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The prognostic value of hypoxia-inducible factor-1α in advanced cancer survivors: a meta-analysis with trial sequential analysis

Susu Han, Tao Huang, Fenggang Hou, Liting Yao, Xiyu Wang and Xing Wu

Abstract

Background: Expression of hypoxia-inducible factors (HIFs) has been observed, but their prognostic role in advanced cancers remains uncertain. We conducted a meta-analysis to establish the prognostic effect of HIFs and to better guide treatment planning for advanced cancers.

Methods: Pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Trial sequential analysis (TSA) was also performed. The clinical outcomes included overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), cancer-specific survival (CSS), relapse/recurrence-free survival (RFS), and metastasis-free survival (MFS) in patients with advanced tumors according to multivariate analysis.

Results: A total of 31 studies including 3453 cases who received chemotherapy, radiotherapy, or chemoradiotherapy were identified. Pooled analyses revealed that HIF-1α expression was correlated with worse OS (HR = 1.61, $p < 0.001$), DFS (HR = 1.61, $p < 0.001$), PFS (HR = 1.49, $p = 0.01$), CSS (HR = 1.65, $p = 0.056$), RFS (HR = 2.10, $p = 0.015$), or MFS (HR = 2.36, $p = 0.002$) in advanced cancers. HIF-1α expression was linked to shorter OS in the digestive tract, epithelial ovarian, breast, non-small cell lung, and clear cell renal cell carcinomas. Subgroup analysis by study region showed that HIF-1α expression was correlated with poor OS in Europeans and Asians, while an analysis by histologic subtypes found that HIF-1α expression was not associated with OS in squamous cell carcinoma. No relationship was found between HIF-2α expression and OS, DFS, PFS, or CSS.

Conclusions: Targeting HIF-1α may be a useful therapeutic approach to improve survival for advanced cancer patients. Based on TSA, more randomized controlled trials are strongly suggested.

Keywords: advanced cancer, HIF-1α, HIF-2α, multivariate analysis, prognosis, therapies

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Introduction

Cancer is still a major public health problem throughout the world; cancer is a leading cause of death and has high morbidity rates. According to GLOBOCAN estimates, approximately 14.1 million new cases and 8.2 million deaths occurred due to cancer in 2012 worldwide.[1] Although surgical techniques, chemotherapy/radiotherapy, targeted molecular therapy, and immunotherapy regimens have greatly improved advanced disease management in recent years, the 5-year survival rate of most advanced cancers is still low.[1–3] Combination treatments are commonly used to improve treatment outcomes for most advanced cancers.[4,5] In clinics, current management of cancer patients still relies mainly on clinical staging assessments to guide treatment and determine prognosis, which cannot always be accurately used to classify disease prognosis to target cancers.[6] Thus, an effective indicator needs to be developed to better predict the behavior of advanced cancer patients,

enhance the selection of appropriate treatment and management strategies, and guide necessary clinical trial implementation.

Meta-analysis suggests that the development of strategies against biomarkers may be a cost-effective therapeutic approach in solid tumors.[7] Solid tumors generally exhibit hypoxia, which plays a central role in tumor angiogenesis and cancer metastasis.[8,9] Moreover, hypoxia is associated with metabolism, differentiation, necrosis, rapid tumor growth, and other malignant biological behaviors, leading to resistance to radiotherapy and chemotherapy.[10,11] Two common hypoxia-inducible factors (HIFs) (HIF-1α and HIF-2α) have been identified as key regulators of the response to hypoxic stress.[12] HIFs are involved in the regulation of angiogenesis through vascular endothelial growth factor (VEGF: a potent angiogenic protein) and platelet-derived growth factor, enhancing the transcriptional activity of Notch signaling, mediating cancer metabolic pathways (glucose, lipid, and amino acid metabolism), and exerting a tumor-promoting effect by immunosuppression.[13–15] HIFs also may induce epithelial-to-mesenchymal transition (EMT) *via* the PI3K/AKT/mTOR pathway, regulate proto-oncogene c-Myc activity, and activate stem cell factors such as Oct4 and Nanog.[16–18]

Expression of HIFs in cancer cells contributes to metastasis, but inactivation of HIFs decreases metastasis of cancer cells.[19] HIF-1α and HIF-2α are most frequently reported. Their expression is detected in various human cancers and may be associated with a worse prognosis of many tumors, such as gastric cancer, breast cancer, and non-small cell lung cancer.[13,20] However, the clinical outcomes of HIF-1α and HIF-2α expression according to multivariate analysis are still controversial in advanced cancer. For example, HIF-1α expression was not linked to OS in colorectal cancer,[21,22] but was associated with shorter OS in colorectal cancer by Wilson and colleagues.[23] Additionally, the prognostic impact of HIFs expression in advanced cancers is still unclear when investigated *via* meta-analysis.

Therefore, the purpose of the current meta-analysis is to investigate the relationship between HIF-1α and HIF-2α expression and survival outcomes for advanced/metastatic tumor patients treated with chemotherapy, radiotherapy, or chemoradiotherapy, thereby allowing more effective and rational development of combination therapy strategies to optimize treatment.

Materials and methods

Search strategy

The electronic databases PubMed, Embase, EBSCO, and the Cochrane Library were systematically searched to identify eligible papers published before 23 February 2018. We used the following search terms and text words: 'hypoxia inducible factor OR hypoxia-inducible factors OR HIF OR hypoxia-inducible factor 1 OR hypoxia-inducible factor 2 OR endothelial PAS domain-containing protein 1 OR EPAS1', 'metastatic OR advanced OR metastasized OR recurrent', 'cancer OR tumor OR carcinoma OR neoplasm', 'survival OR outcome OR prognosis OR mortality' (Table S1). We also hand-searched the reference lists of the eligible studies to identify other potential articles. Three authors (S.H., T.H., and F.H.) independently evaluated the publications, and discrepancies were discussed by consensus. The present meta-analysis was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[24]

Study selection

Studies that fulfilled the following selection criteria were included: studies recording patients with advanced/metastatic cancer, stage III cancer, or stage IV cancer; studies published in English reporting patients treated with or without surgery and chemotherapy, radiotherapy, or chemoradiotherapy, etc.; studies reporting the prognostic information of HIF-1α, HIF-2α, and HIF-3α expression regarding the hazard ratio (HR) with the corresponding 95% confidence interval (CI) for overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), cancer-specific survival (CSS), relapse/recurrence-free survival (RFS), or metastasis-free survival (MFS) using multivariate analysis; in the case of insufficient information, such as only HR or 95% CI, HR and 95% CI were calculated to evaluate the prognostic data based on the described methods, if possible,[25,26] or the corresponding author was contacted by sending an email to request useful information. If authors published multiple papers using overlapping sample data, only the most recent publication or the study with the largest study population was included. Those with no

relevant studies, case reports, animal studies, reviews, and no prognostic value of HIFs in advanced cancer for multivariate analysis were mainly excluded.

Data extraction and study reporting quality

The quality of the included studies was assessed using the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria.[27] The REMARK criteria reported 20 items for each eligible study (Introduction: 1 item, Materials and Methods: 10 items, Results: 7 items, and Discussion: 2 items), and each item consists of three possible values (0, 1, and 2), allowing for evaluation of the study objective, method, data analysis, and relevant discussion, with a maximal score of 40. The classifications were as follows; an item was not defined or applicable at all (0 score); an item clearly stated all aspects (2 scores); and an item was incompletely described (1 score). According to the overall scores, studies were divided into two groups: studies with a score of $\geqslant$24 (60% of the maximum score) were considered high quality, and the study with a REMARK score of $<$24 was low quality (Table S2). The following data were extracted from the full texts of the eligible studies, including the first author's surname, year of publication, case number, study source, mean or median age, tumor type, testing method, therapy regime, study design, sample type, cut-off value, survival status, adjusted variables, and clinical outcomes. Any disagreements were resolved by consensus.

Statistical analysis

The pooled HR and 95% CI were calculated to estimate the effect of HIF-1α and HIF-2α expression status on advanced cancer survival (OS, DFS, PFS, CSS, RFS, or MFS of multivariate analysis). An observed HR $>$1 implied a worse prognosis, whereas a HR $<$1 indicated a favorable prognosis. The between-study heterogeneity was determined using Cochran's Q statistic.[28] The random-effects model (DerSimonian-Laird) was applied in the current meta-analysis.[29,30] For substantial heterogeneity ($p<0.1$) in $\geqslant$10 of the included studies, we conducted subgroup analyses based on some of the baseline features of the eligible studies, such as the study region, tumor location, and survival rate, to determine the potential source of heterogeneity and the difference between subgroups. Publication bias was examined using Egger's regression model and Begg's test for the results with more than 10 studies.[31,32]

A meta-analysis included a small number of participants, the associated random errors may cause spurious results.[33,34] Trial sequential analysis (TSA) was performed to avoid type I error rate (α) and estimate the required sample information.[35] A type I error of 5% and type II error (β) of 10% (1–β = 90% power) were set. We used a relative risk reduction (RRR) of 20% and the optimal *a priori* anticipated information size (APIS) method. A sequential monitoring boundary was constructed to determine whether a trial could be terminated early. A cumulative Z-curve that crossed the trial sequential monitoring boundary suggested that the statistical evidence was conclusive. In other cases, additional studies were needed to achieve sufficient evidence. Data were analyzed using Stata software, version 12.0 (Stata Corp., College Station, TX, USA) and R software, version 3.4.2 (The R Foundation for Statistical Computing; Vienna, Austria).

Results

Study characteristics

Figure 1 describes the detailed steps for the literature search, and a total of 28 articles met the eligibility criteria of this meta-analysis. All studies using multivariate analysis were published from 2002 to 2017. Of these, 27 studies[21–23,36–59] evaluated the prognostic effect of HIF-1α expression and included 3056 individuals. Four studies[36,41,55,60] including 397 individuals assessed the prognostic role of HIF-2α expression. Most studies reported the 5-year survival outcome, and HIF-1α and HIF-2α expression were mainly detected using an immunohistochemistry (IHC) method. The antibodies and staining procedure used for the IHC method are listed in Table S3. A total of 13 studies had quality scores $\geqslant$24, and 15 studies had a score of $<$24. The main characteristics of the included studies are listed in Table 1.

Overall survival of HIF-1α expression

A total of 19 studies including 2342 cases were identified in the analysis of HIF-1α expression and OS. Multivariate analysis showed that HIF-1α expression was associated with worse OS in tissue samples (n = 17 studies with 2027 cases,

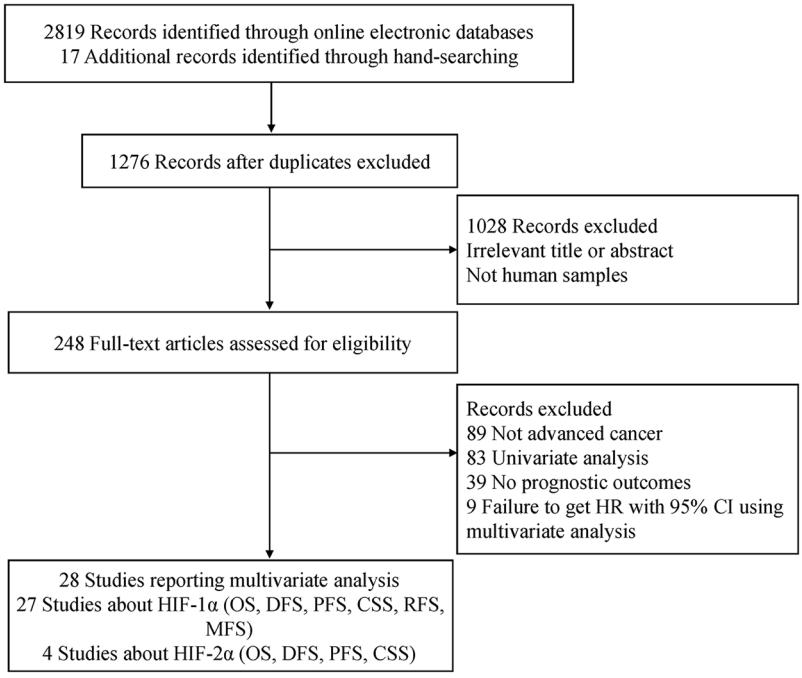

Figure 1. Flow diagram of the study identification process.
95% CI, 95% confidence interval; CSS, cancer-specific survival; DFS, disease-free survival; HIF-1α, hypoxia-inducible factor-1α; HIF-2α, hypoxia-inducible factor-2α; HR, hazard ratio; MFS, metastasis-free survival; OS, overall survival; PFS, progression-free survival; RFS, relapse/recurrence-free survival.

HR = 1.61, 95% CI = 1.28–2.03, $p < 0.001$), but was not correlated with OS in blood samples (n = two studies with 315 cases, HR = 0.79, 95% CI = 0.15–4.07, $p = 0.774$) (Figure 2).

Subgroup analyses were performed based on the available information in tissue samples, and Table 2 lists the results of the subgroup analyses to explain potential sources of heterogeneity for OS. However, all p values for heterogeneity per subgroup were not more than 0.1, suggesting that the subgroup analyses failed to explore the heterogeneity sources.

Stratified analysis by study region showed a poor OS for 12 studies with European subjects (n = 1566 cases, HR = 1.39, $p = 0.002$) and for five studies with Asian subjects (n = 461 cases, HR = 2.14, $p < 0.001$). Stratified analysis by tumor location indicated that a poor OS was found for 12 studies with other cancer types (n = 1377 cases, HR = 1.71, $p < 0.001$), but not colorectal cancer (n = three studies with 423 cases, $p = 0.398$) and cervical cancer (n = two studies with 227 cases, $p = 0.122$). Stratified analysis by histologic subtypes demonstrated that no correlation was found between HIF-1α expression and OS in squamous cell carcinoma (n = four studies with 375 cases), but HIF-1α expression was linked to worse OS in other histotypes (n = 1652 cases, HR = 1.67, $p < 0.001$). Subgroup analysis by survival status showed that HIF-1α expression was significantly associated with worse prognosis for 5-year OS (n = 10 studies with 1566 cases, HR = 1.36, $p < 0.001$) and <3-year OS (n = two studies with 95 cases, HR = 3.47, $p = 0.007$) subgroups, but no relationship was found between the HR of the 3-year OS (n = four studies with 204 cases, $p = 0.064$).

Stratified analysis by the study design determined that HIF-1α expression had a negative prognostic impact on patient OS in prospective and retrospective studies (HR = 1.39, 95% CI = 1.14–1.68, $p = 0.001$, four studies, 734 patients; HR = 1.79, 95% CI = 1.25–2.56, $p = 0.001$, 12 studies, 1149 patients; respectively), but no significant association was noted among a randomized controlled trial ($p = 0.422$, one study, 144 patients). Stratified analysis by age (years) showed that patients aged less than 60 years had worse OS (HR = 1.44, 95% CI = 1.21–1.71, $p < 0.001$, eight studies, 1017 patients), and patients older than 60 years had a prognostic impact on OS (HR = 2.10, 95%

Table 1. Baseline characteristics of the included studies investigating the prognosis.

Gene	First author	Country	Age	Method	Histology	Study design	Specimen type	Cases	Therapy	Method patterns	Cut off	Survival status	Adjusted variables	Prognosis reported	Scores
HIF-1α	Schindl[59]	Austria	52.3	IHC	Advanced breast cancer	Prospective, multicenter	Paraffin-embedded tumor specimens	206	Surgery and combined chemotherapy with tamoxifen	Clone monoclonal antibody H1 67, NB 100–105; Novus Biologicals, Littleton, CO; Dilution: 1:60	Nuclear 10%	5 years	HER-2 staining intensity, patient's age at time of diagnosis, menopausal status, histological grading, estrogen receptor density, and tumor stage	OS, DFS	29
HIF-1α	Bachtiar[57]	Austria	NA	IHC, blind	Advanced cervical cancer	Retrospective, single-center	Paraffin-embedded tumor specimens	67	Radiotherapy	No. H72320; BD Transduction Laboratories, Franklin Lakes; Dilution: 1:25	Nuclear 10%	3 years	Tumor size, patients' age, nodal status, FIGO stage, and histological grading	CSS, PFS	29
HIF-1α	Burri[58]	Switzerland	64	IHC, blind	Advanced cervical cancer	Retrospective, NA	Paraffin-embedded tumor specimens	78	Radiotherapy and chemotherapy	H1α67, Novus Biologicals, Littleton, CO; Dilution: 1:5000	Nuclear 0%	5 years	Tumor stage, nodal status, histology, anemia, and median total dose	OS	28
HIF-1α	Theodoropoulos[56]	Greece	68	IHC, blind	Advanced rectal cancer	Retrospective, multicenter	Tissue	92	Surgery, chemotherapy and radiotherapy	Mab H1α67, IgG2b isotype; StressGene, Victoria, British Columbia, Canada; Dilution: 1:1200	Nuclear 10%	3 years	Tumor grade, pattern of tumor growth, vascular invasion, and lymph node status	OS, DFS	24
HIF1α	Winter[55]	UK	NA	IHC	Advanced head and neck squamous cell carcinoma	Retrospective, single-center	Paraffin-embedded tumor specimens	140	Surgery and radiotherapy	ESEE122; Dilution: 1:30	Nuclear 10%	5 years	Advanced disease, anemia, gender, age, smoking history, lymph node status, tumor subsite, and tumor grade	CSS, OS, DFS	22
HIF-1α	Generali[54]	UK	NA	IHC, blind	Advanced breast cancer	Prospective randomized clinical trial, single-center	Paraffin-embedded tumor specimens	187	Surgery and chemoendocrine therapy	ESEE 122, IgG1 monoclonal antibody; Dilution: 1:40	Weak-strong	5 years	T stage, N status, steroid hormone receptor status, c-erb2, bcl2, p53, and Ki67	DFS	29

(Continued)

Table 1. (Continued)

Gene	First author	Country	Age	Method	Histology	Study design	Specimen type	Cases	Therapy	Method patterns	Cut off	Survival status	Adjusted variables	Prognosis reported	Scores
HIF-1α	Klatte[53]	USA	NA	IHC, blind	Metastatic clear cell RCC	Retrospective, single-center	Paraffin-embedded tumor specimens	141	Immunotherapy	IgG2b, cloneH1α67-sup, final concentration, 6 Ag/mL; Novus Biologicals; NA	Nuclear 35%	5 years	ECOG PS, T stage, concomitant lymph node metastases, Fuhrman grade, and number of metastatic sites	CSS	23
HIF-1α	Dellas[52]	Germany	58.4	IHC	Advanced cervical cancer	Retrospective, NA	Paraffin-embedded tumor specimens	44	Radiotherapy	Ab463; Abcam, UK; NA	Nuclear, weak-intensive	5 years	Tumor stage	CSS	15
HIF-1α	Koo and Kim[51]	Korea	53.2	IHC	Metastatic squamous cell carcinoma	Retrospective, single-center	Paraffin-embedded tumor specimens	17	Chemo/radiation therapy	EP1215Y, Biocare, CA, USA; Dilution: 1:100	Nuclear or cytoplasmic (or both) 10%	3 years	NDR	OS	11
HIF-1α	Shioya[48]	Japan	59	IHC, blind	Advanced rectal cancer	Retrospective, NA	Paraffin-embedded tumor specimens	50	Surgery and hyperthermo-chemo-radiotherapy	Neomarkers, Fremont, CA; Dilution: 1:20000	Nuclear 40%	3 years	Radiation dose, chemotherapy course, treatment time of hyperthermia, age, gender, and stage	RFS, MFS	21
HIF-1α	Xiang[50]	China	50	IHC, blind	Hepatocellular carcinoma with abdominal LN metastases	Retrospective, single-center	Paraffin-embedded tumor specimens	69	Radiotherapy	Santa Cruz Biotechnology, Santa Cruz, CA; NA	Nuclear or cytoplasmic (or both) 10%	3 years	Hb, intrahepatic tumor number, vascular invasion, child-Pugh score, cumber of metastatic LN, and intrahepatic tumor control etc.	OS, RFS	25
HIF-1α	Wan[47]	China	43.1	IHC, blind	Advanced nasopharyngeal carcinoma	Randomized controlled trial	Tissue	144	Chemotherapy and radiotherapy	Millipore, Billerica, MA, USA; Dilution: 1:200	Nuclear or cytoplasmic (or both) 5 scores	5 years	Age, gender, histological style, TNM stage, and Aurora-A	OS, MFS, PFS	25
HIF-1α	Fraga[49]	Brazil	NA	IHC	Upper aerodigestive tract cancer with cervical lymph nodes	Retrospective, single-center	Paraffin-embedded tumor specimens	26	Surgery and radiotherapy	Clone HIF-1α 67, Sigma-Aldrich, St. Louis, USA; NA	NA	3 years	NDR	OS	17

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Table 1. (Continued)

Gene	First author	Country	Age	Method	Histology	Study design	Specimen type	Cases	Therapy	Method patterns	Cut off	Survival status	Adjusted variables	Prognosis reported	Scores
HIF-1α	Shim[46]	Korea	62	IHC, blind	Advanced rectal cancer	Retrospective, single-center	Paraffin-embedded tumor specimens	104	Surgery and chemoradiotherapy	Novus Biologicals, Littleton, CO; Dilution: 1:50	2 scores	3 years	Age and stage	RFS	22
HIF-1α	Shimomura[45]	Japan	62	IHC	Colorectal liver metastasis	Retrospective, single-center	Paraffin-embedded tumor specimens	64	Surgery and chemotherapy	Novus Biologicals, Littleton, CO; Dilution: 1:50	Cytoplasm 5 scores	5 years	N stage, no. of liver tumors, and CEA level etc.	DFS	22
HIF-1α	Wu[44]	China	NA	IHC	Advanced non-small cell lung cancer	Retrospective, single-center	Frozen tissues	162	Chemotherapy	Millipore Corporation®, USA; Dilution: 1:150	6 scores	NA	Age, sex, smoking status, histology, stage, chemotherapy regimens, and response status	OS	21
HIF-1α	Wilson[23]	USA	62	qRT-PCR	Metastatic colorectal cancer	Retrospective, multi-center	Paraffin-embedded tumor specimens	42	FOLFOX4 chemotherapy plus the VEGFR inhibitor PTK787/ZK 222584 (vatalanib)	Applied Biosystems, Foster City, CA, USA; NA	mRNA 1.84 ratio	<3 years	Performance status and serum LDH level	OS	25
HIF-1α	Zhang[43]	China	NA	IHC, blind	Metastastic esophageal squamous cell carcinoma	Retrospective, single-center	Paraffin-embedded tumor specimens	69	Surgery and radiotherapy/chemotherapy	Clone H1α67; Novus Biologicals, Inc., Littleton, CO; NA	Nucleus and cytoplasm 44%	5 years	NDR	OS, DFS	16
HIF-1α	Braicu[42]	Germany, Belgium, Austria	58	ELISA	Advanced epithelial ovarian cancer	Prospective, multi-center	Tissue	275	Surgery and platinum-based chemotherapy	ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA; NA	80 pg/mg protein	5 years	Age at first diagnosis, FIGO stage, histological subtype, histological grade, presence and volume of ascites, residual tumor mass after surgery, peritoneal dissemination, and responses to platinum-based chemotherapy	OS	26

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Table 1. (Continued)

Gene	First author	Country	Age	Method	Histology	Study design	Specimen type	Cases	Therapy	Method patterns	Cut off	Survival status	Adjusted variables	Prognosis reported	Scores
HIF-1α	Xie 2015[41]	China	63	ELISA, blind	Metastatic renal cell carcinoma	Prospective, open-label, single-arm, multicenter, phase II trial	Serum	86	Second-line treatment with pazopanib after failure of first-line sunitinib treatment	(ELISA) kit (R&D Systems, Minneapolis, MN; NA	80.2 pg/mg protein	<3 years	Previous nephrectomy, six IMDC (International Metastatic RCC Database Consortium) risk factors (anaemia, neutrophilia, Karnofski performance status (KPS) <80%, thrombocytosis, hypercalcaemia, and time from diagnosis to treatment interval <1 year), number of organs involved, and best response to prior sunitinib therapy	OS, PFS	25
HIF-1α	Berk[21]	Turkey	55	IHC	Metastatic colorectal cancer	Retrospective, single-center	Paraffin-embedded tumor specimens	53	Chemotherapy combinations with Bevacizumab	Thermo scientificAb-4, (Clone H1α67, U.K; Dilution: 1:50	Cytoplasm 5 scores	<3 years	Age, gender, K-ras status, dose reduction, dose delay, ECOG PS, metastases, and chemotherapy regimen	OS, PFS	21
HIF-1α	Goos[22]	The Netherlands	NA	IHC	Colorectal cancer liver metastasis	Retrospective, multicenter	Paraffin-embedded tumor specimens	328	Surgery and systemic therapy	BD Transduction Laboratories, Breda, The Netherlands; Dilution: 1:500	Nuclear 25%	5 years	Primary tumor-to-CRCLM interval of less than 12 months, lymph node positivity at the time of diagnosis of the primary tumor, maximal CRCLM diameter of greater than 5.0 cm, number of CRCLM greater than 1, and serum CEA level greater than 200 ng/mL	OS	28

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Table 1. (Continued)

Gene	First author	Country	Age	Method	Histology	Study design	Specimen type	Cases	Therapy	Method patterns	Cut off	Survival status	Adjusted variables	Prognosis reported	Scores
HIF-1α	Shultz[40]	USA	NA	Proximity ligation assay/ quantitative PCR	Advanced or metastatic pancreatic cancer	Randomized control trial, multicenter	Plasma	229	Gemcitabine and erlotinib	Model 7500, Applied Biosystems; NA	Protein	<3 years	Age, sex, race, ECOG performance status, pain intensity, and disease stage	OS	26
HIF-1α	Chen[39]	China	53	IHC, blind	Advanced pharyngeal cancer	Retrospective, single-center	Paraffin-embedded tumor specimens	57	Chemoradiotherapy/ radiotherapy	Not clear; NA	Nuclear 80%	<3 years	TNM classification, volumetric parameters, texture indices, and primary tumor origin	CSS, RFS	22
HIF-1α	Nyström[38]	Sweden	71	IHC	Advanced soft tissue sarcoma of extremities and trunk wall	Retrospective, single-center	Tissue	73	Surgery and radiotherapy/chemotherapy	Clone 54, BD Biosciences, Sweden; Dilution: 1:50	Nuclear 10%	5 years	Size, vascular invasion, necrosis, and tumor depth	OS, MFS	22
HIF-1α	Moreno-Acosta[37]	Colombia	46.3	IHC	Advanced squamous cell cervical carcinoma	Prospective, National Cancer Institute	Fresh tissue	149	Chemo-radiotherapy and brachytherapy	ESEE122, ab8366, abcam; Dilution: 1:400	10%	5 years	FIGO, differentiation degree, treatment type, and anemia	OS, PFS	21
HIF-1α	Beuselinck[36]	France, Belgium	59	qRT-PCR	Metastatic clear cell renal cell carcinoma	Prospective, multicenter	Tissue	104	Sunitinib	Fluidigm, South San Francisco, CA; NA	mRNA	5 years	Arcomatoid dedifferentiation >25% of tumor volume, neutrophil count, bone metastasis, liver metastasis, and Karnofsky performance status	OS	23
HIF-2α	Winter[55]	United Kingdom	NA	IHC	Advanced head and neck squamous cell carcinoma	Retrospective, single-center	Paraffin-embedded tumor specimens	140	Surgery and radiotherapy	ESEE122; Dilution: 1:30	Nuclear 10%	5 years	Advanced disease, anemia, gender, age, smoking history, lymph node status, tumor subsite, and tumor grade	CSS, OS, DFS	22

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Table 1. (Continued)

Gene	First author	Country	Age	Method	Histology	Study design	Specimen type	Cases	Therapy	Method patterns	Cut off	Survival status	Adjusted variables	Prognosis reported	Scores
HIF-2α	Garcia-Donas[60]	Spain	66	IHC	Advanced clear cell renal cell carcinoma	Prospective, multicenter	Paraffin-embedded tumor specimens	67	Sunitinib	Polyclonal Novus Biologicals NB100–122; Dilution: 1:200	5%	<3 years	MSKCC prognostic classification and gender	OS, PFS	26
HIF-2α	Xie 2015[41]	China	63	ELISA, blind	Metastatic renal cell carcinoma	Prospective, open-label, single-arm, multicenter, phase II trial	Serum	86	Second-line treatment with pazopanib after failure of first-line sunitinib treatment	(ELISA) kit (R&D Systems, Minneapolis, MN): NA	80.2 pg/mg protein	<3 years	Previous nephrectomy, six IMDC (International Metastatic RCC Database Consortium) risk factors (anaemia, neutrophilia, Karnofski performance status (KPS) <80%, thrombocytosis, hypercalcaemia, and time from diagnosis to treatment interval <1 year), number of organs involved, and best response to prior sunitinib therapy	OS, PFS	25
HIF-2α	Beuselinck[36]	France	59	qRT-PCR	Metastatic clear cell renal cell carcinoma	Prospective, multicenter	Tissue	104	Sunitinib	Fluidigm, South San Francisco, CA; NA	mRNA	5 years	Arcomatoid dedifferentiation >25% of tumor volume, neutrophil count, bone metastasis, liver metastasis, and Karnofsky performance status	OS, PFS	23

CEA, carcino embryonic antigen; CRCLM, colorectal cancer liver metastasis; CSS, cancer-specific survival; DFS, disease-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; FIGO, International Federation of Gynecology and Obstetrics; Hb, hemoglobin; HIF-1α, hypoxia inducible factor-1α, HIF-2α, hypoxia inducible factor-2α; IHC, immunohistochemistry; LDH, lactate dehydrogenase; LN, lymph nodes; MFS, metastasis-free survival; MSKCC, Memorial Sloan-Kettering Cancer Center; NA, not applicable; NDR, not detailed report; OS, overall survival; PFS, progression-free survival; qRT-PCR, quantitative reverse transcription polymerase chain reaction; enzyme-linked immunosorbent assay; RCC, renal cell carcinoma; RFS, relapse/recurrence-free survival; TNM, tumor node metastasis.

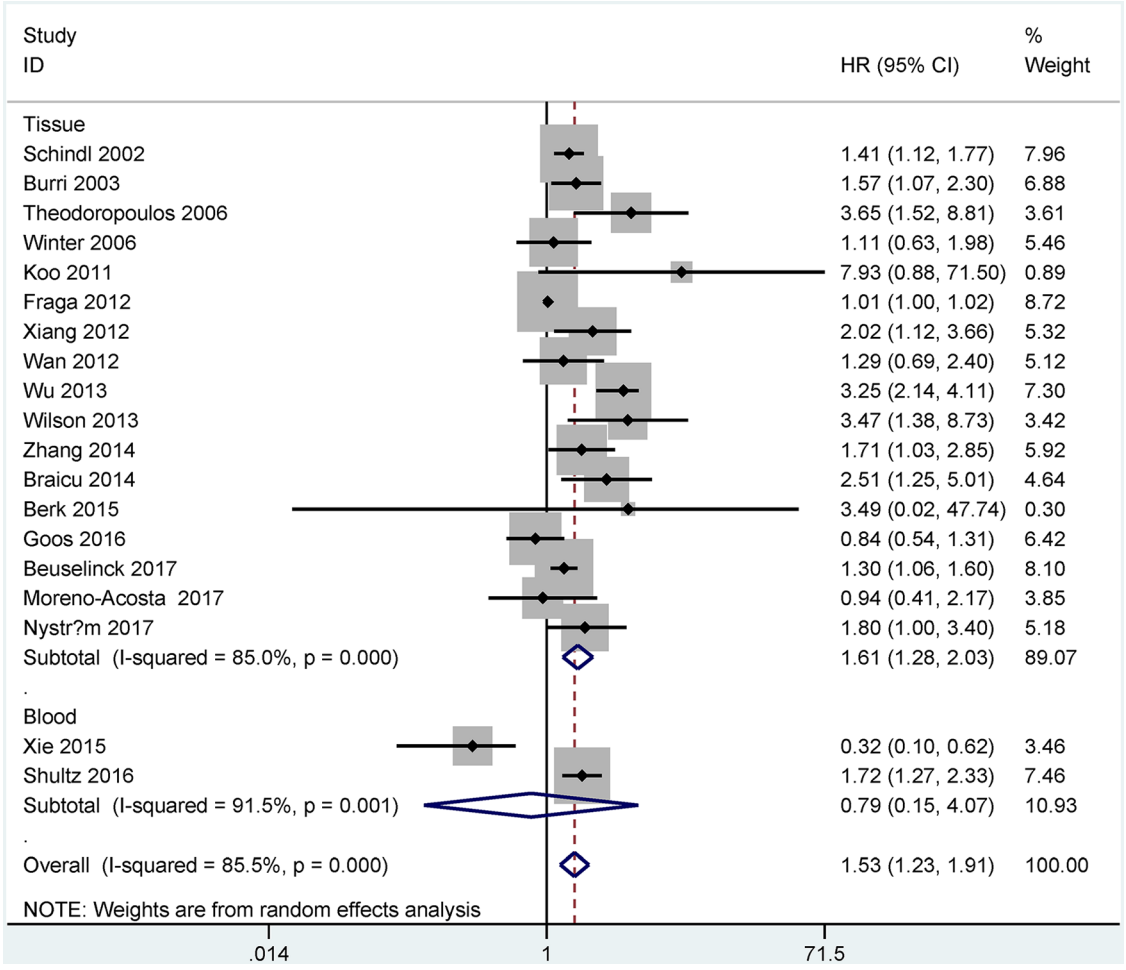

Figure 2. Forest plot for the relationship between HIF-1α expression and OS.
HIF-1α, hypoxia-inducible factor-1α; OS, overall survival

CI = 1.40–3.15, $p < 0.001$, four studies, 285 patients). Subgroup analysis by treatment regimen showed that HIF-1α expression was associated with worse OS in patients receiving surgery and nonsurgical treatment (HR = 1.40, 95% CI = 1.08–1.82, $p = 0.012$) and patients receiving the nonsurgical treatment such as chemotherapy, radiotherapy, or chemoradiotherapy (HR = 1.85, 95% CI = 1.29–2.64, $p = 0.001$). We also noted a negative prognostic impact of HIF-1α expression on patient OS in the other three features (center design, sample size, and study reporting quality) (Table 2).

OS of HIF-1α expression in various cancer systems

Among various cancer systems, HIF-1α expression was associated with shorter OS in the digestive tract (n = eight studies with 908 cases, HR = 1.61, 95% CI = 1.13–2.28, $p = 0.008$), gynecological (n = three studies with 502 cases, HR = 1.60, 95% CI = 1.03–2.48, $p = 0.035$), breast (n = one study with 206 cases, HR = 1.41, 95% CI = 1.12–1.77, $p = 0.003$), non-small cell lung (n = one study with 162 cases, HR = 3.25, 95% CI = 2.35–4.50, $p < 0.001$), and clear cell renal cell carcinomas (n = one study with 104 cases, HR = 1.30, 95% CI = 1.06–1.60, $p = 0.011$), but there was no association in nasopharyngeal ($n = 144$ cases, $p = 0.422$) and head and neck cancers ($n = 140$ cases, $p = 0.721$) (Figure 3).

DFS, PFS, CSS, RFS, and MFS of HIF-1α expression

Data suggested that HIF-1α expression was also correlated with worse survival in DFS (HR = 1.61, 95% CI = 1.32–1.96, $p < 0.001$, six studies, 758 patients), PFS (HR = 1.49, 95% CI = 1.10–2.01,

Table 2. Subgroup analyses of HIF-1α expression with OS in tissue samples.

Variables	HR with 95% CI	Heterogeneity (*p*)	*p* value	Studies	Cases	TSA
Study region						
Asian	2.14 (1.40–3.28)	0.036	<0.001	5	461	More studies
European	1.39 (1.13–1.71)	<0.001	0.002	12	1566	More studies
Tumor location						
Metastatic colorectal cancer	1.70 (0.50–5.84)	0.021	0.398	3	423	More studies
Advanced cervical cancer	1.40 (0.91–2.13)	0.273	0.122	2	227	More studies
Others	1.71 (1.30–2.24)	<0.001	<0.001	12	1377	More studies per cancer type
Histologic subtype						
Squamous cell carcinoma	1.38 (0.87–2.17)	0.214	0.171	4	375	More studies
Others	1.67 (1.29–2.16)	<0.001	<0.001	13	1652	No need
Survival status						
5 years	1.36 (1.18–1.57)	0.264	<0.001	10	1566	No need
3 years	2.05 (0.96–4.39)	0.001	0.064	4	204	More studies
<3 years	3.47 (1.41–8.52)	0.998	0.007	2	95	More studies
Study design						
Randomized controlled trial	1.29 (0.69–2.40)	NA	0.422	1	144	More studies
Prospective	1.39 (1.14–1.68)	0.263	0.001	4	734	More studies
Retrospective	1.79 (1.25–2.56)	<0.001	0.001	12	1149	More studies
Age (years)						
>60	2.10 (1.40–3.15)	0.192	<0.001	4	285	More studies
⩽60	1.44 (1.21–1.71)	0.315	<0.001	8	1017	No need
Not clear	1.40 (0.83–2.34)	<0.001	0.204	5	725	More studies
Study quality						
⩾24	1.66 (1.24–2.21)	0.015	0.001	8	1234	More studies
<24	1.54 (1.09–2.18)	<0.001	0.014	9	793	More studies
Center design						
Multicenter	1.50 (1.12–2.02)	0.008	0.007	7	1196	More studies
Single-center	1.80 (1.10–2.95)	<0.001	0.019	8	609	More studies
Not clear	1.49 (1.07–2.06)	0.598	0.017	2	222	More studies
Sample size						
>100	1.45 (1.06–1.99)	<0.001	0.019	8	1508	More studies
⩽100	1.89 (1.28–2.77)	<0.001	0.001	9	519	More studies
Treatment regimen						
Surgery and nonsurgical treatment	1.40 (1.08–1.82)	<0.001	0.012	8	1209	More studies
Nonsurgical treatment	1.85 (1.29–2.64)	<0.001	0.001	9	818	More studies

Nonsurgical treatment such as chemotherapy, radiotherapy, or chemoradiotherapy etc. was used.
95% CI, 95% confidence interval; HIF-1α, hypoxia inducible factor-1α; HR, hazard ratio; OS, overall survival; TSA, trial sequential analysis.

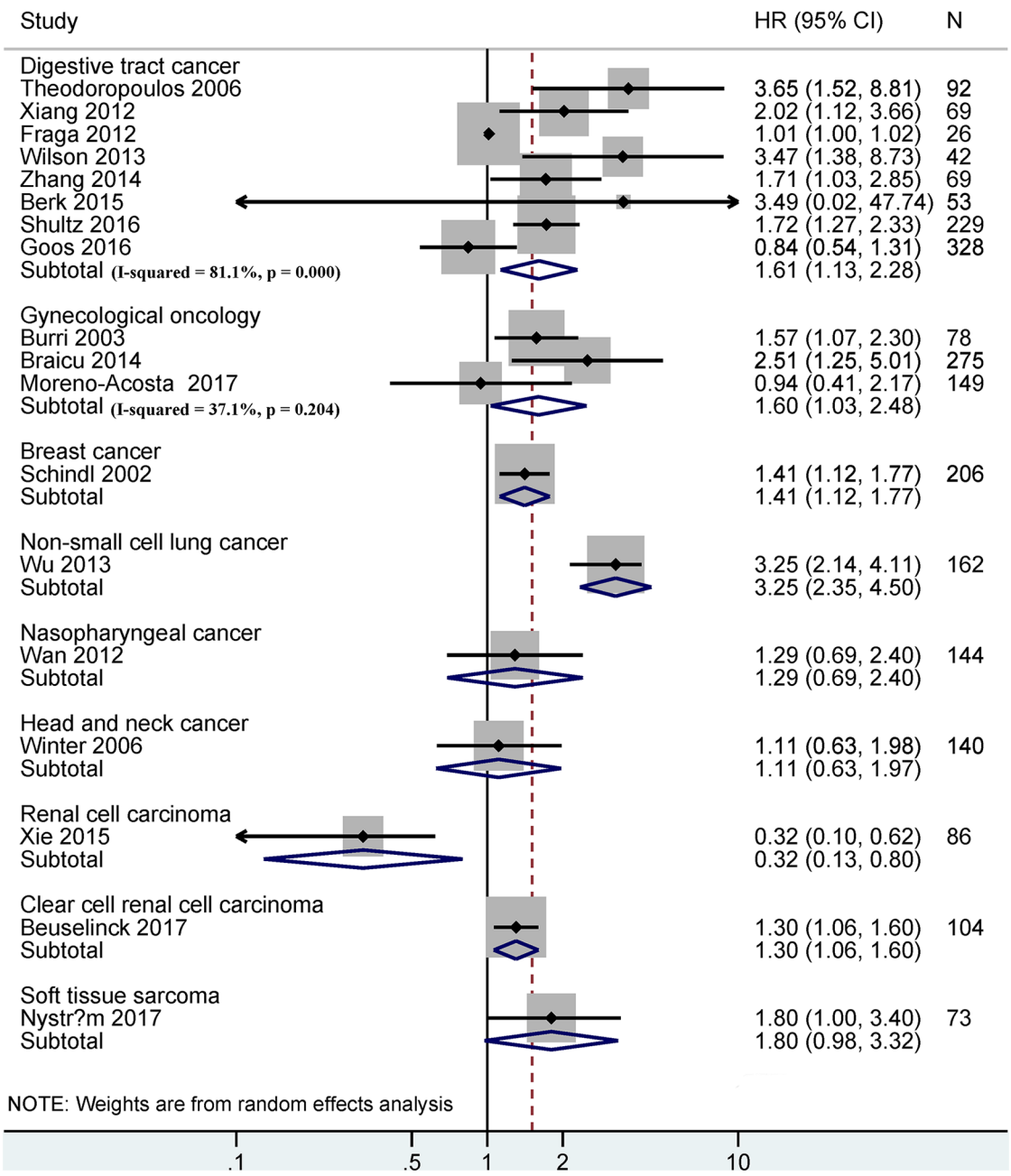

Figure 3. Forest plot for the relationship between HIF-1α expression and OS in different cancer systems.
HIF-1α, hypoxia-inducible factor-1α; OS, overall survival

$p=0.01$, five studies, 499 patients), CSS (HR = 1.65, 95% CI = 0.99–2.77, $p=0.056$, five studies, 449 patients), RFS (HR = 2.10, 95% CI = 1.15–3.81, $p=0.015$, four studies, 280 patients), and MFS (HR = 2.36, 95% CI = 1.38–4.03, $p=0.002$, three studies, 267 patients) (Figure 4).

The prognostic role of HIF-1α expression was also performed based on sample collection (Table 3), the results showed that HIF-1α expression was associated with worse OS (HR = 1.70, 95% CI = 1.31–2.20, $p<0.001$) and DFS (HR = 1.47, 95% CI = 1.22–1.76, $p<0.001$) in patients without previously received therapy prior to testing.

Publication bias

Egger's and Begg's tests were used to detect the potential publication bias for OS of HIF-1α

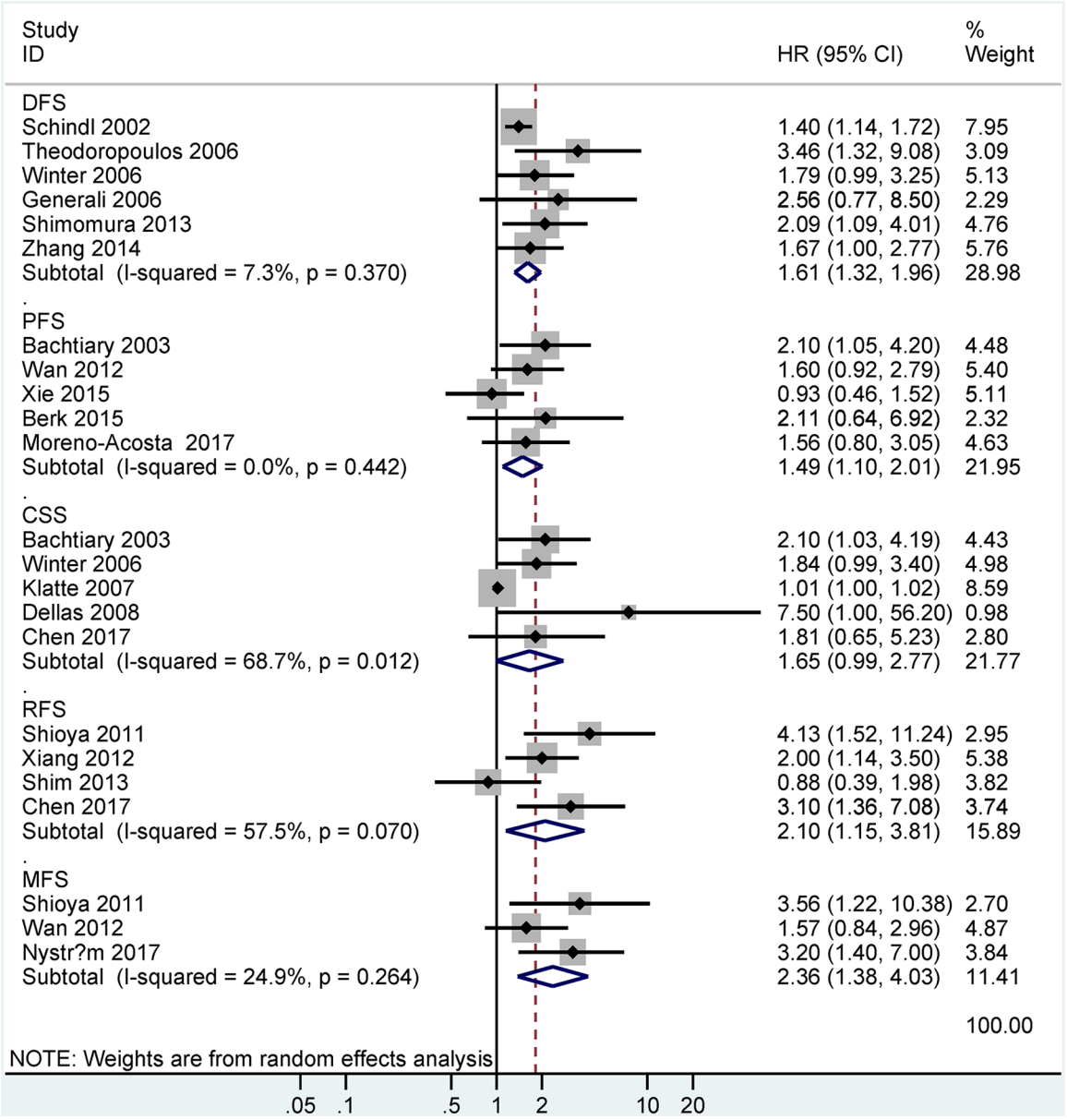

Figure 4. Forest plot for the relationship between HIF-1α expression and prognosis in DFS, PFS, CSS, RFS, or MFS.
DFS, disease-free survival; PFS, progression-free survival; CSS, cancer-specific survival; RFS, relapse/recurrence-free survival; MFS metastasis-free survival.

expression (Figure S1). No evidence of publication bias was found using Begg's test ($p = 0.484$), while there was obvious evidence of publication bias based on Egger's test ($p = 0.002$). When we removed this study by Fraga and colleagues,[49] the recalculated result from the remaining 18 studies remained significant (HR = 1.59, 95% CI = 1.28–1.98, $p < 0.001$), with no evidence of publication bias ($p = 0.582$).

Prognosis of HIF-2α expression

No relationship was found between HIF-2α expression and prognosis in OS (HR = 0.75, 95% CI = 0.38–1.47, $p = 0.399$, four studies, 396 patients), DFS (HR = 1.57, 95% CI = 0.82–3.01, one study, 139 patients), PFS (HR = 0.64, 95% CI = 0.27–1.53, three studies, 257 patients), and CSS (HR = 1.39, 95% CI = 0.66–2.89, one study, 139 patients) (Figure S2).

Table 3. The prognostic role of HIF-1α expression based on sample collection.

Sample collection	HR with 95% CI	Heterogeneity (*p*)	*p* value	Studies	Cases	TSA
OS						
Samples without previously received therapy	1.70 (1.31–2.20)	0.003	<0.001	9	1447	No need
Samples with previously received therapy	0.69 (0.18–2.73)	0.003	0.602	2	190	More studies
DFS						
Samples without previously received therapy	1.47 (1.22–1.76)	0.645	<0.001	3	415	More studies
Samples with previously received therapy	NA	NA	NA	NA	NA	NA

95% CI, 95% confidence interval; DFS, disease-free survival; HIF-1α, hypoxia inducible factor-1α; HR, hazard ratio; NA, not applicable; OS, overall survival; TSA, trial sequential analysis.

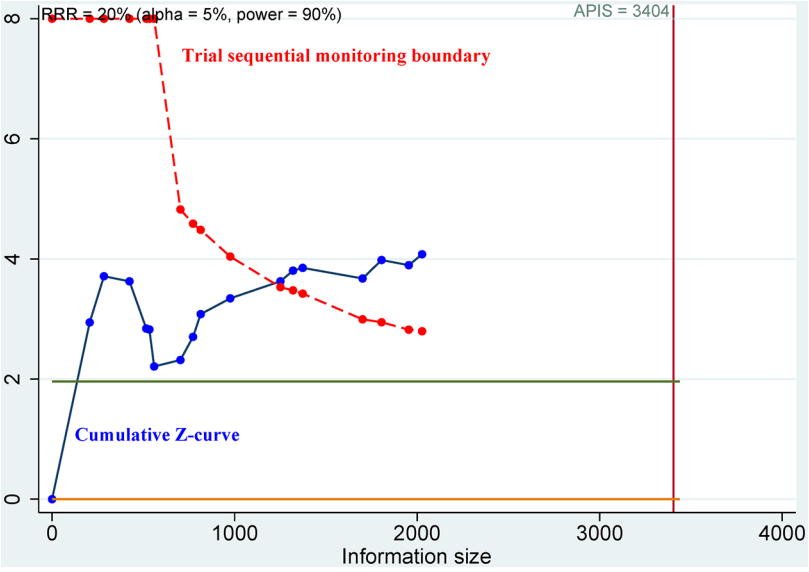

Figure 5. Trial sequential analysis between HIF-1α expression and OS.
HIF-1α, hypoxia-inducible factor-1α; OS, overall survival

Trial sequential analysis

The required sample information was quantified by TSA. The cumulative Z-curve significantly crossed the trial sequential monitoring boundary for OS of HIF-1α expression in tissue samples (Figure 5) and its subgroups such as 5-year OS and patients aged less than 60 years, and thus, additional studies were not required (Table 2). The cumulative Z-curve did not obviously cross the trial sequential monitoring boundary for DFS, PFS, CSS, RFS, or MFS of HIF-1α expression (Table S4); the remaining subgroups of HIF-1α expression in OS (Table 2); and the clinical outcomes of HIF-2α expression (Table S4), which indicated that further studies were needed.

Discussion

Traditional chemoradiotherapeutic regimens generally cannot eradicate cancer cells. Drug resistance and cancer recurrence are common obstacles for improving the long-term survival of cancer patients.[61,62] HIF-1α and HIF-2α are two of the most significant transcription factors

regulating cellular adaptation to hypoxia, have been found in the etiology of a number of human cancers, and have an adverse impact on the efficacy of radiotherapy and chemotherapy.[19,63] The expression of HIF-1α and HIF-2α in human cancers has been reported and detected.[13] HIF-1α and HIF-2α expression may be associated with poor prognoses in many cancers.[13,19,64] However, the prognostic significance of HIF-1α and HIF-2α expression in advanced cancer patients remains unclear based on a meta-analysis.

Activation of HIF transcription leads to the upregulation of many HIF-targeted genes, and HIFs regulate these targeted genes, which encode proteins such as Oct4 and Nanog in cancer stem cells.[65,66] HIFs also play roles in therapy resistance by activating the multidrug resistance 1 (MDR1) gene and ATP-binding cassette sub-family G member 2 (ABCG2)[13,67] and inflammation and immunity by activating the expression of ligands such as programmed death ligand 1 (PD-L1) and increasing cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expression on CD8+ T cells,[68,69] which are involved in decreasing the effectiveness of anticancer therapies, such as radiotherapy, chemotherapy, and immunotherapy.

To the best of the authors' knowledge, our study is the first comprehensive meta-analysis of 27 studies including a total of 3056 cases (HIF-1α) and four studies including a total of 397 cases (HIF-2α). We assessed the prognostic significance of HIF-1α and HIF-2α expression in advanced cancer patients receiving chemotherapy, radiotherapy, chemoradiotherapy, or immunotherapy. Our analyses did not find data associated with hypoxia-targeting agents and inhibitors of HIF activity for advanced cancer using multivariate analysis in preclinical and clinical studies.

The expression of HIF-2α was not linked to prognosis according to OS, DFS, PFS, or CSS. The pooled data indicated that the expression of HIF-1α was associated with reduced OS (HR = 1.61, $p < 0.001$), DFS (HR = 1.61, $p < 0.001$), PFS (HR = 1.49, $p = 0.01$), CSS (HR = 1.65, $p = 0.056$), RFS (HR = 2.10, $p = 0.015$), or MFS (HR = 2.36, $p = 0.002$). Moreover, evidence from some of the previous studies published is consistent with the current results, where HIF-1α expression was reported to be correlated with poor OS,[23,36,38,40,42–44,49,50,56,58,59] DFS,[43,45,56,59] PFS,[57] CSS,[52,53,57] RFS,[39,48,50] and MFS[38,48] in advanced cancers. These results were further confirmed using TSA, and TSA suggested that additional trials were necessary to validate these conclusions, including the association between HIF-1α expression and inferior DFS, PFS, CSS, RFS, and MFS and that there was no association between HIF-2α expression and survival. Additionally, based on different cancer systems, we found that HIF-1α expression was linked to shorter OS in digestive tract (HR = 1.61, $p = 0.008$), gynecological (HR = 1.60, $P = 0.035$), breast (HR = 1.41, $p = 0.003$), non-small cell lung (HR = 3.25, $p < 0.001$), and clear cell renal cell carcinomas (HR = 1.30, $p = 0.011$), but no correlation was observed in nasopharyngeal and head and neck cancers. Recent research has highlighted that chemotherapeutic treatments such as paclitaxel can induce the expression of HIF-1α.[66] We demonstrated that HIF-1α expression was correlated with poor OS and DFS in patients without previously received therapy.

Stratification by study region showed a worse OS for European and Asian subjects; stratification by tumor location indicated no correlation between HIF-1α expression and OS in colorectal cancer and cervical cancer, but was significantly linked to reduced OS in pancreatic cancer (HR = 1.72, 95% CI = 1.27–2.33) and epithelial ovarian cancer (HR = 2.505, 95% CI = 1.252–5.013). Additionally, evidence from some previously published studies on these specific tumor types is consistent with our analyses, such as colorectal and cervical cancer. [21,22,37] When classified by survival status, HIF-1α expression was linked to worse prognosis for 5-year OS (HR = 1.36, $p < 0.001$) and <3-year OS (HR = 3.47, $p = 0.007$); classification by study design, HIF-1α expression showed a negative prognostic impact on OS in four prospective studies (HR = 1.39, $p = 0.001$)[36,37,42,59] and 12 retrospective studies (HR = 1.79, $p = 0.001$). Classification by age subgroup showed that HIF-1α expression was related to worse OS in patients aged less than 60 years (HR = 1.44, $p < 0.001$) and older than 60 years (HR = 2.10, $p < 0.001$). Finally, we further applied TSA to obtain more meaningful results. TSA showed that there was sufficient data to draw reliable conclusions regarding the 5-year OS and patients less than 60 years of age subgroups (Table 2). Additional well-designed multicenter randomized controlled trials (RCTs) are needed to provide more accurate and conclusive evidence.

Interestingly, according to histologic subtypes, we found that HIF-1α expression was not associated

with OS in squamous cell carcinoma, whereas the remaining studies with unclear or mixed histotypes showed a significant association. Other histotypes, such as adenocarcinoma, were unclear and lacking; it is possible that HIF-1α expression in other histotypes might affect the prognosis. Additionally, Furukawa and colleagues reported that HIF-1α-regulated glucose transporter (GLUT) 1 in lung adenocarcinoma may promote tumor aggressiveness and serve as a prognostic indicator of worse prognosis, but not in lung squamous cell carcinoma.[70] Additional clinical studies are needed among other histologic subtypes of advanced cancer.

Our study has some important implications. First, HIF-1α expression is associated with worse outcomes, which suggests that HIF-1α may be a key druggable therapeutic target. This is important for advanced cancer patients who are treated with common chemotherapy, radiotherapy, or chemoradiotherapy. Second, a number of subgroup analyses have been conducted. Third, HIF-1α expression is linked to poor OS in European and Asian subjects, which suggests that HIF-1α may play important roles in different ethnic populations. Fourth, HIF-1α expression is related to an unfavorable OS in younger and older cancer patients, which indicates that HIF-1α may be a potential therapeutic target for younger or older cancer stratification. Finally, HIF-1α expression was not related to OS in squamous cell carcinoma, suggesting that additional prospective studies are essential to further validate whether HIF-1α expression has therapeutic implications in other histotypes, such as adenocarcinoma, due to different histological features.

This meta-analysis had several limitations. First, publication bias is present in the current meta-analysis, as indicated using Egger's test because predominantly positive results were published. Articles with other styles, such as papers in other languages, unpublished papers, and conference abstracts, were excluded due to insufficient information, which may lead to potential bias. In addition, sensitivity analysis by omitting an individual study demonstrated a similar trend for the OS of HIF-1α expression results. Second, the number of some eligible studies had small sample sizes between HIF-1α expression and DFS, PFS, CSS, RFS, and MFS, and some subgroups on OS. The number of the included studies and sample sizes was relatively small between HIF-2α and the prognosis. Although all eligible studies were well performed, these results should be interpreted with caution based on TSA. Third, the cut-off values of HIF-1α and HIF-2α expression from the included studies may differ, and, in the future, HIF-1α and HIF-2α expression should be defined as positive or negative based on a standard, such as within a single cancer; for example, for lung cancer, the American Society of Clinical Oncology (ASCO), the College of American Pathologists (CAP), and the Association for Molecular Pathology (AMP) had to come together to standardize results for epidermal growth factor receptor (EGFR) detection.[71] Fourth, the molecular features of various cancer types might differ to some extent and thus represent a complicated network. Biomarkers may also be affected by patient baseline characteristics. Thus, our study only included data adjusted by multivariate survival, and multivariable survival analysis adjusted factors are more valuable than the study that used univariable survival analysis. Fifth, even within a single cancer (colorectal), different treatment regimens were also found because the data are not an individual patient data analysis. In addition, only two RCTs evaluated the prognostic significance of HIF-1α expression in advanced cancer. We lacked sufficient RCTs to further prove our findings, and more trials that include subgroup analyses are warranted. Finally, the different sample types employed in these studies, including paraffin-embedded tumor tissue specimens, fresh tissue, serum, and plasma may be a potential source of heterogeneity. A detailed investigation of the best sample processing was not possibly performed in this meta-analysis. Therefore, the development of a stable high-performance assay with good sensitivity can be a good method for HIF-1α and HIF-2α detection and may help overcome this issue in the future.

In conclusion, the current study showed that HIF-1α expression was associated with a worse prognosis for advanced cancer patients treated with chemotherapy, radiotherapy, or chemoradiotherapy, which suggested that targeting HIF-1α may be a useful therapeutic approach to improve survival in advanced cancer patients. Based on the REMARK criteria, further large-scale prospective clinical trials including training and validation sets are strongly suggested to confirm our findings and help stratify the clinical treatment of patients into specific cancer types.

Author contributions

Susu Han and Tao Huang contributed to the conception and design of this research. Susu Han,

Xing Wu, Xiyu Wang, Liting Yao, and Tao Huang contributed to the drafting of the article and final approval of the submitted version. Susu Han, Tao Huang, Xing Wu, Xiyu Wang, Liting Yao, and Fenggang Hou contributed to data analyses and the interpretation and completion of the figures and tables. All authors read and approved the final manuscript.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

Ethical review from patients

Our study was not primary research involving human samples, but rather a secondary analysis of human subject data published in public databases.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Availability of data and materials

All data supporting our findings are listed in this manuscript.

Supplemental material

Supplemental material for this article is available online.

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