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The prognostic value of cyclin D1 expression in the survival of cancer patients: A meta-analysis

Maryam Moradi Binabaj[1,2*], Afsane Bahrami[3*], Majid Khazaei[4,5], Mikhail Ryzhikov[6], Gordon A Ferns[7], Amir Avan[5,8,9], Seyed Mahdi Hassanian[1,5#]

1) Department of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2) Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3) Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.
4) Department of Medical Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5) Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
6) Division of Pulmonary and Critical Care Medicine, Washington University, School of Medicine, Saint Louis, MO, USA.
7) Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.
8) Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
9) Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

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* Equally contributed as first author.

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 # Corresponding Author:
Seyed Mahdi Hassanian, Ph.D.
Department of Medical Biochemistry
Faculty of Medicine, Mashhad University of Medical Sciences
Mashhad, Iran.
Phone: (+98) 5138002375, Fax: (+98) 5138002389
E-mail: hasanianmehrm@mums.ac.ir

Abstract

The association between cyclin D1 over-expression with cancer prognosis and outcomes is controversial in different malignancies. In the presented meta-analysis we aim to comprehensively assess the relationship between tissue cyclin D1 expression levels and overall survival (OS) in diverse cancers. PubMed, EMBase, Scopus, Web of Sciences and Cochrane Library database were searched to explore eligible studies. For prognostic meta-analysis, study-specific hazard ratios (HRs) of tissue cyclin D1 for survival were obtained. One hundred and fifteen studies with a total of 20,253 patients with 10 different cancer types were included in this meta-analysis. In pooled analysis, high expression of cyclin D1 was significantly associated with poor OS with a combined HR of 1.09 (95% CI: 1.01-1.19, P=0.034; random-effects). However, the sub-group analysis showed that elevated cyclin D1 expression was only associated with worse OS of head and neck cancers (HR=2.08, 95% CI: 1.75-2.47; P<0.001) but not in breast (HR=1.11, 95% CI: 0.93-1.32, P= 0.241), gastrointestinal (HR = 0.92, 95% CI:0.76-1.11; P=0.390), bladder (HR=0.94, CI: 0.84-1.04; P=0.225) and in lung cancer patients (HR=1.08, CI: 0.80-1.45; P=0.613). Further large, prospective, and well-designed trials are warranted to determine the exact clinical significance of cyclin D1 over-expression for prognosis of cancer patients receiving treatment regimens.

Keywords: Cyclin D1, Prognosis, Cancer, Survival

Introduction

The transition from the different check-points of the cell cycle is controlled by cyclin-dependent kinases (cdks), which are bound to their cyclin co-partners (1). Cyclin D1 (CycD1) as one of the D cyclins amplified in the G1 phase, coordinates the G1/S phase transition and DNA synthesis which in complex with cdk4 enhances phosphorylation of the retinoblastoma protein (pRb). Dephosphorylated Rb binds to transcription factors including that of the E2F family and inhibits their functions, whereas hyper-phosphorylated Rb dissociates from E2F, enhancing G1 to S transition (2). CycD1 is composed of 295 amino acids and was initially isolated as the PRAD-1 putative oncogene. The *CCND1* gene, encoding human cyclin D, is located at chr.11q13 and is frequently deregulated through chromosomal rearrangements, inversion, translocation, and over-expression.

There are several studies showing that amplification of the *CCND1* gene and/or CycD1 over-expression is closely associated with outcome of patients with multiple types of malignant tumors (3). Consistent amplification of *CCND1* affects the regulation of CycD1, leading to cell cycle disruption, growth promotion and carcinogenesis *CCND1* amplification has been found in numerous human tumor types, and it is a key determinant of the behavior of malignancies, such as aggressiveness and high proliferative activity of the tumor (4). *CyclinD1* gene is amplified in nearly 22-58% of several human malignancies and is closely related to overall survival in cancer patients, supporting the prognostic value of CycD1 in cancer patients (5, 6). However, the published findings are somehow controversial which could be due to relatively small study populations, low statistical power, and clinic-pathological heterogeneity. Thus, we conducted a meta-analysis to quantify prognostic value of CycD1 over-expression in patients with various solid tumors based on all eligible published studies to clarify this question.

Materials and Methods

To find all related articles investigating the prognostic value of CycD1 over-expression in human cancers, we conducted a computerized literature search of PubMed, EMBase, Scopus, Web of Sciences and Cochrane Library database using the terms (Cyclin D1 OR Cyclin D) AND (prognosis OR prognostic OR outcome OR mortality OR survival) AND (cancer OR tumor OR malignancy OR neoplasm). The search was limited to human studies published in English. Reference lists of potential studies were also checked again to make sure that no relevant publications are missed. A flow diagram of the literature research is presented in Figure 1.

Inclusion and exclusion criteria

Studies were identified eligible if they met the following criteria: (1) they studied the patients with different solid tumors; (2) they measured the expression of CycD1 in tissue samples; (3) they explored the association between CycD1 expression levels and cancer survival. *In vitro* studies, duplicated or unqualified data, reviews, letters, conference abstracts, case reports or articles which do not provide sufficient information to calculate the HR about overall survival were excluded.

Data extraction

Data were carefully assessed and extracted independently from the eligible articles by two investigators (M.M.B. and A.B.). The following data were gathered: first author's name, publication year, country, cancer type, sample type, stage, sample size, follow ups and hazard ratios (HRs) of CycD1 for overall survival (OS) with their 95% confidence intervals (CIs) and P value. When disagreement occurred between the two researchers, corresponding author (S.M.H) was invited to discuss and reach consensus.

Statistical methods

Heterogeneity was assessed using Q statistics and Higgins I^2 statistic (I^2 > 50% or P < 0.05 was considered heterogeneous) (7). If heterogeneity among the studies was not significant, fixed-effects model was applied. Otherwise, significant heterogeneity was resolved by using the random-effects model (8). The effect of CycD1 expression on OS was estimated by forest plots. Sub-group analysis of pooled HRs of cancer patients with high CycD1 expression was investigated with regard to the cancer type. In pooled HR, association between CycD1 over-expression with prognosis was considered statistically significant if the 95% CI did not encompass 1. Publication bias was assessed through the symmetry of a funnel plot and Egger's test, P>0.05 was considered representative of no publication bias (9). All analyses were conducted using the comprehensive meta-analysis software version 2 (Biostat, Inc., Englewood, NJ) (10).

Results

Study descriptions

An initial search retrieved 565 potentially relevant publications, and after checking eligibility and exerting inclusion and exclusion criteria, a total of 115 qualified articles remained for the final analysis. These eligible articles were published between 1995 to 2017. The minimum and maximum numbers of patients in these studies were 40 and 1785, respectively.

Study characteristics

One hundred and fifteen publications involving 20,253 subjects with different types of solid tumors were analyzed. The baseline characteristics of all studied populations are presented in Table 1. By cancer type, 10 different tumors including 7 oral cancer (OC), 4 head and neck squamous cell carcinoma (HNSCC), 11 esophageal cancer (EC), 24 lung cancer (LC), 34 breast cancer (BC), 22 (CRC), 10 bladder cancer (BIC), 1 hepatocellular carcinoma (HCC), 1

gastric cancer (GC) and 1 melanoma were studied. Regionally, study groups were conducted in 20 different countries including Switzerland (n=3), Italy (n=6), Korea (n=6), Greece (n=4), Spain (n=5), USA (n=12), Japan (n=21), China/Taiwan (n=2), Germany (n=5), China (n=11), Austria (n=3), Poland (n=4), Canada (n=1), Sweden (n=4), Netherlands (n=2), Norway (n=3), Finland (n=2), France (n=1), Taiwan (n=3), and Egypt (n=1). By detection method, various techniques were used including 104 immunohistochemistry (IHC), 6 fluorescence in situ hybridization (FISH), 6 PCR, 1 western blotting (WB), 1 chromogenic in situ hybridization (CISH), 1 slot blot hybridization (SBH), and 1 microarray. Based on sample size, studies were conducted in <100 patients (n=40), 100-200 patients (n=50), and >200 (n=25).

Overall association

In the pooled analysis, high expression of CycD1 was significantly associated with poor OS with a combined HR of 1.09 (95% CI: 1.01-1.19, P=0.034; Figure 2). The random-effects model was used due to the high heterogeneity [I^2 = 75.2%; Q = 471.8; degrees of freedom (df) = 117; P<0.001] in the pooled analysis,

Sub-group analysis

Because of the significant heterogeneity between the association of CycD1 expression level and OS of cancer patients, we conducted sub-group meta-analysis to explore whether the heterogeneity is due to different cancer types or population size. In the stratified analysis by cancer types (Figure 3), CycD1 over-expression was associated with worse OS of head and neck cancers (11 studies; HR=2.08, 95% CI: 1.75-2.47; P<0.001; random effect model). However, CycD1 over-expression was not significantly associated with OS among breast cancer patients (34 studies; HR=1.11, 95% CI:0.93-1.32, P= 0.241; random effect), gastrointestinal cancer patients (35 studies; HR = 0.92, 95% CI:0.76-1.11; P=0.390; random effect), bladder cancer patients (10 studies; HR=0.94, 95%CI:0.84-1.04; P=0.225; random

effect) and lung cancer patients (24 studies; HR=1.08, 95% CI:0.80-1.45; P=0.613; random effect). The result of Egger's test indicated no publication bias among studies (Figure 4).

Discussion

The CCND1/pRb pathway is one of the crucial pathways regulating the cell cycle in human tumors. Over-expression of CycD1 contracts the G1 phase, reduces cell size, and decreases cellular dependency on mitogens *in vitro* and *in vivo* (11). CycD1 expression is altered in human malignancies, suggesting that its deregulation is implicated in tumorgenesis. The presence of both contradicting findings addressing the significance of CycD1 over-expression in different cancers made it necessary to conduct a quantitative assessment of the survival results. Our findings indicated that patients with CycD1-positive tumors had a lower survival rate compared to those with CycD1 negative tumors. The sub-group analysis suggested that elevated cyclin D1 expression was significantly associated with worse OS of head and neck cancers. However, CycD1 over-expression was not significantly associated with OS among patients with BC, LC, Blc and gastrointestinal cancers.

Consistent with our findings, it has been shown that the CycD1 gene is amplified in 12% to 68% and protein overexpressed in up to 80% of all HNSCC cases. Importantly, in a large number of tumors, over-expression of CycD1 does not reveal DNA amplification. It is believed that protein expression of CycD1 is more directly affected by increased CycD1 mRNA level than CycD1 gene amplification. Similarly, in a transgenic mice model, increased expression of CycD1 induces formation and development of dysplasic lesions in the oral cavity and esophagus region, suggesting that CycD1 over-expression could be associated with local invasiveness and a relatively aggressive behavior of head and neck tumors (12). Moreover, Nakashima *et al.* demonstrated that administration of anti-sense CycD1 cDNA suppresses cell growth and invasiveness of head and neck tumors in *in vivo* and *in vitro* models (13). Furthermore, it has

been shown that anti-tumor activities of flavopiridol are at least partially mediated by down-regulation of CycD1 in head and neck squamous cell carcinoma (14).

The significance of CycD1 in the head and neck tumors supports the therapeutic potency of CDK inhibitors in this cancer group. In line with this, several clinical trials have been completed or ongoing using cdks inhibitors in different advanced cancer patients (NCT00141297, NCT00147485, NCT00020189, and etc).

This meta-analysis has several limitations. First, only English language published studies were enrolled in our study. Second, our findings were based on study-level and not on individual case information. Individual case information could indicate more reliable estimates of the association. Third, in this meta-analysis, a random-effects model was predominantly used due to their significant heterogeneity. Variations in countries, tumor types, disease stages, therapeutic strategy, the cutoff values for CycD1 over-expression, small population size or mixed cancer analysis, variations in methodology and other variables might involve relatively high heterogeneity in the present study. Although IHC was the most frequently used method for detecting cyclin D1 in situ, other methods including RT-qPCR, FISH, PCR, WB, CISH, SBH, and microarray have also been used for the quantification of the levels of CycD1 mRNA expression in tumor tissue sections.

In conclusion, despite the limitations mentioned above, our meta-analysis demonstrates that the over-expression of CycD1 is associated with a poor prognosis in head and neck cancer patients and could be a potential prognostic indicator for this cancer type. However, several issues should be well resolved before using CycD1 as diagnostic and prognostic indicators in the clinical setting. Acquisition of specimens from non-invasive circulating biomarkers (plasma, serum or other body fluid) is more convenient than tissue samples. Moreover, the prognostic value of CycD1 in cancer patients should be assessed in the context of other related molecular biomarkers. For instance, compared to CycD1 alone, co-expression of CycD1, p21, EGFR, Bcl-2, PCNA and p53 could be a more reliable independent prognostic marker for predicting survival

(15). Well-designed investigations focused on specific cancer types and large target population sizes are recommended to confirm the prognostic importance of higher CycD1 levels in various malignancies.

References

1. Kenny FS, Hui R, Musgrove EA, Gee JM, Blamey RW, Nicholson RI, et al. Overexpression of cyclin D1 messenger RNA predicts for poor prognosis in estrogen receptor-positive breast cancer. Clinical Cancer Research. 1999;5(8):2069-76.

2. Chen W, Luo Y, Liu L, Zhou H, Xu B, Han X, et al. Cryptotanshinone inhibits cancer cell proliferation by suppressing Mammalian target of rapamycin-mediated cyclin D1 expression and Rb phosphorylation. Cancer Prev Res (Phila). 2010;3(8):1015-25.

3. Ortiz AB, Garcia D, Vicente Y, Palka M, Bellas C, Martin P. Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PloS one. 2017;12(11):e0188068.

4. Huang S-F, Cheng S-D, Chuang W-Y, Chen I-H, Liao C-T, Wang H-M, et al. Cyclin D1 overexpression and poor clinical outcomes in Taiwanese oral cavity squamous cell carcinoma. World journal of surgical oncology. 2012;10(1):40.

5. Loden M, Stighall M, Nielsen NH, Roos G, Emdin SO, Östlund H, et al. The cyclin D1 high and cyclin E high subgroups of breast cancer: separate pathways in tumorogenesis based on pattern of genetic aberrations and inactivation of the pRb node. Oncogene. 2002;21(30):4680.

6. Tut V, Braithwaite K, Angus B, Neal D, Lunec J, Mellon J. Cyclin D1 expression in transitional cell carcinoma of the bladder: correlation with p53, waf1, pRb and Ki67. British Journal of cancer. 2001;84(2):270.

7. Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in medicine. 2002;21(11):1539-58.

8. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled clinical trials. 1986;7(3):177-88.

9. Terrin N, Schmid CH, Lau J, Olkin I. Adjusting for publication bias in the presence of heterogeneity. Statistics in medicine. 2003;22(13):2113-26.

10. Borenstein M, Hedges L, Higgins J, Rothstein H. Comprehensive meta-analysis version 2. Englewood, NJ: Biostat. 2005;104.

11. Hitchings A, Kumar M, Jordan S, Nargund V, Martin J, Berney D. Prediction of progression in pTa and pT1 bladder carcinomas with p53, p16 and pRb. British journal of cancer. 2004;91(3):552.

12. Mueller A, Odze R, Jenkins TD, Shahsesfaei A, Nakagawa H, Inomoto T, et al. A transgenic mouse model with cyclin D1 overexpression results in cell cycle, epidermal growth factor receptor, and p53 abnormalities. Cancer research. 1997;57(24):5542-9.

13. Nakashima T, Clayman GL. Antisense inhibition of cyclin D1 in human head and neck squamous cell carcinoma. Archives of Otolaryngology–Head & Neck Surgery. 2000;126(8):957-61.

14. Patel V, Senderowicz AM, Pinto D, Igishi T, Raffeld M, Quintanilla-Martinez L, et al. Flavopiridol, a novel cyclin-dependent kinase inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis. The Journal of clinical investigation. 1998;102(9):1674-81.

15. McKay JA, Douglas JJ, Ross VG, Curran S, Murray GI, Cassidy J. Cyclin D1 protein expression and gene polymorphism in colorectal cancer. International journal of cancer. 2000;88(1):77-81.

16. Seiler R, Thalmann GN, Rotzer D, Perren A, Fleischmann A. CCND1/CyclinD1 status in metastasizing bladder cancer: a prognosticator and predictor of chemotherapeutic response. Modern pathology. 2014;27(1):87.

17. Galmozzi F, Rubagotti A, Romagnoli A, Carmignani G, Perdelli L, Gatteschi B, et al. Prognostic value of cell cycle regulatory proteins in muscle-infiltrating bladder cancer. Journal of cancer research and clinical oncology. 2006;132(12):757-64.

18. Lee K, Jung ES, Choi Y-J, Lee KY, Lee A. Expression of pRb, p53, p16 and cyclin D1 and their clinical implications in urothelial carcinoma. Journal of Korean medical science. 2010;25(10):1449-55.

19. Levidou G, Saetta AA, Karlou M, Thymara I, Pratsinis H, Pavlopoulos P, et al. D-type cyclins in superficial and muscle-invasive bladder urothelial carcinoma: correlation with clinicopathological data and prognostic significance. Journal of cancer research and clinical oncology. 2010;136(10):1563-71.

20. Lopez-Beltran A, Luque RJ, Alvarez-Kindelan J, Quintero A, Merlo F, Requena MJ, et al. Prognostic factors in survival of patients with stage Ta and T1 bladder urothelial tumors: the role of G1-S modulators (p53, p21Waf1, p27Kip1, cyclin D1, and cyclin D3), proliferation index, and clinicopathologic parameters. American journal of clinical pathology. 2004;122(3):444-52.

21. Sgambato A, Migaldi M, Faraglia B, De Aloysio G, Ferrari P, Ardito R, et al. Cyclin D1 expression in papillary superficial bladder cancer: Its association with other cell cycle-associated proteins, cell proliferation and clinical outcome. International journal of cancer. 2002;97(5):671-8.

22. Shariat SF, Bolenz C, Karakiewicz PI, Fradet Y, Ashfaq R, Bastian PJ, et al. p53 expression in patients with advanced urothelial cancer of the urinary bladder. BJU international. 2010;105(4):489-95.

23. Takagi Y, Takashi M, Koshikawa T, Sakata T, Ohshima S. Immunohistochemical demonstration of cyclin D1 in bladder cancers as an inverse indicator of invasiveness but not an independent prognostic factor. International Journal of Urology. 2000;7(10):366-72.

24. Yurakh AO, Ramos D, Calabuig-Fariñas S, López-Guerrero JA, Rubio J, Solsona E, et al. Molecular and immunohistochemical analysis of the prognostic value of cell-cycle regulators in urothelial neoplasms of the bladder. european urology. 2006;50(3):506-15.

25. Goto H, Kawano K, Kobayashi I, Sakai H, Yanagisawa S. Expression of cyclin D1 and GSK-3β and their predictive value of prognosis in squamous cell carcinomas of the tongue. Oral oncology. 2002;38(6):549-56.

26. Kuo MYP, Lin CY, Hahn LJ, Cheng SJ, Chiang CP. Expression of cyclin D1 is correlated with poor prognosis in patients with areaca quid chewing-related oral squamous cell carcinomas in Tiwan. Journal of oral pathology & medicine. 1999;28(4):165-9.

27. Mineta H, Miura K, Takebayashi S, Ueda Y, Misawa K, Harada H, et al. Cyclin D1 overexpression correlates with poor prognosis in patients with tongue squamous cell carcinoma. Oral oncology. 2000;36(2):194-8.

28. Shah NG, Trivedi TI, Tankshali RA, Goswami JV, Jetly DH, Shukla SN, et al. Prognostic significance of molecular markers in oral squamous cell carcinoma: a multivariate analysis. Head & neck. 2009;31(12):1544-56.

29. Shiraki M, Odajima T, Ikeda T, Sasaki A, Satoh M, Yamaguchi A, et al. Combined expression of p53, cyclin D1 and epidermal growth factor receptor improves estimation of prognosis in curatively resected oral cancer. Modern pathology. 2005;18(11):1482.

30. Vora HH, Shah NG, Patel DD, Trivedi TI, Chikhlikar PR. Prognostic significance of biomarkers in squamous cell carcinoma of the tongue: multivariate analysis. Journal of surgical oncology. 2003;82(1):34-50.

31. Anayama T, Furihata M, Ishikawa T, Ohtsuki Y, Ogoshi S. Positive correlation between p27Kip1 expression and progression of human esophageal squamous cell carcinoma. International journal of cancer. 1998;79(4):439-43.

32. Fukuchi M, Fukai Y, Kimura H, Sohda M, Miyazaki T, Nakajima M, et al. Prolyl isomerase Pin1 expression predicts prognosis in patients with esophageal squamous cell carcinoma and correlates with cyclinD1 expression. International journal of oncology. 2006;29(2):329-34.

33. Güner D, Sturm I, Hemmati P, Hermann S, Hauptmann S, Wurm R, et al. Multigene analysis of Rb pathway and apoptosis control in esophageal squamous cell carcinoma identifies patients with good prognosis. International journal of cancer. 2003;103(4):445-54.

34. Imamura M, Shimada Y, Ide H, Ozawa S, Kuwano H, Kato H, et al. Prognostic significance of CyclinD1 and E-cadherin in patients with esophageal squamous cell carcinoma: Multiinstitutional retrospective analysis. JOURNAL OF THE AMERICAN COLLEGE OF SURGEONS. 2001;192(6):708-18.

35. Kuwahara M, Hirai T, Yoshida K, Yamashita Y, Hihara J, Inoue H, et al. p53, p21 (Waf1/Cip1) and cyclin D1 protein expression and prognosis in esophageal cancer. Diseases of the Esophagus. 1999;12(2):116-9.

36. Nagasawa S, Onda M, Sasajima K, Makino H, Yamashita K, Takubo K, et al. Cyclin D1 overexpression as a prognostic factor in patients with esophageal carcinoma. Journal of surgical oncology. 2001;78(3):208-14.

37. Sarbia M, Stahl M, Fink U, Heep H, Dutkowski P, Willers R, et al. Prognostic significance of cyclin D1 in esophageal squamous cell carcinoma patients treated with surgery alone or combined therapy modalities. International journal of cancer. 1999;84(1):86-91.

38. Shinohara M, Aoki T, Sato S, Takagi Y, Osaka Y, Koyanagi Y, et al. Cell cycle-regulated factors in esophageal cancer. Diseases of the Esophagus. 2002;15(2):149-54.

39. Li H, Xiao W, Ma J, Zhang Y, Li R, Ye J, et al. Dual high expression of STAT3 and cyclinD1 is associated with poor prognosis after curative resection of esophageal squamous cell carcinoma. International journal of clinical and experimental pathology. 2014;7(11):7989.

40. Wang M-T, Chen G, An S-J, Chen Z-H, Huang Z-M, Xiao P, et al. Prognostic significance of cyclinD1 amplification and the co-alteration of cyclinD1/pRb/ppRb in patients with esophageal squamous cell carcinoma. Diseases of the Esophagus. 2012;25(7):664-70.

41. Yu Z, Weinberger PM, Haffty BG, Sasaki C, Zerillo C, Joe J, et al. Cyclin d1 is a valuable prognostic marker in oropharyngeal squamous cell carcinoma. Clinical Cancer Research. 2005;11(3):1160-6.

42. Anton RC, Coffey DM, Gondo MM, Stephenson MA, Brown RW, Cagle PT. The expression of cyclins D 1 and E in predicting short-term survival in squamous cell carcinoma of the lung. Modern Pathology. 2000;13(11):1167.

43. Jiping Z, Like Y, Ping Z, Yong S, Qin W. The relationships between cyclin D1 expression and prognosis of non-small cell lung cancer. Chinese Journal of Lung Cancer. 2010;13(8).

44. Sterlacci W, Fiegl M, Hilbe W, Jamnig H, Oberaigner W, Schmid T, et al. Deregulation of p27 and cyclin D1/D3 control over mitosis is associated with unfavorable prognosis in non-small cell lung cancer, as determined in 405 operated patients. Journal of thoracic oncology. 2010;5(9):1325-36.

45. Grossi F, Spizzo R, Bordo D, Cacitti V, Valent F, Rossetto C, et al. Prognostic stratification of stage IIIA pN2 non-small cell lung cancer by hierarchical clustering analysis of tissue microarray immunostaining data: an Alpe Adria Thoracic Oncology Multidisciplinary Group study (ATOM 014). Journal of thoracic oncology. 2010;5(9):1354-60.

46. Khoury T, Alrawi S, Ramnath N, Li Q, Grimm M, Black J, et al. Eukaryotic initiation factor-4E and cyclin D1 expression associated with patient survival in lung cancer. Clinical lung cancer. 2009;10(1):58-66.

47. Dworakowska D, Jassem E, Jassem J, Karmoliński A, Łapiński M, Tomaszewski D, et al. Prognostic value of the apoptotic index analysed jointly with selected cell cycle regulators and proliferation markers in non-small cell lung cancer. Lung Cancer. 2009;66(1):127-33.

48. Sánchez-Mora N, Presmanes MC, Monroy V, Moreno N, Lara-Martínez JM, Aladro MH, et al. Micropapillary lung adenocarcinoma: a distinctive histologic subtype with prognostic significance. Case series. Human pathology. 2008;39(3):324-30.

49. Li R, An S-J, Chen Z-H, Zhang G-C, Zhu J-Q, Nie Q, et al. Expression of cyclin D1 splice variants is differentially associated with outcome in non-small cell lung cancer patients. Human pathology. 2008;39(12):1792-801.

50. Gupta VK, Feber A, Xi L, Pennathur A, Wu M, Luketich JD, et al. Association between CCND1 G/A870 polymorphism, allele-specific amplification, cyclin D1 expression, and survival in esophageal and lung carcinoma. Clinical Cancer Research. 2008;14(23):7804-12.

51. Esposito V, Baldi A, De Luca A, Tonini G, Vincenzi B, Santini D, et al. Cell cycle related proteins as prognostic parameters in radically resected non-small cell lung cancer. Journal of clinical pathology. 2005;58(7):734-9.

52. Dworakowska D, Jassem E, Jassem J, Boltze C, Wiedorn KH, Dworakowski R, et al. Prognostic value of cyclin D1 overexpression in correlation with pRb and p53 status in non-small cell lung cancer (NSCLC). Journal of cancer research and clinical oncology. 2005;131(7):479-85.

53. Jian-qun Y, Jing-yao X, Jing Z, Qi-cai H, Jia Z, Cai-xia S. Expression and significance of cyclin D1, p27kip1 protein in bronchioloalveolar carcinoma. Journal of Zhejiang University-SCIENCE A. 2004;5(2):235-41.

54. Shah L, Walter KL, Borczuk AC, Kawut SM, Sonett JR, Gorenstein LA, et al. Expression of syndecan-1 and expression of epidermal growth factor receptor are associated with survival in patients with nonsmall cell lung carcinoma. Cancer. 2004;101(7):1632-8.

55. Au N, Cheang M, Huntsman D, Yorida E, Coldman A, Elliott W, et al. Evaluation of immunohistochemical markers in non-small cell lung cancer by unsupervised hierarchical clustering analysis: a tissue microarray study of 284 cases and 18 markers. The Journal of pathology. 2004;204(1):101-9.

56. Ratschiller D, Heighway J, Gugger M, Kappeler A, Pirnia F, Schmid R, et al. Cyclin D1 overexpression in bronchial epithelia of patients with lung cancer is associated with smoking and predicts survival. Journal of clinical oncology. 2003;21(11):2085-93.

57. Liang R, Liao Z, Jiang S, Zhang W, Li J, Zheng D. Expression of cyclin D1 and vascular endothelial growth factor (VEGF) in non-small cell lung carcinoma and their association with the prognosis. Ai zheng= Aizheng= Chinese journal of cancer. 2003;22(1):86-90.

58. Jin M, Inoue S, Umemura T, Moriya J, Arakawa M, Nagashima K, et al. Cyclin D1, p16 and retinoblastoma gene product expression as a predictor for prognosis in non-small cell lung cancer at stages I and II. Lung cancer. 2001;34(2):207-18.

59. Dosaka-Akita H, Hommura F, Mishina T, Ogura S, Shimizu M, Katoh H, et al. A risk-stratification model of non-small cell lung cancers using cyclin E, Ki-67, and ras p21: different roles of G1 cyclins in cell proliferation and prognosis. Cancer research. 2001;61(6):2500-4.

60. Nguyen VN, Miřejovský P, Miejovský T, Melínová L, Mandys V. Expression of cyclin D1, Ki-67 and PCNA in non-small cell lung cancer: prognostic significance and comparison with p53 and bcl-2. Acta histochemica. 2000;102(3):323-38.

61. Mishina T, Dosaka-Akita H, Kinoshita I, Hommura F, Morikawa T, Katoh H, et al. Cyclin D1 expression in non-small-cell lung cancers: its association with altered p53 expression, cell proliferation and clinical outcome. British journal of cancer. 1999;80(8):1289.

62. Keum J, Kong G, Yang S, Shin D, Park S, Lee J, et al. Cyclin D1 overexpression is an indicator of poor prognosis in resectable non-small cell lung cancer. British journal of cancer. 1999;81(1):127.

63. Nishio M, Koshikawa T, Yatabe Y, Kuroishi T, Suyama M, Nagatake M, et al. Prognostic significance of cyclin D1 and retinoblastoma expression in combination with p53 abnormalities in primary, resected non-small cell lung cancers. Clinical cancer research. 1997;3(7):1051-8.

64. Kawabuchi B, Moriyama S, Hironaka M, Fujii T, Koike M, Moriyama H, et al. p16 inactivation in small-sized lung adenocarcinoma: Its association with poor prognosis. International journal of cancer. 1999;84(1):49-53.

65. Caputi M, De LL, Papaccio G, D'Aponte A, Cavallotti I, Scala P, et al. Prognostic role of cyclin D1 in non small cell lung cancer: an immunohistochemical analysis. European journal of histochemistry: EJH. 1997;41(2):133-8.

66. Mylona E, Tzelepis K, Theohari I, Giannopoulou I, Papadimitriou C, Nakopoulou L. Cyclin D1 in invasive breast carcinoma: favourable prognostic significance in unselected patients and within subgroups with an aggressive phenotype. Histopathology. 2013;62(3):472-80.

67. Ahlin C, Lundgren C, Embretsén-Varro E, Jirström K, Blomqvist C, Fjällskog M-L. High expression of cyclin D1 is associated to high proliferation rate and increased risk of mortality in women with ER-positive but not in ER-negative breast cancers. Breast cancer research and treatment. 2017;164(3):667-78.

68. Pelosio P, Barbareschi M, Bonoldi E, Marchetti A, Verderio P, Caffo O, et al. Clinical significance of cyclin D1 expression in patients with node-positive breast carcinoma treated with adjuvant therapy. Annals of oncology. 1996;7(7):695-703.

69. Michalides R, Hageman P, Van Tinteren H, Houben L, Wientjens E, Klompmaker R, et al. A clinicopathological study on overexpression of cyclin D1 and of p53 in a series of 248 patients with operable breast cancer. British Journal of Cancer. 1996;73(6):728.

70. Guo L-L, Gao P, Wu Y-G, Jian W-C, Hao C-Y, Li H, et al. Alteration of cyclin D1 in Chinese patients with breast carcinoma and its correlation with Ki-67, pRb, and p53. Archives of medical research. 2007;38(8):846-52.

71. Bukholm IR, Bukholm G, Nesland JM. Over-expression of cyclin a is highly associated with early relapse and reduced survival in patients with primary breast carcinomas. International journal of cancer. 2001;93(2):283-7.

72. Lee A, Park WC, Yim HW, Lee MA, Park G, Lee KY. Expression of c-erbB2, cyclin D1 and estrogen receptor and their clinical implications in the invasive ductal carcinoma of the breast. Japanese journal of clinical oncology. 2007;37(9):708-14.

73. Lim S-C. Role of COX-2, VEGF and cyclin D1 in mammary infiltrating duct carcinoma. Oncology reports. 2003;10(5):1241-9.

74. McIntosh G, Anderson J, Milton I, Steward M, Parr A, Thomas M, et al. Determination of the prognostic value of cyclin D1 overexpression in breast cancer. Oncogene. 1995;11(5):885-91.

75. Millar E, Dean J, McNeil C, O'toole S, Henshall S, Tran T, et al. Cyclin D1b protein expression in breast cancer is independent of cyclin D1a and associated with poor disease outcome. Oncogene. 2009;28(15):1812.

76. Van Diest PJ, Michalides R, Jannink L, Van der Valk P, Peterse HL, De Jong JS, et al. Cyclin D1 expression in invasive breast cancer. Correlations and prognostic value. The American journal of pathology. 1997;150(2):705.

77. Lin S-Y, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y, et al. β-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proceedings of the National Academy of Sciences. 2000;97(8):4262-6.

78. Takano Y, Takenaka H, Kato Y, Masuda M, Mikami T, Saegusa M, et al. Cyclin D1 overexpression in invasive breast cancers: correlation with cyclin-dependent kinase 4 and oestrogen receptor overexpression, and lack of correlation with mitotic activity. Journal of cancer research and clinical oncology. 1999;125(8-9):505-12.

79. Umekita Y, Ohi Y, Sagara Y, Yoshida H. Overexpression of cyclinD1 predicts for poor prognosis in estrogen receptor-negative breast cancer patients. International journal of cancer. 2002;98(3):415-8.

80. Utsumi T, Yoshimura N, Maruta M, Takeuchi S, Ando J, Mizoguchi Y, et al. Correlation of cyclin D1 mRNA levels with clinico-pathological parameters and clinical outcome in human breast carcinomas. International journal of cancer. 2000;89(1):39-43.

81. García V, García JM, Peña C, Silva J, Domínguez G, Lorenzo Y, et al. Free circulating mRNA in plasma from breast cancer patients and clinical outcome. Cancer letters. 2008;263(2):312-20.

82. Peurala E, Koivunen P, Haapasaari K-M, Bloigu R, Jukkola-Vuorinen A. The prognostic significance and value of cyclin D1, CDK4 and p16 in human breast cancer. Breast Cancer Research. 2013;15(1):R5.

83. Rudas M, Lehnert M, Huynh A, Jakesz R, Singer C, Lax S, et al. Cyclin D1 expression in breast cancer patients receiving adjuvant tamoxifen-based therapy. Clinical Cancer Research. 2008;14(6):1767-74.

84. Choschzick M, Heilenkötter U, Lebeau A, Jaenicke F, Terracciano L, Bokemeyer C, et al. MDM2 amplification is an independent prognostic feature of node-negative, estrogen receptor-positive early-stage breast cancer. Cancer Biomarkers. 2011;8(2):53-60.

85. Wachter DL, Fasching PA, Haeberle L, Schulz-Wendtland R, Dimmler A, Koscheck T, et al. Prognostic molecular markers and neoadjuvant therapy response in anthracycline-treated breast cancer patients. Archives of gynecology and obstetrics. 2013;287(2):337-44.

86. Bonnefoi H, Diebold-Berger S, Therasse P, Hamilton A, Van De Vijver M, MacGrogan G, et al. Locally advanced/inflammatory breast cancers treated with intensive epirubicin-based neoadjuvant chemotherapy: are there molecular markers in the primary tumour that predict for 5-year clinical outcome? Annals of oncology. 2003;14(3):406-13.

87. Chen S, Chen CM, Yu KD, Yang WT, Shao ZM. A prognostic model to predict outcome of patients failing to achieve pathological complete response after anthracycline-containing neoadjuvant chemotherapy for breast cancer. Journal of surgical oncology. 2012;105(6):577-85.

88. Hwang TS, Han HS, Hong YC, Lee HJ, Paik NS. Prognostic value of combined analysis of cyclin D1 and estrogen receptor status in breast cancer patients. Pathology international. 2003;53(2):74-80.

89. Elsheikh S, Green AR, Aleskandarany MA, Grainge M, Paish CE, Lambros MB, et al. CCND1 amplification and cyclin D1 expression in breast cancer and their relation with proteomic subgroups and patient outcome. Breast cancer research and treatment. 2008;109(2):325-35.

90. Husdal A, Bukholm G, Bukholm I. The prognostic value and overexpression of cyclin A is correlated with gene amplification of both cyclin A and cyclin E in breast cancer patient. Analytical Cellular Pathology. 2006;28(3):107-16.

91. Rodriguez C, Hughes-Davies L, Vallès H, Orsetti B, Cuny M, Ursule L, et al. Amplification of the BRCA2 pathway gene EMSY in sporadic breast cancer is related to negative outcome. Clinical cancer research. 2004;10(17):5785-91.

92. Al-Kuraya K, Schraml P, Torhorst J, Tapia C, Zaharieva B, Novotny H, et al. Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer research. 2004;64(23):8534-40.

93. Seshadri R, Lee C, Hui R, McCaul K, Horsfall DJ, Sutherland RL. Cyclin DI amplification is not associated with reduced overall survival in primary breast cancer but may predict early relapse in patients with features of good prognosis. Clinical cancer research. 1996;2(7):1177-84.

94. Reis-Filho JS, Savage K, Lambros MB, James M, Steele D, Jones RL, et al. Cyclin D1 protein overexpression and CCND1 amplification in breast carcinomas: an immunohistochemical and chromogenic in situ hybridisation analysis. Modern pathology. 2006;19(7):999.

95. Kirkegaard T, Nielsen K, Jensen L, Campbell F, Müller S, Tovey S, et al. Genetic alterations of CCND1 and EMSY in breast cancers. Histopathology. 2008;52(6):698-705.

96. Massidda B, Sini M, Budroni M, Atzori F, Deidda M, Pusceddu V, et al. Molecular alterations in key-regulator genes among patients with T4 breast carcinoma. BMC cancer. 2010;10(1):458.

97. Jirström K, Stendahl M, Rydén L, Kronblad Å, Bendahl P-O, Stål O, et al. Adverse effect of adjuvant tamoxifen in premenopausal breast cancer with cyclin D1 gene amplification. Cancer research. 2005;65(17):8009-16.

98. Feng Z, Guo W, Zhang C, Xu Q, Zhang P, Sun J, et al. CCND1 as a predictive biomarker of neoadjuvant chemotherapy in patients with locally advanced head and neck squamous cell carcinoma. PLoS One. 2011;6(10):e26399.

99. Kyomoto R, Kumazawa H, Toda Y, Sakaida N, Okamura A, Iwanaga M, et al. Cyclin-D1-gene amplification is a more potent prognostic factor than its protein over-expression in human head-and-neck squamous-cell carcinoma. International journal of cancer. 1997;74:576-81.

100. Åkervall JA, Michalides RJ, Mineta H, Balm A, Borg Å, Dictor MR, et al. Amplification of cyclin D1 in squamous cell carcinoma of the head and neck and the prognostic value of chromosomal abnormalities and cyclin D1 overexpression. Cancer. 1997;79(2):380-9.

101. Bova RJ, Quinn DI, Cole IE. Cyclin D1 expression predicts reduced survival in early stage carcinoma of the anterior tongue. Australian Journal of Oto-Laryngology. 2001;4(1):41.

102. Lu J-W, Lin Y-M, Chang J-G, Yeh K-T, Chen R-M, Tsai JJ, et al. Clinical implications of deregulated CDK4 and Cyclin D1 expression in patients with human hepatocellular carcinoma. Medical oncology. 2013;30(1):379.

103. Oba J, Nakahara T, Abe T, Hagihara A, Moroi Y, Furue M. Expression of c-Kit, p-ERK and cyclin D1 in malignant melanoma: an immunohistochemical study and analysis of prognostic value. Journal of dermatological science. 2011;62(2):116-23.

104. Shan YS, Hsu HP, Lai MD, Hung YH, Wang CY, Yen MC, et al. Cyclin D1 overexpression correlates with poor tumor differentiation and prognosis in gastric cancer. Oncology letters. 2017;14(4):4517-26.

105. Holland TA, Elder J, McCloud JM, Hall C, Deakin M, Fryer AA, et al. Subcellular localisation of cyclin D1 protein in colorectal tumours is associated with p21WAF1/CIP1

expression and correlates with patient survival. International journal of cancer. 2001;95(5):302-6.

106. Bahnassy AA, Zekri A-RN, El-Houssini S, El-Shehaby AM, Mahmoud MR, Abdallah S, et al. Cyclin A and cyclin D1 as significant prognostic markers in colorectal cancer patients. BMC gastroenterology. 2004;4(1):22.

107. Balcerczak E, Pasz-Walczak G, Kumor P, Panczyk M, Kordek R, Wierzbicki R, et al. Cyclin D1 protein and CCND1 gene expression in colorectal cancer. European journal of surgical oncology. 2005;31(7):721-6.

108. Eric JT, Brosens RP, Delis-van Diemen PM, Bril H, Tijssen M, van Essen DF, et al. Cell cycle proteins predict recurrence in stage II and III colon cancer. Annals of surgical oncology. 2012;19(3):682-92.

109. Bhatavdekar JM, Patel DD, Chikhlikar PR, Shah NG, Vora HH, Ghosh N, et al. Molecular markers are predictors of recurrence and survival in patients with Dukes B and Dukes C colorectal adenocarcinoma. Diseases of the colon & rectum. 2001;44(4):523-33.

110. Bondi J, Husdal A, Bukholm G, Nesland J, Bakka A, Bukholm I. Expression and gene amplification of primary (A, B1, D1, D3, and E) and secondary (C and H) cyclins in colon adenocarcinomas and correlation with patient outcome. Journal of clinical pathology. 2005;58(5):509-14.

111. Fang Y, Lu Z, Wang G, Pan Z, Zhou Z, Yun J, et al. Elevated expressions of MMP7, TROP2, and survivin are associated with survival, disease recurrence, and liver metastasis of colon cancer. International journal of colorectal disease. 2009;24(8):875.

112. Hilska M, Collan YU, Laine VJO, Kössi J, Hirsimäki P, Laato M, et al. The significance of tumor markers for proliferation and apoptosis in predicting survival in colorectal cancer. Diseases of the colon & rectum. 2005;48(12):2197-208.

113. Jang KY, Kim YN, Bae JS, Chung MJ, Moon WS, Kang MJ, et al. Expression of cyclin D1 is associated with β-catenin expression and correlates with good prognosis in colorectal adenocarcinoma. Translational oncology. 2012;5(5):370-8.

114. Lyall MS, Dundas SR, Curran S, Murray GI. Profiling markers of prognosis in colorectal cancer. Clinical Cancer Research. 2006;12(4):1184-91.

115. Maeda K, Chung YS, Kang SM, Ogawa M, Onoda N, Nakata B, et al. Overexpression of cyclin D1 and p53 associated with disease recurrence in colorectal adenocarcinoma. International journal of cancer. 1997;74(3):310-5.

116. Mao Y, Li Z, Lou C, Zhang Y. Expression of phosphorylated Stat5 predicts expression of cyclin D1 and correlates with poor prognosis of colonic adenocarcinoma. International journal of colorectal disease. 2011;26(1):29-35.

117. McKay JA, Douglas JJ, Ross VG, Curran S, Loane JF, Ahmed FY, et al. Analysis of key cell-cycle checkpoint proteins in colorectal tumours. The Journal of pathology. 2002;196(4):386-93.

118. Moore HG, Shia J, Klimstra DS, Ruo L, Mazumdar M, Schwartz GK, et al. Expression of p27 in residual rectal cancer after preoperative chemoradiation predicts long-term outcome. Annals of surgical oncology. 2004;11(11):955-61.

119. Ogino S, Nosho K, Irahara N, Kure S, Shima K, Baba Y, et al. A cohort study of cyclin D1 expression and prognosis in 602 colon cancer cases. Clinical Cancer Research. 2009;15(13):4431-8.

120. Palmqvist R, Stenling R, Öberg Å, Landberg G. Expression of cyclin D1 and retinoblastoma protein in colorectal cancer. European Journal of Cancer. 1998;34(10):1575-81.

121. Pasz-Walczak G, Kordek R, Faflik M. P21 (WAF1) expression in colorectal cancer: correlation with P53 and cyclin D1 expression, clinicopathological parameters and prognosis. Pathology-Research and Practice. 2001;197(10):683-9.

122. Theocharis S, Giaginis C, Parasi A, Margeli A, Kakisis J, Agapitos E, et al. Expression of peroxisome proliferator-activated receptor-γ in colon cancer: correlation with histopathological parameters, cell cycle-related molecules, and patients' survival. Digestive diseases and sciences. 2007;52(9):2305-11.

123. Tsai HL, Yeh YS, Chang YT, Yang I-p, Lin CH, Kuo CH, et al. Co-existence of cyclin D1 and vascular endothelial growth factor protein expression is a poor prognostic factor for UICC stage I–III colorectal cancer patients after curative resection. Journal of surgical oncology. 2013;107(2):148-54.

124. Stockmar-Von Wangenheim V, Caroline A, Mönig SP, Schneider PM, Landsberg S, Drebber U, et al. p16, cyclin D1 and Rb expression in colorectal carcinomas: Correlations with clinico-pathological parameters and prognosis. Molecular medicine reports. 2008;1(1):27-32.

125. Wang Y, Xie C, Li Q, Xu K, Wang E. Clinical and prognostic significance of Yes-associated protein in colorectal cancer. Tumor Biology. 2013;34(4):2169-74.

126. Saridaki Z, Papadatos-Pastos D, Tzardi M, Mavroudis D, Bairaktari E, Arvanity H, et al. BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients' outcome. British journal of cancer. 2010;102(12):1762.

Figure legends:

Figure1. Flowchart of search strategy and selection process

Figure 2. Forest plot investigating the association between cyclin D1 expression and overall survival in all patients with different solid tumors

Figure 3. Forest plot studying the association between cyclin D1 expression and overall survival in patients with A) gastrointestinal cancer, B) breast cancer, C) head and neck cancers, D) bladder cancer, and E) lung cancer.

Figure 4. Funnel plot showing the association between cyclin D1 expression and overall survival of all patients with different solid tumors.

Table 1. Characteristics of eligible studies

Study number	Year	Country	Sample size	Stage	Follow-up duration (month)	Overall Survival	Technique	Sample
Bladder								
(16)	2014	Switzerland	152	cN0cM0	86.4	1.37(0.8-2.32)	FISH IHC	FFPE
(17)	2006	Italy	82	Muscle-invasive	21	0.73(0.37-1.45)	IHC	FFPE
(18)	2010	Korea	103	0-IV	31.5	1.98 (0.92-4.23)	IHC	FFPE
(19)	2010	Greece	157	Muscle-invasive	44.95	0.95(0.91-0.99)	IHC	FFPE
(20)	2004	Spain	159	Superficial	74.8	1.87(0.78-4.44)	IHC	FFPE
(21)	2002	Italy	96	Superficial	50	0.39(0.14-1.12)	IHC	FFPE
(22)	2007	USA	74	Superficial	42.3	0.62(0.26-1.47)	IHC	FFPE
(23)	2000	Japan	102	I-IV	41	0.46(0.21-0.99)	IHC	FFPE
(6)	2001	UK	150	I-IV	35	0.42(0.22-0.80)	IHC	FFPE
(24)	2006	Spain	84	I-IV	36.4	0.96(0.92-1.01)	IHC	Frozen tissue
Oral								
(4)	2012	China/ Taiwan	264	I-IV	46.5	1.70(1.17-2.48)	IHC	FFPE
(25)	2002	Japan	41	I-IV	36.3	1.87(0.14-24.24)	IHC	FFPE
(26)	1999	China/ Taiwan	88	I-IV	>60	2.06(1.48-2.62)	IHC	FFPE
(27)	2000	Japan	94	I-IV	>60	3.03(1.09-8.47)	IHC	FFPE
(28)	2009	India	135	I-IV	>24	1.28(0.65-2.52)	IHC	FFPE
(29)	2005	Japan	140	I-IV	66	1.68(0.84-3.34)	IHC	FFPE
(30)	2003	India	84	I-IV	60	2.04(1.09-3.83)	IHC	FFPE
Esophageal cancer								
(31)	1998	Japan	77	I-IV	60	2.44(1.16-5.12)	IHC	FFPE
(32)	2006	Japan	119	I-IV	60	2.07(1.12-3.83)	IHC	FFPE
(33)	2003	Germany	63	I-IV	48	2.70(1.279-5.714)	IHC	FFPE
(34)	2001	Japan	416	I-IV	24	1.42(1.04-1.94)	IHC	FFPE
(35)	1999	Japan	88	0-IV	60	2.47(1.01-6.06)	IHC	Tissue
(36)	2001	Japan	86	0-IV	<60	0.40(0.17-0.93)	IHC	FFPE
(37)	1999	Germany	172	I-IV	19	2.14(1.34-3.42)	IHC	FFPE
(38)	2002	Japan	144	0-IV	<80	0.69(0.39-1.23)	IHC	FFPE
(39)	2014	China	82	I-IV	1-39	2.11(1.029-4.347)	IHC	FFPE
(40)	2012	China	100	I-IV	NR	2.259(1.15-4.439)	IHC	Frozen tissue
(41)	2005	USA	63	I-IV	35	0.3(0.12-0.71)	IHC	FFPE
Lung								
(42)	2000	USA	130	I-IV	76	0.53(0.29-0.98)	IHC	FFPE

(43)	2010	China	115	I-IV	22	1.59(1.04-2.43)	IHC	FFPE
(44)	2010	Austria	390	I-IV	39.6	1.58(1.09-2.28)	IHC	FFPE
(45)	2010	Italy	87	IIIA pN2	140.4	0.22(0.12-0.42)	IHC	FFPE
(46)	2009	USA	147	I-IV	39.4	0.56(0.35-0.88)	IHC	FFPE
(47)	2009	Poland	166	I-IV	60	0.82(0.26-1.38)	IHC	FFPE
(48)	2008	Spain	67	I	3-120	4.05(1.46-11.25)	IHC	FFPE
(49)	2008	China	102	I-IV	23.5	2.79(1.47-5.28)	IHC	FFPE
(50)	2008	USA	69	I-IV	27.3	1.14(0.34-3.84)	RT-PCR	FFPE
(51)	2005	Austria	105	I-III	25	1.75(1.25-2.45)	IHC	FFPE
(52)	2005	Poland	111	I-IV	62	1.02(0.44-1.59)	IHC	FFPE
(53)	2004	China	43	I-IV	4-95	0.42(0.06-2.80)	IHC	FFPE
(54)	2004	USA	63	I-III	44	0.97(0.44-2.20)	IHC	FFPE
(55)	2004	Canada	284	NA	31.2	0.39(0.20-0.76)	IHC	FFPE
(56)	2003	UK	48	I-IV	18	0.13(0.03-0.62)	IHC	FFPE
(57)	2003	China	55	I-IV	4-80	1.57(1.02-2.46)	IHC	FFPE
(58)	2001	Japan	106	I-II	>18	3.93(1.68-9.22)	IHC	FFPE
(59)	2001	Japan	104	p-stage I	6-132	1.25(0.61-2.58)	IHC	FFPE
(60)	2000	1Czech	88	I-IV	24-36	2.21(1.30-3.75)	IHC	FFPE
(61)	1999	Japan	111	I-IV	6-84	0.16(0.02-1.22)	IHC	FFPE
(62)	1999	Korea	69	I-IIIA	0.5-108	2.13(1.28-3.56)	IHC	FFPE
(63)	1997	Japan	208	I-IIIB	<84	0.63(0.42-0.95)	IHC	FFPE
(64)	1999	Japan	51	I-IIIA	52.5	1.05(0.42-2.61)	IHC	FFPE
(65)	1997	Italy	60	NA	50	1.78(1.03-3.10)	IHC	FFPE
BC								
(66)	Total ER+ ER-	2013	Greece	364 242 115	NR	75	0.54(0.32-0.91) 3.1(1.3-7.1) 0.3(0.1-2.4)	IHC	FFPE
(67)	2017		Sweden	357	T1-2N0M0		0.94(0.63-1.4)	IHC FISH	FFPE
(3)	2017		Spain	179	I-IV	<120	0.12(0.035-0.44)	IHC	FFPE
(68)	1996		Italy	180	II-III	72	0.67(0.39-1.14)	IHC	FFPE
(69)	1996		Netherland	248	I-II	106	1.03(0.7-1.52)	IHC	FFPE
(70)	2007		China	140	I-III	60	2.05(1.08-3.89)	IHC	FFPE
(71)	2001		Norway	170	NR	58.8	0.76(0.33-1.78)	IHC	FFPE
(72)	2007		Korea	333	I-III	57	0.83(0.54-1.29)	IHC	FFPE
(73)	2003		Korea	128	I-III	1-116	0.31(0.14-0.69)	IHC	FFPE
(74)	1995		UK	93	NR	NR	1.1(0.3-4.05)	IHC	FFPE
(75)	2009		USA	150	NR	75	2.38(1.25-4.52)	IHC	Tissue
(76)	1997		USA	148	I-II	83	0.76(0.43-1.34)	IHC	FFPE
(77)	2000		USA	123	NR	48	1.72(1.01-2.94)	IHC	Tissue

(78)	1999	Japan	117	NR	<80	2.2(0.64-7.59)	IHC	FFPE
(79)	ER+ 2002	Japan	173	NR	86	3.45(1.22-9.81)	IHC	FFPE
	ER-					1.33(0.48-3.71)		
(1)	1999	UK	253	I-II	75	1.22(0.70-2.11)	NB	FFPE
(80)	2000	Japan	97	NR	40.8	0.43(0.16-1.19)	PCR	Frozen tissues
(81)	2008	Spain	129	I-III	50	2.89(0.92-9.04)	PCR	Plasma
(82)	2013	Finland	102	I-IV	<120	3.93(1.23-12.6)	IHC	FFPE
(83)	2008	Austria	253	NR	NR	2.47(1.08-5.03)	IHC	Tissue
(84)	2010	Germany	661	I-II	NR	1.38(0.87-2.2)	Microarray	Tissue
(85)	2012	Germany	100	NR	120	1.06(0.89-1.28)	IHC	FFPE
(86)	2003	Switzerland	187	III	66	0.91(0.58-1.43)	IHC	FFPE
(87)	2012	China	199	II-III	60	2.50(1.20-6.40)	IHC	FFPE
(88)	2003	Korea	175	I-III	>60	0.79(0.32-1.94)	IHC	FFPE
(89) ER+	2009	UK	301	I-IV	87	2.57(1.04-6.35)	IHC	FFPE
(90)	2006	Norway	82	NR	120	0.34(0.10-1.00)	RT-PCR IHC	FFPE
(91)	2004	France	296	NR	84	1.60(0.70-3.60)	SB	Tissue
(92)	2004	Switzerland	1785	I-IV	68	1.26(1.01-1.57)	FISH	FFPE
(93)	1996	Australia	1014	I-III	66	1.00(0.60-1.50)	SBH	Tissue
(94)	2006	UK	206	I-III	67	1.60(0.57-4.49)	IHC CISH	FFPE
(95)	2008	UK	115	I-III	77.4	1.80(1.05-3.07)	FISH	Tissue
(96)	2010	Italy	53	IIIB	125	0.75(0.26-2.12)	FISH	FFPE
(97)	2005	Sweden	280	II	168	0.41(0.22-0.75)	FISH IHC	FFPE
HNSCC								
(98)	2011	USA	100	III-IV	107	3.64(2.2-6.03)	IHC	FFPE
(99)	1997	Japan	45	I-IV	44.5	1.16(0.37-3.59)	PCR IHC	FFPE
(100)	1996	Sweden	75	I-IV	18	3(1.2-7.4)	IHC	FFPE
(101)	1999	Australia	147	I-IV	57	3.89(1.37-11.07)	IHC	FFPE
HCC								
(102)	2013	Taiwan	59	I-IV	51.6	0.69(0.38-1.26)	PCR IHC	frozen tissue
Melanoma								
(103)	2011	Japan	78	I-IV	40	1.10(0.29-4.13)	IHC	FFPE
GC								
(104)	2017	Taiwan	32	I-IV	NR	1.29(1.07-1.55)	WB	frozen tissue
CRC								

(105)	2001	UK	126	A-D*	28.56	0.24(0.07-0.81)	IHC	FFPE
(106)	2004	Egypt	60	I-IV	30	10.87(1.05-86.25)	IHC	FFPE
(107)	2005	Poland	111	A-D*	NR	0.74(0.33-1.68)	IHC	FFPE
(108)	2012	Netherland	386	II-III	NR	0.68(0.45-1.02)	IHC	FFPE
(109)	2001	India	98	B , C *	60	0.96(0.41-2.27)	IHC	FFPE
(110)	2004	Norway	219	A-D*	60	1.10(0.47-2.57)	IHC	FFPE
(111)	2009	China	620	I-IV	52	0.60(0.41-0.88)	IHC	FFPE
(112)	2005	Finland	363	A-D*	NR	1.09(0.67-1.78)	IHC	FFPE
(113)	2012	Korea	217	I-IV	NR	0.63(0.36-1.09)	IHC	FFPE
(114)	2006	UK	90	C*	60-100	0.84(0.37-1.95)	IHC	FFPE
(115)	1997	Japan	101	NR	60	0.73(0.22-2.38)	IHC	FFPE
(116)	2010	China	169	I-IV	92.1	0.47(0.25-0.87)	IHC	FFPE
(117)	2002	UK	249	A-D*	35	1.17(0.70-1.93)	IHC	FFPE
(118)	2004	USA	40	T3–4 or N1	69	0.83(0.13-5.30)	IHC	FFPE
(119)	2009	USA	602	I-IV	every 2 Years	0.67(0.48-0.92)	IHC	FFPE
(120)	1998	Sweden	90	A-C*	42	0.34(0.10-1.23)	IHC	FFPE
(121)	2001	Poland	122	I-IV	44.5	1.29(0.63-2.66)	IHC	FFPE
(122)	2007	Greece	86	A-D*	43	0.77(0.30-2.00)	IHC	FFPE
(123)	2013	Taiwan	100	I-III	30.5	0.37(0.16-0.84)	IHC	FFPE
(124)	2008	Germany	200	I-IV	>60	1.18(0.67-2.10)	IHC	FFPE
(125)	2013	China	139	I-IV	NR	0.41(0.19-0.90)	IHC	FFPE
(126)	2010	Greece	144	I-IV	NR	0.51(0.21-1.22)	IHC	FFPE

NR=Not reported, FISH=Fluorescence In situ Hybridization, WB=western blotting, CISH= Chromogenic In Situ Hybridization, HNSCC= Head and Neck Squamous Cell Carcinoma, HCC= hepatocellular carcinoma, GC= gastric cancer, ESCC = esophageal squamous cell carcinoma
SBH= Slot blot hybridization. PCR=Polymerase Chain Reaction, FFPE= formalin-fixed paraffin embedded, IHC=Immunohistochemistry.