent tissue and distant lung tissue
[g] Classification based on International Association for the Study of Lung Cancer 2009
[h] Classification based on universal grading system for ovarian epithelian cancer
[i] *PRDM2* expression level is decreased when compared to control
[j] Chi-square test; $X^2 = 12.00$
[k] Median fold difference = 0.08 (interquartile range 0.03-0.50)
[l] Wilcoxon matched pair test
[m] Student's t-test or one-way analysis of variance, followed by Newman-Keuls test

Table 1: Study characteristics of studies included in systematic review.

F = Female. IHC = Immunohistochemistry. LAC = Lung adenocarcinoma. LSCC = Lung squamous cell carcinoma. M = Male. ND = Not determined. PRDM2 = Positive Regulatory/Su(var)3-9, Enhancer-of-zeste and Trithorax Domain 2. qRT-PCR = Quantitative reverse transcription-polymerase chain reaction. RT-PCR = Reverse transcription-polymerase chain reaction.

Table 2(on next page)

Study characteristics of studies included in meta-analysis.

FN = False negative. FP = False positive. IHC = Immunohistochemistry. LAC = Lung adenocarcinoma. LSCC = Lung squamous cell carcinoma. qRT-PCR = Quantitative reverse transcription-polymerase chain reaction. TN = True negative. TP = True positive.

Study	Method	No. of sample	No. of control	Cancer type	TP	FP	FN	TN
Akahira et al. (2007)	IHC	164	6	Ovarian cancer	110	0	54	6
Dong et al. (2012)	IHC	12	12	Esophageal squamous cell carcinoma	12	4	0	8
Geli et al. (2005)	qRT-PCR	11	6	Pheochromocytoma (n=4) Abdominal paraganglioma (n=7)	9	0	2	6
Jiang et al. (1999)	qRT-PCR	4	3	Hepatoma	4	1	0	2
Tan et al. (2018)	IHC	75	150	LSCC (n=52) LAC (n=23)	22	10	53	140

Table 2: Study characteristics of studies included in meta-analysis.

FN = False negative. FP = False positive. IHC = Immunohistochemistry. LAC = Lung adenocarcinoma. LSCC = Lung squamous cell carcinoma. qRT-PCR = Quantitative reverse transcription-polymerase chain reaction. TN = True negative. TP = True positive.

Table 3(on next page)

Quality Assessment of Diagnostic Accuracy Studies – 2 (QUADAS-2) risk of bias assessment.

Table 3: Quality Assessment of Diagnostic Accuracy Studies – 2 (QUADAS-2) risk of bias assessment.

Study	Quality Assessment of Diagnostic Accuracy Studies - 2 (QUADAS-2)						
	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Akahira et al. (2007)	Low	Low	Low	Low	Low	Low	Low
Dong et al. (2012)	Low	Low	Low	Low	Low	Low	Low
Ge et al. (2015)	Low	Unclear	Low	Low	Low	Low	Low
Geli et al. (2005)	Low	Unclear	Low	Low	Low	Low	Low
Jiang et al. (1999)	Unclear	Unclear	Low	Low	Low	Low	Low
Tan et al. (2018)	Low	Unclear	Low	Low	Low	Low	Low

Table 4(on next page)

Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) evidence profile for the studies included in the meta-analysis.

ND = Not determined. QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies – 2.

TSA = Trial sequential analysis.

Table 4: Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) evidence profile for the studies included in the meta-analysis.

			Grading of Recommendations, Assessment, Development, and Evaluations (GRADE)					
Outcome	**No. of studies**	**Design**	**Risk of bias**	**Indirectness**	**Inconsistency**	**Imprecision**	**Publication bias**	**Quality of evidence**
True positives (patients/samples with solid tumor)	5 studies (443 patients/samples)	Cross-sectional studies	Not serious	Serious[1]	Not serious	Serious[2]	ND[3]	⨁◯◯◯ Very low
False negatives (patients/samples incorrectly classified as not having solid tumor)	5 studies (443 patients/samples)	Cross-sectional studies	Not serious	Serious[1]	Not serious	Serious[2]	ND[3]	⨁◯◯◯ Very low
True negatives (patients/samples without solid tumor)	5 studies (443 patients/samples)	Cross-sectional studies	Not serious	Serious[1]	Not serious	Serious[2]	ND[3]	⨁◯◯◯ Very low
False positives (patients/samples incorrectly classified as having solid tumor)	5 studies (443 patients/samples)	Cross-sectional studies	Not serious	Serious[1]	Not serious	Serious[2]	ND[3]	⨁◯◯◯ Very low

[1] All samples undergo both index test and reference standard, introducing indirectness into the studies.
[2] Most of the individual studies have a wide confidence interval and inconclusive TSA results.
[3] Publication bias could not be evaluated as the number of studies is <10.

ND = Not determined. QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies – 2. TSA = Trial sequential analysis.