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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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**# 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 (BlC), 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.

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**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 | 2013 | Greece | 364 | NR | 75 | 0.54(0.32-0.91) | IHC | FFPE |
| | ER+ | | | 242 | | | 3.1(1.3-7.1) | | |
| | ER- | | | 115 | | | 0.3(0.1-2.4) | | |
| (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** | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|
| (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.