# *KRAS* Codon 12 and Codon 13 Mutations in Relation to Overall Survival among Colorectal Cancer Patients: A Systematic Review and Meta-Analysis

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## Abstract

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Oncogenic mutations in *KRAS* codon 12 and codon 13 within exon 2 are frequently occurring mutations in colorectal cancer (CRC). However, the existing published data on the association of *KRAS* codon 12 and codon 13 mutations on the survival of CRC patients remains inconclusive. To address this, we conducted a systematic PubMed search and meta-analysis. Random-effects models were employed to quantify pooled association estimates and to investigate heterogeneity. Meta-regression was performed to examine sources of heterogeneity followed by subgroup analyses stratified by variables that significantly impact survival estimates. In total, this study involved a review of 502 articles, with 46 studies included in the meta-analysis. Compared to individuals with *KRAS* wild-type CRC, those CRC exhibiting a *KRAS* codon 12 or codon 13 mutation experienced significantly poorer overall survival (OS) [pooled hazard ratio (HR) = 1.40, 95% confidence interval (CI): 1.32–1.49], with heterogeneity in point estimates across studies (p-het = 0.0006). Meta-regression showed that whether or not study-specific estimates were adjusted for microsatellite

instability (MSI) status explained part of this heterogeneity ($p < 0.01$) and revealed that associations were more modest in the presence of adjustment for MSI status (pooled HR = 1.21, 95% CI: 1.07–1.36, 9 studies, p-het = 0.09) and stronger in the absence of MSI status adjustment with evidence of remaining heterogeneity (pooled HR = 1.45, 95% CI: 1.37–1.54, 37 studies, p-het = 0.03). Further meta-regression among these 37 studies not adjusted for MSI status showed that whether or not study-specific estimates were adjusted for stage explained part of the heterogeneity ($p = 0.01$) and showed that the associations were stronger among studies that adjusted for stage (pooled HR = 1.61, 95% CI: 1.45–1.79, 10 studies, p-het = 0.40) than among the studies that did not adjust for stage (pooled HR = 1.39, 95% CI: 1.30–1.48, 27 studies, p-het = 0.11). Overall, somatic *KRAS* codon 12 and codon 13 mutations were significantly associated with poorer OS, and results varied depending on whether studies adjusted for MSI status and stage, supporting the prognostic significance of these mutations in patients with CRC.

## Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer type, with approximately 2 million cases diagnosed globally in 2020, and the second most common cause of cancer death, responsible for 1 million deaths annually [1]. In the United States, CRC remains the third leading cause of cancer-related death in both men and women, with an estimated 52,550 deaths expected in 2023 [2]. The stages of CRC are categorized into stages I to IV according to the TNM system by the American Joint Committee on Cancer (AJCC). In the United States, the 5-year overall survival for CRC is 63% and 68% for colon cancer and rectal cancer, respectively [2]. However, survival differs substantially according to stage at diagnosis. When CRC is confined to a local area (stage I-II), 5-year survival is about 88%; however, when it spreads to the lymph nodes (stage III) or metastasizes to distant organs (stage IV), 5-year survival drops to roughly 71% and 16%, respectively [3].

Cancer originates through a gradual accumulation of genetic mutations, which ultimately results in an increase of cell proliferation [4-6]. Approximately 60-65% of CRC cases are thought to occur sporadically due to acquired somatic mutations and approximately 15-33% of cases may be linked to an inherited susceptibility to CRC [7-9]. *RAS* gene mutations are among the most frequent oncogenic alterations in cancers. In particular, *KRAS*, a key member of the *RAS* family, is the most frequently mutated gene, constituting 86% of all *RAS* mutations [10-12]. *KRAS*, under the regulation of tyrosine kinase receptors, activates the mitogen activated protein kinase (MAPK) signaling pathway, which is primarily responsible for cell proliferation. Thus, mutations in *KRAS* can enable cancer cells to thrive in lower glucose concentration than non-mutated cells [13, 14].

Mutations located in *KRAS* codon 12 and codon 13 of exon 2 are the most frequent oncogenic mutations in *KRAS*, accounting for nearly 90% of all *KRAS* mutations [15-17] and are observed among about 40% of CRC patients [18, 19]. Despite being highly mutated in CRC, targeting of *KRAS* codon 12 and/or 13 mutations for therapeutic intervention has proven to be challenging [20]. Unraveling the role of *KRAS* codon 12 and codon 13 mutations and their impact on CRC patient survival outcomes is of paramount importance, and has been the subject of numerous

publications to date [21-92]. However, the magnitude of the association of *KRAS* codon 12 and codon 13 mutations with patient survival outcomes remains inconclusive. Effect sizes may be inconsistent due to varying subgroup analyses (such as stage-, site-, or sex-stratified), as well as differences in confounder adjustment (such as adjustment for mutational burden or microsatellite instability (MSI) status, or factors like stage that may be on the causal pathway between mutation and survival). Additionally, some studies had small sample size that lacked statistical power and studies may have accounted for multiple comparisons differently when investigating more than one gene or conducting several stratified analyses. Therefore, a comprehensive assessment and analysis of the prognostic value of *KRAS* codon 12 and codon 13 mutations is necessary to determine what the overall association is between *KRAS* codon 12 and codon 13 mutations and CRC patient survival.

Our aim was to perform a systematic literature review and meta-analysis of the association of *KRAS* codon 12 and codon 13 mutation status and overall survival (OS) among CRC patients. We included 46 studies, including retrospective studies, prospective studies, clinical trials, and others [21-92]. We also determined what factors that are associated with observed heterogeneity across studies.

## Materials and Methods

### ***Search Strategy***

The literature review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [93]. A comprehensive search was conducted on PubMed. The two queries used were as follows:

Query 1: (KRAS) AND ((codon 12) OR (codon 13)) AND ((mutation) OR (mutant) OR (variant)) AND ((colorectal cancer) OR (rectal cancer) OR (colon cancer)) AND (survival), using the filters of “Humans” and “English”, encompassing records until May 13, 2023. As this search did not find all studies that we were aware of, we conducted a second query which included those studies in Query 2.

Query 2: (KRAS) AND ((mutation) OR (mutant) OR (variant)) AND ((colorectal cancer) OR (rectal cancer) OR (colon cancer)) AND (survival), using the filters of "Free full text", "Full text", "Classical Article", "Clinical Study", "Clinical Trial", "Clinical Trial, Phase I", "Clinical Trial, Phase II", "Clinical Trial, Phase III", "Clinical Trial, Phase IV", "Clinical Trial, Veterinary", "Comparative Study", "Controlled Clinical Trial", "Corrected and Republished Article", "Electronic Supplementary Materials", "Evaluation Study", "Introductory Journal Article", "Multicenter Study", "Observational Study", "Observational Study, Veterinary", "Randomized Controlled Trial", "Validation Study", "Humans", "English", excluding Preprints, and encompassing records until July 17, 2023.

***Eligibility Criteria***

Inclusion criteria were: 1) full-text articles on the effect of *KRAS* codon 12 and/or codon 13 mutations on CRC patient survival outcomes with a comparison of wild-type vs mutated tumors, or mutated vs wild-type tumors, including OS; (2) any study design, including retrospective, prospective observational studies, clinical trials, comparative study, evaluation study, or others; (3) the language of the publication was English; (4) subjects are human.

Exclusion criteria were: studies that were (1) unrelated to the association of *KRAS* codon 12 and/or codon 13 mutations and survival outcome; (2) lacked reporting on hazard ratio (HR), odds ratio (OR), or risk ratio (RR); (3) were systematic reviews; (4) were meta-analyses; (5) used the same patient cohort and data as another included study (i.e., duplicate data); (6) were irrelevant to the topic; and (7) were preprints.

***Data Extraction***

In addition to the aforementioned inclusion and exclusion criteria, the following information was extracted from the included publications: (1) country and data source, (2) years of CRC diagnoses among participants, (3) CRC stage at diagnosis, (4) number of participants, (5) study-specific frequency of *KRAS* mutation, (6) Cox proportional hazards model information [reference, model type (univariable or multivariable), HR, 95% CI, p-value, adjusted variables] for OS, (7) first author, (8) year of publication. The data extraction process involved utilizing a

pre-defined shared form, followed by its transfer into an Excel spreadsheet. Wild-type *KRAS* status was employed as the reference in all included studies, and any HR, OR, or RR originally based on codon 12/13 mutations as the reference was converted to utilize wild-type *KRAS* as the reference instead.

***Primary Outcomes***

In this study, we only focus on the association of *KRAS* codon 12 and codon 13 somatic mutations on OS among individuals with CRC. For the selection of appropriate OS for analysis, certain criteria were followed: (1) when a study presented both univariate and multivariate associations with OS, priority was given to the adoption of multivariate analysis results; (2) in instances where OS were reported for both codon 12 and codon 13 mutations separately, the preference was given to the mutation with higher occurrence, predominantly codon 12 mutation.

***Statistical Analysis***

We converted HR estimates to the log scale and calculated beta = ln(HR). We calculated the standard error (SE) based on SD/SE = (ln(upper limit) - ln(lower limit)) / (2*1.96). We created a regression table for all studies, using variables extracted from the adjusted multivariate regression models of all included studies for further quantitative analysis (Supplemental Table 1). We conducted initial quantitative analysis for all 48 included studies with OS. We used funnel plot visual asymmetry to assess possible publication bias, plotting effect sizes against study precision. We removed outliers according to visual inspection before proceeding with further analysis. For meta-regression analyses, we only considered variables present in three or more articles, including “year”, “adjust”, “stage”, “msi”, “rct”, “sex”, “age”, “tumor_type”, “tumor_size”, “site”, “chemotherapy”, “treatment”, “BRAF”, “patient_clinical_status”, “resection”, and “metastasis”. We conducted univariate regression analysis to identify what variable(s) were likely responsible for between-study heterogeneity in effect estimates. We performed subgroup analysis by stratifying any variables that showed significant impact on OS ($p < 0.05$).

We employed the random-effects model with restricted maximum likelihood to obtain pooled

HR estimates and confidence intervals. Heterogeneity was evaluated utilizing p-het. The statistical significance of the pooled estimates was determined based on the Wald test P value; a significance level of $p < 0.05$ (two-sided) was adopted to determine statistical significance. Data heterogeneity was visualized using Forest plots. We performed all analyses using RStudio 4.3.1 and RevMan Web.

## Results

### ***Study Selection***

Using PubMed Query 1, we retrieved 237 articles published before May 13, 2023 (Figure 1). After excluding articles without full text and evaluating those with full text, we excluded 193 articles unrelated to *KRAS* codon 12 or codon 13 mutation and survival outcomes among CRC patients, leaving 44 studies for further screening. With PubMed Query 2, we found 265 articles published before July 17, 2023 (Figure 1). After assessing full texts, we excluded 230 articles not specifically examining *KRAS* codon 12/13 mutation status in relation to OS. Removing 7 studies that overlapped with Query 1 left 28 articles for further screening. Thus, together, Query 1 and Query 2 yielded a total of 72 articles that underwent further screening. Following the removal of 7 duplicated studies from various trials or databases, we evaluated 65 studies for inclusion eligibility: after excluding 17 studies that lacked OS data, we included 48 studies in the initial qualitative and quantitative synthesis.

We employed funnel plot analysis to assess heterogeneity across the 48 initial studies (Figure 2). Li-Chen Yen et al.'s study (2010), where the ln(HR) was 1.91 (HR = 6.76), and Malapelle et al.'s study (2012), where the ln(HR) was 2.21 (HR = 9.09), were identified as outliers based on visual inspection of the funnel plot, and were consequently excluded from further analysis.

### ***Analysis for Effect of KRAS codon 12/13 Mutations on OS***

Within the primary meta-analysis, we included 46 studies to analyze OS in relation to *KRAS* codon 12 and codon 13 mutations (Figure 3). The results from the 46 studies indicated a significant association between *KRAS* codon 12 and codon 13 mutations and poorer OS, yielding a pooled HR = 1.40 (95% CI: 1.32–1.49), with statistically significant heterogeneity

among studies (p-het = 0.0006).

***Meta-regression Analysis***

To further explore the possible determinants of this heterogeneity, we performed a meta-regression analysis focusing on the potential factors influencing the observed magnitude of association between *KRAS* codon 12 or codon 13 mutation status and OS (Table 2). From the 46 studies, 16 variables were assessed in the univariate regression analysis. Notably, the analysis indicated that whether studies adjusted for MSI status or not was the only significant factor impacting the association between *KRAS* mutations and OS ($p < 0.01$).

***Subgroup Analysis***

We conducted a subgroup analysis stratified on whether studies adjusted for MSI or not (Figure 4). In the subgroup analysis, among the 9 studies that adjusted for MSI status, the associated heterogeneity was not statistically significant (p-het = 0.09) and the pooled HR was lower in magnitude, although still statistically significant (HR = 1.21, 95% CI: 1.07–1.36); among the 37 studies that did not adjust for MSI status, the associated heterogeneity was remained statistically significant (p-het = 0.03) and the pooled HR was similar to that in unstratified analyses (HR = 1.45, 95% CI: 1.37–1.54).

We further performed a univariate meta-regression analysis for these 37 studies to investigate if additional factors explain some of the remaining heterogeneity (Table 3). The results indicated that whether or not studies adjusted for CRC stage was the most influential factor on observed associations between *KRAS* mutations and OS ($p = 0.01$). We further conducted subgroup analysis among the 37 studies that did not adjust for MSI status according to whether they adjusted for stage (Figure 5). In the subgroup analysis of the 10 studies that adjusted for stage, the pooled HR was greater in magnitude (pooled HR = 1.61, 95% CI: 1.45–1.79, p-het = 0.40) than among the 27 studies that did not adjust for stage or MSI (pooled HR = 1.39, 95% CI: 1.30–1.48, p-het = 0.11). Upon stratification by stage, the heterogeneities of both subgroups were not statistically significant.

## Discussion

In this meta-analysis, we included 46 studies focusing on the association of somatic *KRAS* codon 12 and codon 13 mutation status in CRC with OS from diverse article types. We observed considerable heterogeneity among these studies (pooled HR = 1.40, 95% CI: 1.32–1.49, p-het = 0.0006). Univariate meta-regression analysis revealed that adjustment for MSI status was the most influential factor with respect to between-study heterogeneity ($p < 0.01$). Although associations with *KRAS* mutation status were stronger among studies not adjusting for MSI status (pooled HR = 1.45, 95% CI: 1.37–1.54, p-het = 0.03), *KRAS* mutation status was still significantly associated with poorer OS in studies that did adjust for MSI (pooled HR = 1.21, 95% CI: 1.07–1.36, p-het = 0.09). Further univariate meta-regression analysis indicated that adjustment for stage was the most influential factor with respect to between-study heterogeneity among those studies that did not adjust for MSI status ($p = 0.01$). Associations with *KRAS* mutation status were stronger among studies that did not adjust for MSI status but did adjust for stage (pooled HR = 1.61, 95% CI: 1.45–1.79, p-het = 0.40), compared to studies that did not adjust for MSI status or stage (pooled HR = 1.39, 95% CI: 1.30–1.48, p-het = 0.11).

Two previous meta-analyses have examined the relationship of *KRAS* mutation status with OS among individuals with CRC (Supplemental Table 2). Formica et al. (2022) analyzed 9 studies involving Phase III clinical trials for patients with stage II-III colon cancer (pooled HR = 1.33, 95% CI: 1.03–1.71, p-het = 0.03 for studies adjusting for MSI; pooled HR = 1.09, 95% CI: 0.70–1.69, p-het = 0.70 for studies not adjusting for MSI) [94]. Their meta-analysis showed a smaller unadjusted MSI estimate, which contrasts with our findings in this analysis. This variation between their and our results might be attributed to discrepancies in the number and composition of included studies. Specifically, the meta-analysis by Formica encompassed only 9 phase III clinical trials, while our analysis encompassed all stages of CRC patients, incorporating 46 studies. Kwak et al. (2017) analyzed 8 diverse studies, including observational, randomized controlled, and retrospective/prospective cohort designs, to assess the impact of *KRAS* codon 13 mutation on OS (unadjusted pooled HR = 1.37, 95% CI: 1.03–1.81, p-het = 0.002), with heterogeneity attributed to anti-EGFR drug administration [95], compared to our results (unadjusted pooled HR = 1.40 , 95% CI: 1.32–1.49, p-het = 0.0006) with heterogeneity

impacted by MSI status and stage at diagnosis.

Our study possesses several strengths. To begin with, our study focused solely on the most prevalent and oncogenic *KRAS* codon 12 and codon 13 mutations in CRC, preventing the dilution of effect size due to a mixture of other *KRAS* mutation types. Our meta-analysis also included more studies and has a larger sample size than prior meta-analyses, which can increase the statistical power of the results and provide a more comprehensive assessment of the impact of *KRAS* mutations on OS among individuals with CRC. Through meta-regression, we were also able to identify MSI status adjustment and stage adjustment as key factors in driving between-study heterogeneity.

Nevertheless, our study does come with certain limitations. We only reported pooled associations with OS in this study and did not examine associations with disease-specific survival due to the more limited availability of data on this more sensitive outcome in published studies. Based on the strength of observed associations between *KRAS* codon 12 or codon 13 mutation status and OS, and the fact that these somatic mutations are unlikely to impact risk from other causes of death, it is expected that associations would likely be stronger when based on these more sensitive outcomes.

We assessed the consistency of currently published work on *KRAS* codon 12 and codon 13 mutations in relation to OS among CRC patients. Overall, *KRAS* codon 12 and codon 13 mutations were significantly associated with poorer OS; however, this association may be impacted by heterogeneity, particularly MSI status and CRC stage.

**Table 1. Characteristics of Selected Studies**

| First Author | Year | Mutated Genes | Country & Data | Sample Period | Stage | Total # of Patient Included | Mutation Frequency (%) | Cox Proportional Hazards Models | Confounder |
|---|---|---|---|---|---|---|---|---|---|
| Pablo Gajate [21] | 2012 | *KRAS* p.G13D | (RCT) (Spain) Hospital Clínico San Carlos (HCSC) | Sept. 2003 - Oct. 2008 | 3-4 | 110 patients | NA | multivariable | age, sex, ECOG (Eastern Cooperative Oncology Group), treatment line, treatment |
| Tamuro Hayama [22] | 2019 | *KRAS* G12V or G12C | (Japan) Teikyo University Hospital | 2014 - 2016 | 1-3 | 200 samples | 37.50% | multivariable | stage |
| Luisa Foltran [23] | 2015 | *KRAS* 12/13 | (Italy) University Hospital, Udine | 1 Jan. 2004 - 31 Jan. 2010 | 4 | 194 tumors | 47.40% | multivariable | age at diagnosis, gender, cancer location, tumor stage and grade, use of radio- or chemotherapy, and treatment |
| Julien Taieb [24] | 2016 | *KRAS* codon 12 and p.G13D | (France) PETACC8 trial (EUDRACT 2005-003463-23), (USA) North Central Cancer Treatment Group (NCCTG) N0147 trials | | 3 | 4411 patients | 34.50% | multivariable | BRAF, sex, age, tumor, site, stage, treatment, ECOG |
| Jing Chen [26] | 2014 | *KRAS* | (China) Zhongda Hospital Affiliated to Southeast University | 2007 - 2012 | | 214 samples | 44.90% | univariable | |
| Neda Amini [28] | 2022 | *KRAS* | 10 tertiary academic centers in the United States, Europe, and | 2000 - 2018 | 4 | 482 patients | 38.00% | multivariable | clinical risk score, R1 resection, chemo |
| Robert P. Jones [29] | 2017 | *KRAS* | UK Cheshire and Merseyside Cancer Network | 2010 - 2015 | 1 - 4 | 392 patients | 42.90% | multivariable | stage and grade of tumor, curative intent surgery, pattern of metastasis |
| Tatsuki Ikoma [30] | 2021 | *KRAS* 12/13 | (Japan) | Aug. 2018 - July 2019 - July 2020 | 4 | 152 patients | 37.50% | | |
| Georgios A. Margonis [32] | 2015 | *KRAS* 12/13 | (USA) Johns Hopkins | 2003 - 2013 | | 331 patients | 27.50% | multivariable codon 13 | regional lymph node status, tumor size, number of lesions, resection margin status |
| J. Smeby [34] | 2018 | *KRAS* | (Germany) five first-line trials in mCRC: FIRE-1, FIRE-3 (only bevacizumab-arm), AIO KRK 0604, AIO KRK 0207, and RO91 | 2000 - 2013 | 3/4 | 664 tumors | 37% | Multivariate | age, MSI, stage, tumor, BRAF |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Matthew G. Summers [35] | 2017 | *KRAS* 12/13 | (UK) MRC clinical trials COIN (NCT00182715) and COIN-B (NCT00640081) | | | 2,157 patients | 40.00% | multivariable codon 13 | sex, age, site, metastasis |
| Aasma Shaukat [37] | 2012 | *KRAS* 12/13 | (USA) Minneapolis Veterans Administration Medical Center (MVAMC) | Jan. 1991 – Dec. 2004 | | 1,323 cases | 38% | | age, tumor grade, TNM Stage, MSI |
| Jing Hu [38] | 2016 | *KRAS* 12/13 | (China) Affiliated Drum Tower Hospital of Nanjing University Medical School | 2013 - 2015 | | 551 patients | 45% | multivariable | prognostic influence of tumor grade, the depth of the tumor, TNM stage |
| Susan D. Richman [41] | 2009 | *KRAS* 12/13 | (UK) FOCUS trial | 2000 - 2003 | | 711 patients | 40.5% | multivariable | thymidylate synthase and deoxyuridine triphosphatase immunohistochemistry, presence of liver metastases, tumor grade, baseline alkaline phosphatase, and age |
| Myung Hee Chang [43] | 2010 | *KRAS* 12/13 | (Korea) | Jan. 2004 - Oct. 2007 | 2 - 3 | 66 patients | 42.4% | | |
| Marta Schirripa [46] | 2014 | *KRAS* | (Italy) University Hospital of Pisa | 2009 - 2012 | 3-4 | 786 patients | 50.00% | multivariable | ECOG PS, site of primary tumor, liver-only disease, resected primary tumor (Y/N) and time to metastases |
| Hae Su Kim [47] | 2016 | *KRAS* 12/13 | (Korea) | Jan. 2008 - Jun. 2014 | | 82 patients | 37.80% | univariable G12/G13 | age, sex, stage, chemo |
| D. J. Hartman [49] | 2012 | *KRAS* p.G13D | (USA) University of Pittsburgh Medical Center | Jan. 2007 - May 2011 | 1 - 4 | 1,250 patients | | multivariable | clinical stage, tumor site, MSI |
| A. Fariña-Sarasqueta [51] | 2010 | *KRAS* 12/13 | (The Netherlands) four different regional hospitals | 1996 - 2004 | 2-3 | 364 patients | 33%-35% | multivariable | T stage, N stage, age, sex, tumor location, differentiation grade, BRAF, MSI |
| Li-Chen Yen [53] | 2010 | *KRAS* 12/13 | Taiwan | Jan. 2005 - Jan. 2008 | 3-4 | 95 patients | 24.2% | multivariable | tumors with EGFR overexpression |
| Klaus Kaczirek [54] | 2015 | *KRAS* 12/13 | (RCT) CECOG/CORE 1.2.002 study 22 sites in 12 countries | Sept. 2007 - Sept. 2009 | | 163 patients | | multivariable | Karnofsky performance status, age, sum of the longest diameter, prior therapy |
| John C. Smith [55] | 2013 | *KRAS* 12/13 | (UK) HORIZON I, trial | | | 623 patients | 43.00% | multivariable | liver function, chemotherapy type, WHO performance status |
| Thomas Winder [56] | 2009 | *KRAS* G12v | (Austria) Academic Teaching Hospital Feldkirch | Jan. 2003 - Oct. 2006 | 1 - 4 | 342 patients | 18.80% | multivariable | age, gender, tumor stage |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ya-Sian Chang [57] | 2009 | *KRAS* 12/13 | (Taiwan) | NA | 1 - 4 | 228 cases | 34.60% | multivariable 12/13 | tumor size, histologic grade, and lymph node metastasis |
| Zhizun Lin [58] | 2021 | *KRAS* 12/13 | (China) Fujian Provincial Hospital | Jun. 2010 - Jan. 2018 | 1 - 4 | 93 patients | 39.80% | multivariable 12/13 | resection, number of live metastases |
| Byeong Seok Sohn [60] | 2009 | *KRAS* 12/13 | (Korea) | Mar. 2005 - Jan. 2008 | 4 | 66 patients | 27.00% | multivariable | skin toxicity grade, prior chemotherapy lines |
| Amanda I Phipps [65] | 2013 | *KRAS* | (USA) (SEER) cancer registry serving Western Washington State | Apr. 2002 - July 2007 | 1-4 | 1989 cases | 31% | multivariable | age at diagnosis, sex, study population, body mass index, and history of cigarette smoking |
| Rafael G. Amado [66] | 2008 | *KRAS* 12/13 | (USA) (RCT) phase III mCRC trial | | | 427 patients | 43% | multivariable | sex, age, race, site, ECGO, chemotherapy |
| Shuji Ogino [67] | 2009 | *KRAS* 12/13 | (USA) (RCT) randomized adjuvant chemotherapy trial 1999–2001 (CALGB 89803) | Apr. 1999 - May 2001 | 3 | 508 cases | 35.00% | multivariable | age, sex, tumor location, tumor/node stage, performance status, adjuvant chemotherapy arm, MSI |
| Christophe Rosty [68] | 2013 | *KRAS* 12/13 | (Australia) The Melbourne Collaborative Cohort Study | 1990 - 1994 | | 776 patients | 28.00% | multivariable | mucinous differentiation, presence of a contiguous polyp, MSI, MGMT methylation |
| Keigo Chida [70] | 2021 | *KRAS* 12/13 | (Japan) four centers | Jan. 2005 - Dec. 2017 | | 1717 patients | 40.90% | multivariable | time of the first metastasis, metastatic site |
| Jian-Ming Xu [71] | 2014 | *KRAS* 12/13 | (China) Affiliated Hospital Cancer Center of the Academy of Military Medical Sciences | Jan. 2007 - Jun. 2011 | | 658 samples | 30.00% | multivariable | sex, age, ECOG, metastatic site |
| Tae-Woo Kim [72] | 2023 | *KRAS* 12/13 | (Korean) 3 hospitals | 2011 - 2013 | | 904 patients | 35.20% | multivariable | differentiation, MMR-D, age, stage |
| Janja Ocvirk [73] | 2010 | *KRAS* 12/13 | (RCT) phase II study involving 28 participating centers across 13 countries (CECOG/CORE1.2.001) | July 2005 - July 2006 | | 117 patients | 47.00% | univariable | |
| D. Klingbiel [74] | 2015 | *KRAS* 12/13 | (Belgium) (RCT) stage II and III PETACC-3 trial | | | 1254 patients | 38.30% | multivariable | stage, MSI, BRAF |
| Enric Domingo [75] | 2018 | *KRAS* 12/13 | (RCT) (Australia, Austria, Czech Republic, New Zealand, Serbia, Slovenia, and the UK) a phase 3 clinical trial (QUASAR 2)<br>(Australia) community-based validation cohort | Jan 1, 1993 - Dec 31, 2009 | 2/3 | | | multivariable | T stage, N stage, and treatment group |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Amanda I. Phipps [76] | 2020 | *KRAS* 12/13 | pooled data from the Cancer Prevention Study-II Nutrition cohort (CPS-II), the German Darmkrebs: Chancen der Verhutung durch Screening Study (DACHS), the Diet Activity and Lifestyle Study (DALS), the Health Professionals Follow-up Study (HPFS), the Melbourne Collaborative Cohort Study (MCCS), the Nurses' Health Study (NHS), and population-based sites from the Colon Cancer Family Registry (CCFR) | | | 5010 patients | 33.00% | multivariable | Set 1: age, sex, and study population<br>Set 2: stage, MSI, BRAF |
| U Malapelle [77] | 2012 | *KRAS* 12/13 | (Italy) | | | 50 patients | | univariable | |
| Beatrix Bencsikova [78] | 2015 | *KRAS* 12/13 | | | | 1310 patients | 58.30% | multivariable | multiple metastatic sites |
| Tae Won Kim [80] | 2018 | *KRAS* 12/13 | (Korea) (RCT) | | | 377 patients | | univariable | |
| Line S Tarpgaard [81] | 2015 | *KRAS* 12/13 | (Denmark) (RCT) The NORDIC VII Study | May 2005 - Oct. 2007 | | 453 patients | | multivariable | plasma, treatment arm, BRAF, serum, WHO PS, metastatic sites, age, sex |
| Efrat Dotan [82] | 2012 | *KRAS* 12/13 | (USA) (RCT) | Jun. 2006 - May 2008 | | 20 patients | 25.00% | univariate | |
| Eduardo Díaz-Rubio [83] | 2012 | *KRAS* 12/13 | (Spain) (RCT) MACRO study | July 2006 - Sept. 2008 | | 394 patients | 44.40% | multivariable | ECOG, metastatic sites, lactate dehydrogenase, alkaline phosphatase, sex, treatment, chemotherapy, resection |
| Jeong Mo Bae [85] | 2015 | *KRAS* 12/13 | (Korea) Seoul National University Hospital | Jan. 2004 - Dec. 2007 | 4 | | | univariate | |
| A Ålgars [86] | 2011 | *KRAS* 12/13 | (Finland) Turku University Hospital | | | 80 patients | 30.00% | univariate | |
| Melanie Poulin-Costello [87] | 2013 | *KRAS* 12/13 | (Canada) (RCT) phase3, randomized, controlled, multicenter trial (20020408) | Jan. 2004 - Jun. 2005 | 3 | 463 patients | | multivariable | age, sex, tumor site, ECOG. |
| Masayuki Okuno [88] | 2020 | unknown | (Japan) (RCT) University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) UMIN000004310 | Mar. 2011 - Aug. 2013 | | 34 patients | | multivariable | sidedness of primary tumor, R0/R1 hepatectomy |
| Melissa Kang [90] | 2015 | *KRAS* 12/13 | (USA) Cancer Care Outcomes Research and Surveillance Consortium (CanCORS) | | | 204 patients | 13.00% | multivariable | age, sex, anatomic site, MSI, chemotherapy |

**Table 2. Univariable Meta-regression for Overall Survival: 46 Studies Included**

| Variable | p-value | |
|---|---|---|
| year | 0.73 | |
| adjust | 0.34 | |
| stage | 0.68 | |
| msi | < **0.01** | *** |
| rct | 0.18 | |
| sex | 0.57 | |
| age | 0.32 | |
| tumor_type | 0.53 | |
| tumor_size | 0.14 | |
| site | 0.96 | |
| chemotherapy | 0.27 | |
| treatment | 0.29 | |
| BRAF | 0.52 | |
| patient_clinical_status | 0.22 | |
| resection | 0.18 | |
| metastasis | 0.93 | |

**Table 3. Univariable Meta-regression for Overall Survival among 37 Studies Without MSI Status Adjustment**

| Variable | p-value | |
|---|---|---|
| year | 0.91 | |
| adjust | 0.11 | |
| stage | **0.01** | *** |
| rct | 0.22 | |
| sex | 0.55 | |
| age | 0.29 | |
| tumor_type | 0.17 | |
| tumor_size | 0.21 | |
| site | 0.94 | |
| chemotherapy | 0.13 | |
| treatment | 0.24 | |
| BRAF | 0.97 | |
| patient_clinical_status | 0.20 | |
| resection | 0.35 | |
| metastasis | 0.19 | |

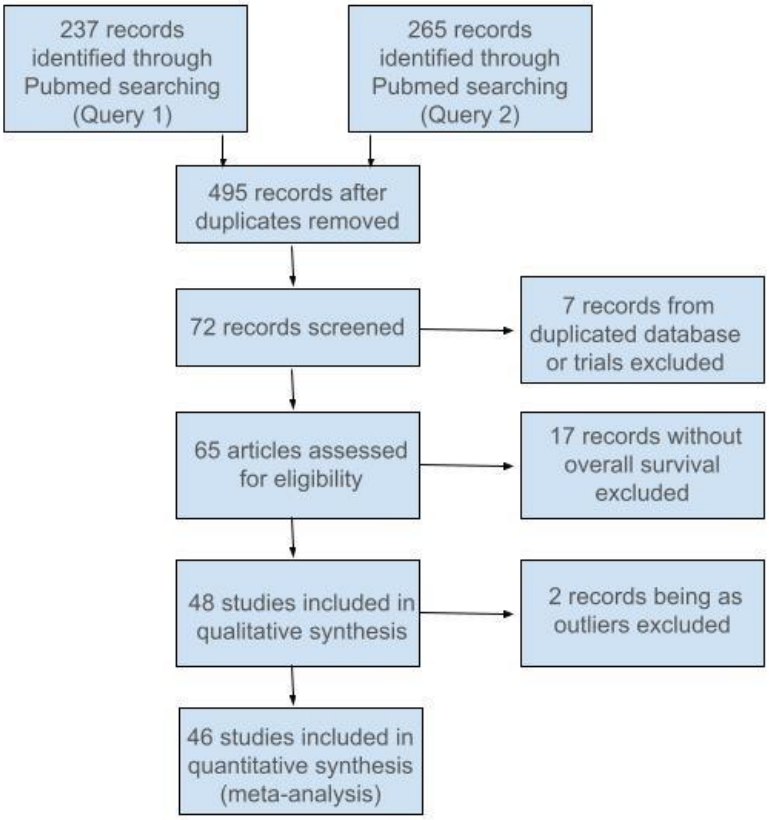


**Figure 1. Flow Diagram Depicting the Process of Study Selection for Meta-analysis**

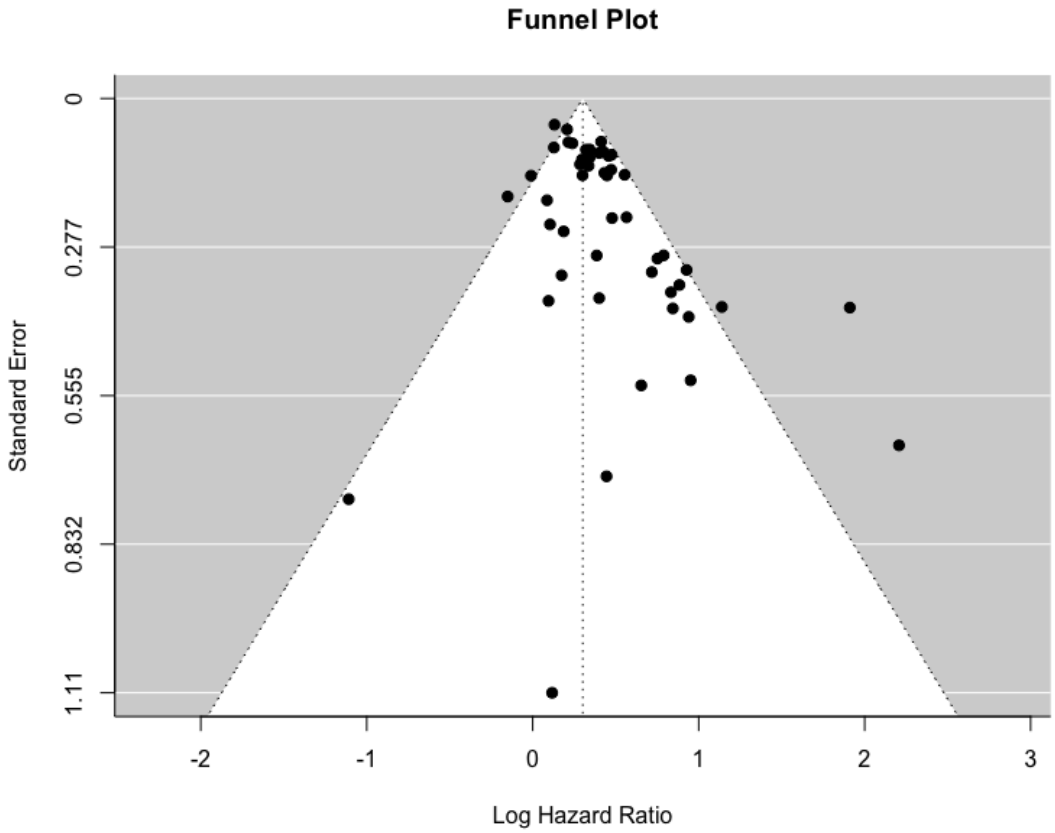


**Figure 2. Funnel Plot: 48 Studies Included**

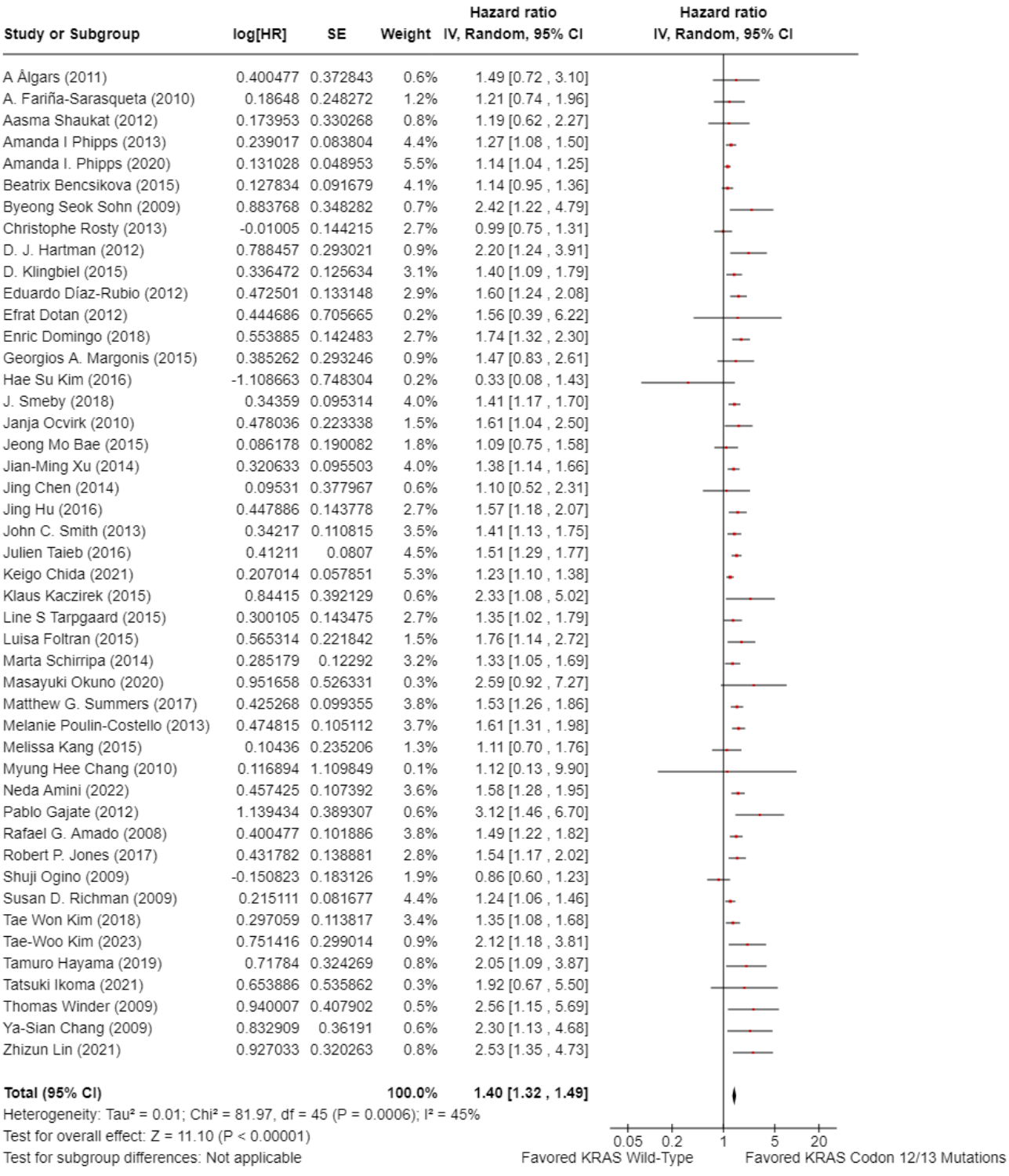


**Figure 3. Forest Plot for Overall Survival: 46 Included Studies**

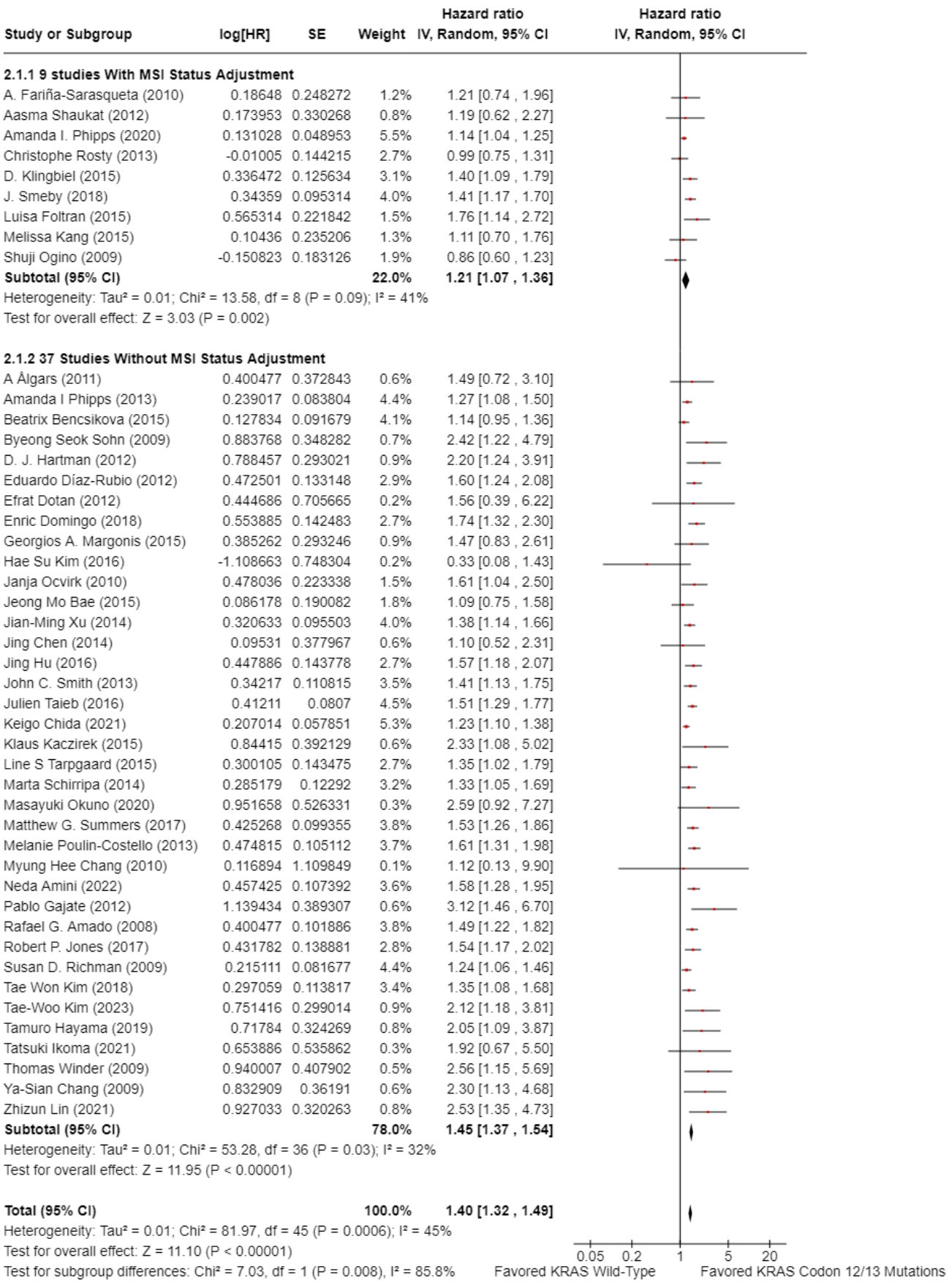


**Figure 4. Forest Plot for Overall Survival Stratified by Adjusting for MSI Status among 46 Studies**

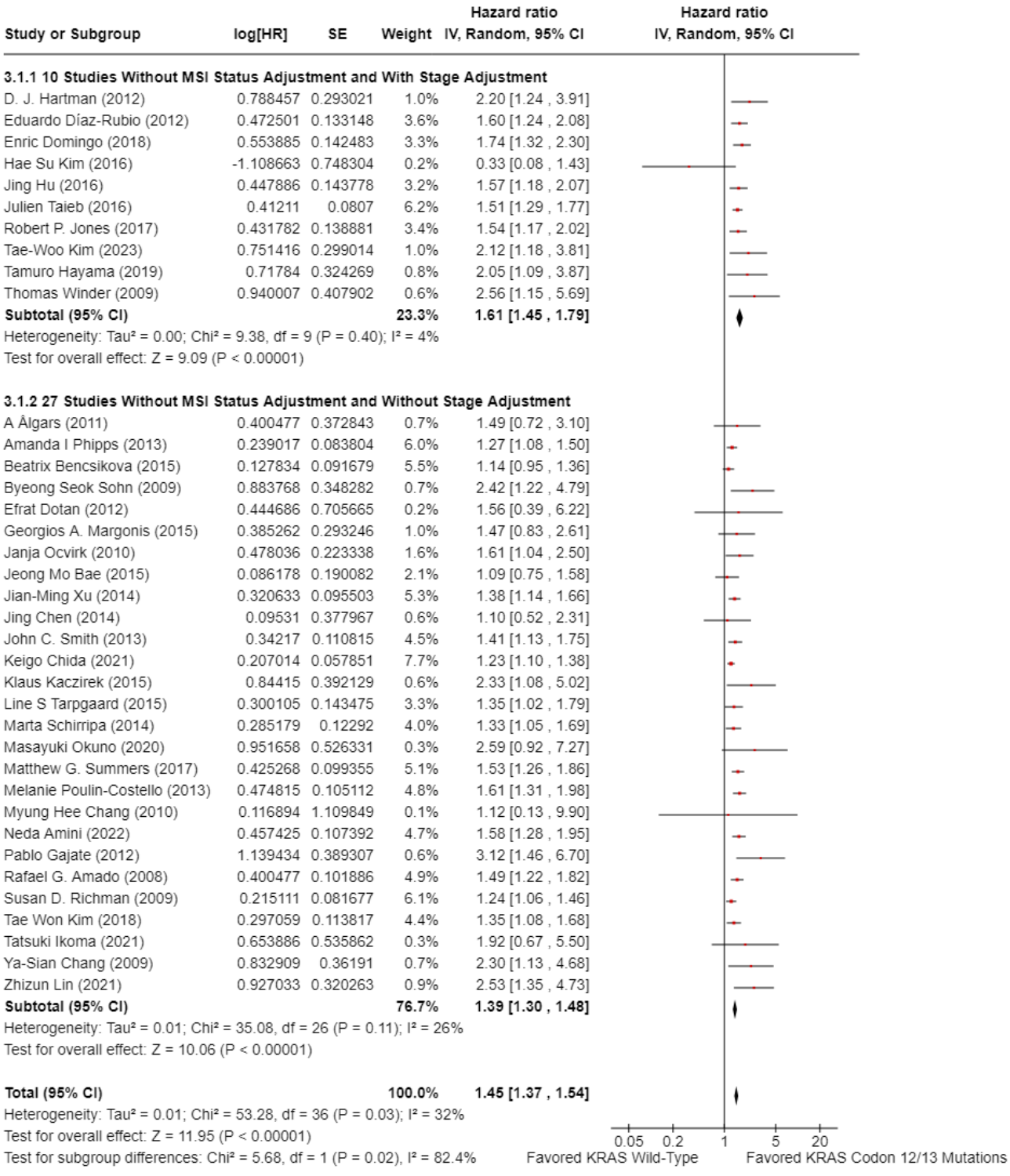


**Figure 5. Forest Plot for Overall Survival Stratified by Adjusting for Stage among 37 MSI-unadjusted Studies**

**Supplemental Table 1. Meta-analysis Coding Book**

| Variable | Type | Explanation | Adjust YES | Adjust NO |
|---|---|---|---|---|
| year | continuous | year of publication | | |
| number | continuous | sample size | | |
| adjust | binary | whether use a multivariate regression model | 1 | 0 |
| stage | binary | whether adjust CRC stage | 1 | 0 |
| stage2 | nominal | CRC stage | | |
| msi | binary | whether adjust for microsatellite instability (MSI) | 1 | 0 |
| rct | binary | whether the study is a randomized controlled trial (RCT) | 1 | 0 |
| sex | binary | whether the study adjust for patients' gender | 1 | 0 |
| age | binary | whether the study adjust for patients' age | 1 | 0 |
| tumor_type | binary | whether the study adjust for patients' tumor subtype | 1 | 0 |
| tumor_size | binary | whether the study adjust for tumor size | 1 | 0 |
| site | binary | whether the study adjust for tumor site in CRC | 1 | 0 |
| chemotherapy | binary | whether the study adjust for patients' chemotherapy status | 1 | 0 |
| treatment | binary | whether the study adjust for patients' status of being treatment | 1 | 0 |
| BRAF | binary | whether the study adjust for patients' BRAF gene mutation | 1 | 0 |
| PICK3 | binary | whether the study adjust for patients' PICK3 gene mutation | 1 | 0 |
| patient_clinical_status | binary | whether the study adjust for clinical risk score, Karnofsky performance status, World Health Organisation (WHO) performance status, or Eastern Cooperative Oncology Group (ECOG) | 1 | 0 |
| resection | binary | whether the study adjust for resection | 1 | 0 |
| metastasis | binary | whether the study adjust for patients' status of metastasis | 1 | 0 |
| number_of_lesions | binary | whether the study adjust for patients' number of CRC | 1 | 0 |
| family | binary | whether the study adjust for patients' family history of CRC | 1 | 0 |
| CIMP | binary | whether the study adjust for patients' CpG island methylator phenotype (CIMP) | 1 | 0 |
| liver_function | binary | whether the study adjust for patients' liver function | 1 | 0 |
| skin_toxicity_grade | binary | whether the study adjust for patients' skin toxicity grade | 1 | 0 |
| BMI | binary | whether the study adjust for patients' body mass index (BMI) | 1 | 0 |
| smoking | binary | whether the study adjust for patients' smoking status | 1 | 0 |
| race | binary | whether the study adjust for patients' race status | 1 | 0 |
| MGMT_methylation | binary | whether the study adjust for patients' level of MGMT methylation | 1 | 0 |
| ldh | binary | whether the study adjust for patients' level of lactate dehydrogenase (LDH) | 1 | 0 |
| alkaline_phosphatase | binary | whether the study adjust for patients' level of alkaline phosphatase | 1 | 0 |
| blood | binary | whether the study adjust for patients' plasma or serum level | 1 | 0 |

**Supplemental Table 2. Meta-Analysis Study Comparison: KRAS Mutation vs. KRAS Wild Type for Overall Survival**

| Study | Year | Population | Number of Included Studies | Adjustment | Pooled HR (95% CI) | P-value |
|---|---|---|---|---|---|---|
| This study | 2023 | Any stages and diverse study types | 46 | MSI = yes<br>MSI = no | 1.21 (1.07–1.36)<br>1.45 (1.37–1.54) | 0.09<br>0.03 |
| Vincenzo Formica [94] | 2022 | Stage II-III colon cancer patients in a Phase III RCT | 9 | MSI = yes<br>MSI = no | 1.33 (1.03–1.71)<br>1.09 (0.70–1.69) | 0.03<br>0.70 |
| Min Seob Kwak [95] | 2017 | Any stages and diverse study types | 8 | Unadjusted | 1.37 (1.03–1.81) | 0.002 |

Note: Wild-type *KRAS* as the reference for pooled HR

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