Vascular Endothelial Growth Factor Pathway Polymorphisms as Prognostic and Pharmacogenetic Factors in Cancer: A Systematic Review and Meta-analysis

Lawson Eng[1,2], Abul Kalam Azad[1], Steven Habbous[1], Vincent Pang[1], Wei Xu[1,2], Anke-Hilse Maitland-van der Zee[4], Sevtap Savas[5], Helen Mackay[2], Eitan Amir[2*], Geoffrey Liu[1-3*]

[1]Ontario Cancer Institute, Toronto, Ontario Canada. [2]Division of Medical Oncology and Hematology, Department of Medicine, Princess Margaret Hospital/University Health Network and University of Toronto, Toronto, Ontario, Canada. [3]Division of Epidemiology, Dalla Lana School of Public Health, Toronto, Ontario, Canada. [4]Division of Pharmacoepidemiology & Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands; and [5]Discipline of Genetics, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Running Title: *VEGF* polymorphisms as predictive and prognostic biomarkers

Category: Experimental Therapeutics

User-Defined Keywords: Outcome, predictive biomarkers, prognostic biomarkers

User-Selected Keywords: Angiogenic factors and receptors, Polymorphisms in genes related to cell growth, differentiation, metastatic potential, and apoptosis in cancer risk, Methodology for SNP data analysis, Pharmacogenetics/pharmacogenomics, EP03 GENETICS OF RISK, OUTCOME, AND PREVENTION

Funding Sources: Alan B Brown Chair in Molecular Genomics, CCO Chair in Experimental Therapeutics and Population studies and Posluns Family Foundation

***Co-Corresponding and Co-Senior Authors:**

Geoffrey Liu, MD, MSc, FRCPC

Princess Margaret Hospital

610 University Ave, Rm 7-124

Toronto, Ontario M5G 2M9

Tel: 416-946-4501 Ext 3428, Fax: 416-946-6546

e-mail: Geoffrey.liu@uhn.ca

Eitan Amir, MB ChB, MRCP (UK)

Princess Margaret Hospital

610 University Ave, Rm 5-124

Toronto, Ontario M5G 2M9

Tel: 416-946-4501 Ext 5181, Fax: 416-946-4563

e-mail: eitan.amir@uhn.ca

Conflicts of Interest: None

Word Count: 3003 Words. Total Number of Figures and Tables: 5 (4 Tables, 1 Figure)

Abstract

Angiogenesis is an important host process that interacts with cancer cells to promote growth, invasion and metastasis. Numerous therapeutic agents targeting the vascular endothelial growth factor (VEGF) pathway have been developed. Host variability in VEGF pathway can influence angiogenesis-dependent signalling, altering sensitivity to anti-angiogenic drugs and prognosis. A systematic review and meta-analysis was conducted (May 1990 - July 2011). Eligible studies involved cancer patients and compared polymorphisms in the VEGF pathway (VEGF and molecules directly interacting with VEGF: KDR, FLT1, FGF, FGF2, FGFR, NRP1, Endostatin (encoded by *COL18A1*)) and reported one of the following outcomes: overall survival, progression-free survival, time-to-recurrence, disease-free survival, response rate or drug toxicity. We identified 48 cancer studies assessing prognosis and 12 cancer studies exploring pharmacogenetics of anti-VEGF therapy across various VEGF pathway polymorphisms. There was marked inter- and intra-disease site heterogeneity in the effect of polymorphisms on both outcome and response to therapy. Meta-analyses of five *VEGF* polymorphisms (+936*C>T,* -460*T>C,* +405*G>C,* -1154*G>A* and -2578*C>A*) identified a significant prognostic relationship: *VEGF* +405*G>C* variants demonstrated a highly statistically significant improvement in OS (HR=0.74, 95% CI = 0.60-0.91, P = 0.004). Variants (heterozygotes and/or homozygotes) of *VEGF +405G>C* were significantly associated with improved survival in a meta-analysis of multiple cancer sites.

Translational Relevance Statement

Anti-angiogenic drugs are part of the clinical armamentarium for numerous solid tumors. Results of clinical trials of anti-angiogenic agents have shown variable results. The need for predictive and prognostic markers for anti-angiogenic therapy is therefore acute. Here we report a systematic review and meta-analysis of single nucleotide polymorphisms in *VEGF* genes and their related protein receptors. Results showed substantial heterogeneity in the design and results of both predictive and prognostic studies. Our meta-analysis revealed a significant improvement in survival for *VEGF* +405*G>C* variants in unselected cancer patients. There did not appear to be an association between germline polymorphisms and benefit or toxicity from anti-angiogeneic agent. However, inconsistency in the definition of these endpoints limited our analysis of predictive power. Future prospective studies should extend this research to include pathway analysis, haplotype analysis and discovery-focused genome-wide association studies, followed by appropriate validation or meta-analytical analyses.

Introduction

Angiogenesis is a critical component in oncogenesis (1). Tumors can promote angiogenesis through the secretion of pro-angiogenic factors such as vascular endothelial growth factor (VEGF and its isoforms) and basic fibroblast growth factor (bFGF) or over-expression of associated receptors: VEGFR, VEGFR2/KDR and NRP2 (2). Tumors utilize this host-mediated process to meet their metabolic needs, to help remove biological waste products of rapidly dividing cells, and to provide a mechanism for metastasis.

Angiogenic factors interact with a multitude of pathways, including matrix metalloproteinases, inflammatory cytokines, chemokines (e.g., interleukins and interferons), integrins, nitric oxide, and other endothelial related proteins. Downstream signalling effectors of angiogenesis receptors also lead to the activation of the phosphatidylinositol 3-kinase/AKT (PI3K-AKT) and mitogen-activated protein kinase (MAPK) pathways.

The VEGF pathway is predominantly driven by host factors. Host genetic variability in this pathway may therefore influence angiogenesis-dependent biological pathways during cancer development and hence influence outcome and sensitivity to various therapies. Associations between genetic polymorphisms in *VEGF* and the *risk* of developing various cancers have been reviewed previously (3). Less is known about the role of potential germline polymorphisms as prognostic markers of *clinical outcomes* or the potential *predictive* or *pharmacogenetic* nature of these polymorphisms in relation to drug efficacy and toxicity.

We performed a systematic review and meta-analysis of the role of *VEGF* pathway polymorphisms as markers of clinical outcome and toxicity in cancer patients. The two aims were: (i) to investigate polymorphisms as pharmacogenetic predictors of drug efficacy and

toxicities; and (ii) to investigate polymorphisms as general prognostic indicators of survival, independent of therapy.

Methods

Search Query

We performed a literature search of MEDLINE (host: PubMed) for the period of May 1990 through July 2011 using the keywords and MeSH terms: {"*angiogenesis*", "*VEGF", "VEGFR1", "FLT1", "KDR", "VEGFR2", "FGF2", "FGF", "FGFR", "NRP1", "Endostatin", "VEGFA", "VEGFB", "Bevacizumab"*} and "*cancer*" and "*polymorphism*". We limited searches to English language articles.

Study Inclusion and Exclusion Criteria

Article eligibility was reviewed independently by 2 reviewers (G.L., L.E.). Eligible articles were original peer-reviewed studies focused on genetic variation in the following angiogenesis pathway genes: *VEGFA*, *VEGFB*, and *FGF2*, and/or genes that code for their direct interactors or receptors: *FLT* (*VEGFR1*), *KDR* (*VEGFR2*), *NRP1* and *FGFR*. These genes were selected from The Pharmacogenomics Knowledgebase website (4). We also included Endostatin, the 20-kDa C-terminal fragment derived from type XVIII collagen that is a broad spectrum anti-angiogenic factor, which can affect both *VEGF* and *FGF2* pathways and is coded by *COL18A1*. Eligible studies must have reported cancer outcome (i.e., not risk alone), and may have included either/both prognostic and pharmacogenetic assessments. The outcomes evaluated were of any of the following: overall survival (OS), progression free survival (PFS), time to progression (TTP), time to recurrence (TTR), disease free survival (DFS), response rate (RR), and association with

treatment toxicity. Finally, all studies must have included genotyping of 25 or more and at least one VEGF pathway polymorphism to warrant inclusion.

Duplicate studies were excluded as were those testing solely the association of polymorphisms and cancer risk/susceptibility, tumor staging/grading or gene expression. We also excluded case reports, opinion pieces (e.g., letters to the editors) and other review papers. We categorized studies as being *pharmacogenetic* (effect of polymorphism on response/outcome/toxicity to therapy where treatment included anti-angiogenic therapy) or *prognostic* (effect of polymorphism on outcome irrespective of therapy); data were analyzed for various disease sites: breast, colorectal, gastro-esophageal, genitourinary, gynecological, lung and other cancers.

Data Extraction and Organization

The following data were extracted from individual publications: title, authors, disease site, country, sample size and/or actual number of patients genotyped, patient inclusion/exclusion criteria, treatment/intervention details, histologic subtypes, grade, stage, tissue used for genotyping (e.g. whole blood versus archival normal tissue) and polymorphisms that were genotyped. Associations between polymorphisms and outcome data were recorded as odds ratios (OR), hazard ratios (HR) or response rate (RR), along with their respective confidence intervals. We noted whether studies were analyzed using only univariate (or crude) analysis or in multivariate (or adjusted) analysis. Haplotype analyses, if performed, were also recorded. A common problem was the use of different nomenclature and names for the same polymorphism. Thus, all identified polymorphisms sharing the same RefSNP (rs) numbers were renamed to

share common names throughout this manuscript (Supplementary Table 1 provides the common and Human Genome Variation Society (HGVS) nomenclature) ((5).

Meta-analysis

Meta-analyses were performed to evaluate the overall prognostic associations of the most commonly evaluated *VEGF* polymorphisms when there were an adequate number of individual studies reporting similar outcomes to justify such an analysis. Only studies reporting HR for OS were included. Analyses were undertaken separately for each polymorphism and were conducted in two phases. First, study data reporting outcome by wild-type or variant polymorphisms irrespective of zygosity were assessed together (i.e., pooled). Second, data were assessed separately for homozygous and heterozygous genotypes. Differences between homozygous and heterozygous subgroups were assessed using methods described by Deeks *et al* (6). Data were analyzed using RevMan 5.1 analysis software (The Cochrane Collaboration, Copenhagen, Denmark). Pooled estimates of HR were computed using generic inverse variance and a random-effects model (7, 8). All statistical tests were two-sided, and statistical significance was defined as $P < 0.01$, which corrected for multiple statistical testing using the Bonferroni method.

Results

Summary of included and excluded studies

A total of 614 studies were initially identified (Figure 1). Of these, 186 were duplicate reports/studies, 130 were related to cancer development risk and 238 further studies were excluded because they did not include at least 25 patients, did not include polymorphisms of

interest, or did not assess any pharmacogenetic or prognostic outcomes of interest. Sixty studies met the inclusion criteria and were included in the final analysis.

Most of the included studies had sample sizes of under 150 patients, and were secondary analyses of Phase I/II clinical trials. Other characteristics of included studies are shown in Table 1. The majority were prognostic (n = 48), which were based primarily on data from case series (n = 33 (69%)). Most pharmacogenetics studies (n = 12) were nested case-control analyses of clinical trials (n = 7 (64%)).

VEGF pathway polymorphisms as predictive markers of bevacizumab efficacy

Nine studies investigated the predictive effect of polymorphisms on response to bevacizumab in numerous cancer sites (Table 2A; additional data in Supplementary Table 2) (9-17). Among those investigating bevacizumab response, Schneider *et al* (n = 341) reported that the *C* allele of *VEGF* -2578*C>A* and *G* allele of *VEGF* -1154*G>A* (alternatively known as -116*G>A*) were independently associated with poorer OS in patients treated with bevacizumab (9). Similarly, a smaller study investigating nine polymorphisms, by Formica *et al* (n = 40), reported the same finding for *VEGF* -1154*G>A* with respect to PFS (corrected for multiple comparisons) and also found the *C* allele for *VEGF* +405*G>C* (alternatively known as +634*G>C*) associated with improved overall radiologic response rate (12). Another small study by Argiris *et al* (n = 28), found the *C/-* genotypes of *VEGF* +405*G>C* significantly associated with OS and TTP in multivariate analysis, but only one patient was present in the *G/G* genotype group (14).

In all other studies, associations have yet to be validated: a) one report by Etienne-Grimaldi *et al* (n = 138), identified the *C* allele of *VEGF* +936*C>T* associated with a shorter

TTP; b) Loupakis *et al* (n = 111), found the *C* allele of *VEGF* -460*C>T* (alternatively known as -1498*T>C*) associated with improved PFS; and c) Schultheis *et al* (n = 70) reported that the *C/T* genotype of *C>T* polymorphism in the 3' UTR of *NRP1* was associated with improved PFS, after adjustment for thirty comparisons (10, 11, 15). In three other studies by Jain *et al* (n = 178), Zhang *et al* (n = 65) (using both univariate and multivariate analyses), and Smerdel *et al* (n = 38), *VEGF*, *KDR* and *NRP1* polymorphisms were found to have no association with survival (13, 16, 17).

VEGF pathway polymorphisms and treatment toxicity

Seven studies investigated bevacizumab toxicity in various cancer sites (9-12, 14, 15, 17). Three additional studies investigated the predictive effect of *VEGF* polymorphisms on toxicity of non-VEGF targeting drugs (Table 2B; additional data in Supplementary Table 3) (18-20). The experimental paclitaxel and bevacizumab arm of a breast cancer study (n = 180) identified the *C* allele in *VEGF* -460*C>T* and the *G* allele in *VEGF* +405*G>C* as being associated with a greater frequency of grade 3 or 4 hypertension (9). In contrast, Etienne-Grimaldi *et al,* found the *G* allele of *VEGF* +405*G>C* associated with decreased toxicity (which included any signs of hypertension, arterial or venous thromboemboli, or hemorrhages) (10). Four smaller studies (n = 28-111) involving various combinations of bevacizumab with chemotherapeutic drugs found no associations of any evaluated polymorphisms with any toxicities (11, 12, 14, 15). In Jain *et al*'s investigation of *KDR* polymorphisms, the *A* allele in *KDR* 1718*T>A* (alternatively known as 1719*A>T*, Q472H, 1416*A>T* or Exon11*T>A*) was found associated with increased frequency of hypertension and hand-foot skin reactions in patients treated with sorafenib and bevacizumab (17).

Three other studies investigated the role of *VEGF* polymorphisms in the toxicities in non-VEGF targeting drugs (18-20). In a 49 patient study focused on drug toxicity of 5-fluorouracil and cisplatin chemotherapy, a multitude of nominally significant *VEGF* polymorphic associations with various toxicities such as cheilitis, leukopenia, and stomatitis was reported (18). Two studies, one investigating danusertib (a multi-kinase aurora inhibitor) and another on telatinib found no associations between any *KDR* or *FLT* polymorphisms and toxicity (19, 20).

VEGF polymorphisms as prognostic biomarkers

Studies that evaluated *VEGF* pathway polymorphisms as prognostic markers of cancer outcome are described in Supplementary Tables 4-7 (21-68).

VEGF +936*C*>*T* was associated with OS, DFS, or TTR in the three largest colorectal cancer studies involving; however, two showed improved outcome by the *T* allele, while one showed worse outcome by the *T* allele (27, 29, 32). Among these three studies, the two showing improved outcome by the *T* allele involved patients from the United States and Greece and both used blood as their tissue source, while the one showing worse outcome was from Korea and used fresh tumor tissue as their tissue source. There was more consistency with *VEGF* +405*G*>*C*, where two of these studies showed improved outcome in patients carrying the *C* allele (a third smaller study reported no significant association) (27, 32). Of the two studies showing improved outcome, both included cases from all stages; Dassoulas *et al* evaluated a Greek population, while Kim *et al* focused on a Korean population that specifically underwent surgical resection.

There was no consistency in the relationship between various *VEGF* polymorphisms and clinical outcome in gastro-esophageal cancers. For example, one gastric cancer study (n = 137)

found a strong relationship between carrying at least one *A* allele in *COL18A1* (*Endostatin*) +4349*G>A* (alternatively known as D104N) and worse outcome (adjusted hazard ratio (aHR) for TTR, 2.28, 95% CI = 1.26-4.11; *P* = 0.004), while a larger 239 patient study in distal esophageal and gastroesophageal junction adenocarcinomas found no relationship (35, 39).

Of five breast cancer studies, *VEGF* polymorphisms, -7*C>T* and +405*G>C*, and the *KDR* polymorphism, +1192*A>G*, were associated with either OS or DFS in single, unvalidated studies (21, 22, 24). In genitourinary and gynaecologic cancers, associations were either of borderline statistical significance or involved crude, unadjusted analyses, with two exceptions: *VEGF* +405*C*/*C* genotype was associated with poorer OS in a cervical cancer study (n = 199) from Korea; and minor alleles of *VEGFC* polymorphisms (rs17697305 and rs1485766) were associated with altered OS outcomes in a single ovarian cancer study that evaluated over 1400 polymorphisms across multiple pathways in 325 patients (52, 54). Separate lung cancer studies reported worse survival with *VEGF* +405*G*/*G*, *VEGF* -1154*A*/-, and *VEGF* -460*T*/*T*; none being validated (55, 56, 58).

Meta-analyses of Prognostic Studies

Because of the large number of studies (n = 42) evaluating five individual *VEGF* polymorphisms (*VEGF* +936*C>T*, *VEGF* -460*T>C*, *VEGF* -1154*G>A*, *VEGF* -2578*C>A* and *VEGF* +405*G>C*; individual details in Supplementary Tables 4-7), meta-analyses were performed separately on these five polymorphisms. When the homozygous and heterozygous polymorphisms were assessed together (i.e., pooled; Table 3A), the *C* allele of *VEGF* +405*G>C* was found significantly associated with improved OS (HR = 0.74, 95% CI = 0.60-0.91, *P* = 0.004). The corresponding forest plot is shown in Table 4A. Among studies in the zygosity-

segregated analysis (Table 3B), the same variant of *VEGF* +405*G>C* also showed a statistically significant improvement in OS (HR = 0.79, 95% CI = 0.67-0.94, *P* = 0.007) that was not different by zygosity (Table 4B). In addition, a non-significant trend was identified where *VEGF* +936*C>T* variants (regardless of zygosity) were associated with worse OS (HR = 1.38, 95% CI = 0.96-1.97, *P* = 0.08); Supplementary Figure 1B). For other polymorphisms, there was no evidence of association with survival in either pooled or zygosity-segregated analyses (Supplementary Figure 2-4). Assessment of funnel plots in both pooled and zygosity-segregated analysis revealed no any evidence of publication bias (data not shown).

Given that two of these five *VEGF* polymorphisms (*VEGF* -460*C>T* and *VEGF* -2578*C>A*) were in strong linkage disequilibrium with each other ((D' > 0.90) based on HapMap data, and with several other polymorphisms (rs833068 *G>A*, rs2146323 *C>A*, and *VEGF* -7*C>T*), a meta-analysis was performed on this haplotype block, pooling results from all studies involving any of these polymorphisms and assigning association direction based on the most common haplotype (69). No significant association with survival outcome was found.

Discussion

Anti-angiogenic drugs are part of the clinical armamentarium for numerous solid tumors. Results of clinical trials of anti-angiogenic agents have shown variable results (70). The need for predictive and prognostic markers for anti-angiogenic therapy is therefore acute. There were too few studies focusing on the same polymorphism and outcome to assess consistency of results across studies. Of the two largest studies, Jain *et al* pooled data from multiple cancer sites across several Phase I and II trials of sorafenib and bevacizumab and found no significant results (17). In contrast, Schneider *et al* evaluated 341 breast cancer patients treated with paclitaxel with or

without bevacizumab and found two variants (*VEGF* -2578*C>A* and -1154*G>A*) associated with improved OS while two different variants (*VEGF* +405G>*C* and -460*C>T*) were significantly associated with hypertension; the *VEGF* -1154*G>A* and -460*C>T* are, in general, highly linked in the Caucasian population (9). Both the *A* allele of *VEGF* -1154*G>A*, and the *C* allele of *VEGF* -460*C>T*, were associated with improved outcome in two separate pharmacogenetics studies (11, 12). However, these relationships await validation. In the future, comprehensive analyses of polymorphisms in either carefully designed observational studies or in larger Phase III trials of bevacizumab and other VEGF-targeting agents in ovarian, colorectal, breast, and other cancers could be substantially more useful in delineating the role of these polymorphisms as pharmacogenetic markers.

We also reviewed VEGF pathway polymorphisms serving as general prognostic markers of outcome in patients not treated with VEGF-targeted agents. In this analysis, a large number of prognostic studies across many different cancers including ovarian, cervical, lung, breast, colorectal, gastric, esophageal/gastro-esophageal junction, renal, lung and liver cancers focused on five specific *VEGF* polymorphic variations. A meta-analysis was performed on these polymorphisms, which identified the variant of *VEGF* +405*G>C* showing a highly significant prognostic role in improving the survival of cancer patients. As expected, given the varied cancer sites there was heterogeneity across individual studies (I^2 = 72%). However, the use of a pooled analysis and random effects modelling has been particularly useful for identifying significant relationships in the face of heterogeneous data.

VEGF +405*G>C* is located in the 5'-untranslated region of the *VEGF* gene and is in modest linkage disequilibrium with *VEGF* +936*C>T* (D' = 0.65 - 0.74); this may help explain the non-significant trend found with *VEGF* +936*C>T* (34, 71). Genotype-phenotype analyses

suggest that the *C* allele *VEGF* +405*G>C* is associated with decreased promoter activity and decreased expression of *VEGF*, as the *C* allele reduces binding of the transcription factor, MZF1, which then subsequently reduces gene expression (72-74). Hence, the *C* allele *VEGF* +405*G>C* may confer a survival advantage by reducing a tumor's ability to stimulate angiogenesis.

The quality of a systematic review and meta-analysis is partly related to the quality of its underlying studies. The included studies have limitations. First, studies were extremely varied in sample size: some studies had as few as 28 patients and the largest involved over 1100 patients. Our meta-analyses included weighting based on sample size. Second, most studies used hypothesis-driven candidate approaches, whereby only specific genes and polymorphisms from a selected list were analyzed. This is a common study design and can be most useful for meta-analyses, but suffers from an inability to identify novel biomarkers. Third, statistical significance of the *VEGF* +405*G>C* polymorphism for cancer prognosis required the pooling of heterogeneous studies across disease sites that had different underlying populations (i.e., race/ethnicity), study designs, sample sources (i.e., blood *versus* tumor based tissue), and statistical analyses (including whether there was adjustment for multiple comparisons, and using multivariate *versus* univariate analyses). However, this heterogeneity has advantages in that the significance of this finding was robust to all these aforementioned issues. Finally, several of the measured phenotypes, such as therapeutic response and drug toxicity, were defined differently across studies, rendering it difficult for cross-comparison, interpretation and evaluation in pooling or meta-analytical analysis. For example, some studies explored each toxicity separately, while other studies analyzed multiple toxicities as a composite measure. Hence, no meta-analyses were performed for the pharamacogenetic arm of the study.

In conclusion, there have been a large number of published studies exploring the association of germline polymorphisms of the VEGF pathway with cancer outcomes and treatment toxicities. These studies have substantial heterogeneity both in terms of methodology and in terms of effect size. When studies using similar methodology and involving similar outcome were assessed in meta-analyses, the variants of *VEGF* +405*G>C* were highly significantly associated with a 26% relative improvement in overall survival across a variety of tumor types, even after accounting for the number of comparisons performed. Future prospective studies should extend this research to include pathway analysis, haplotype analysis and discovery-focused genome-wide association studies, followed by appropriate validation or meta-analytical analyses.

Grant Support

This work was sponsored in part by the Alan B Brown Chair in Molecular Genomics, CCO Chair in Experimental Therapeutics and Population studies and Posluns Family Foundation

References

1. McDougall SR, Anderson AR, Chaplain MA. Mathematical modelling of dynamic adaptive tumour-induced angiogenesis: Clinical implications and therapeutic targeting strategies. J Theor Biol 2006;241(3):564-89.

2. Ulahannan SV, Brahmer JR. Antiangiogenic agents in combination with chemotherapy in patients with advanced non-small cell lung cancer. Cancer Invest 2011;29(4):325-37.

3. Jain L, Vargo CA, Danesi R, Sissung TM, Price DK, Venzon D, et al. The role of vascular endothelial growth factor SNPs as predictive and prognostic markers for major solid tumors. Mol Cancer Ther 2009;8(9):2496-508.

4. McDonagh EM, Whirl-Carrillo M, Garten Y, Altman RB, Klein TE. From pharmacogenomic knowledge acquisition to clinical applications: The PharmGKB as a clinical pharmacogenomic biomarker resource. Biomark Med 2011;5(6):795-806.

5. dbSNP home page [homepage on the Internet]. Bethesda, Maryland, USA: National Center for Biotechnology Information. 2012 03/19/2012 [cited 06/05/2012]. Available from: http://www.ncbi.nlm.nih.gov/projects/SNP/index.html.

6. Deeks JJ. Systematic reviews in health care: Systematic reviews of evaluations of diagnostic and screening tests. BMJ 2001;323(7305):157-62.

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

8. Deeks JJ, Higgins JPT, Altman DG. Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions. Chichester, UK: John Wiley & Sons, Ltd; 2006. p. 243-93.

9. Schneider BP, Wang M, Radovich M, Sledge GW, Badve S, Thor A, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. J Clin Oncol 2008;26(28):4672-8.

10. Etienne-Grimaldi MC, Formento P, Degeorges A, Pierga JY, Delva R, Pivot X, et al. Prospective analysis of the impact of VEGF-A gene polymorphisms on the pharmacodynamics of bevacizumab-based therapy in metastatic breast cancer patients. Br J Clin Pharmacol 2011;71(6):921-8.

11. Loupakis F, Ruzzo A, Salvatore L, Cremolini C, Masi G, Frumento P, et al. Retrospective exploratory analysis of VEGF polymorphisms in the prediction of benefit from first-line FOLFIRI plus bevacizumab in metastatic colorectal cancer. BMC Cancer 2011;11:247,2407-11-247.

12. Formica V, Palmirotta R, Del Monte G, Savonarola A, Ludovici G, De Marchis ML, et al. Predictive value of VEGF gene polymorphisms for metastatic colorectal cancer patients receiving first-line treatment including fluorouracil, irinotecan, and bevacizumab. Int J Colorectal Dis 2011;26(2):143-51.

13. Zhang W, Azuma M, Lurje G, Gordon MA, Yang D, Pohl A, et al. Molecular predictors of combination targeted therapies (cetuximab, bevacizumab) in irinotecan-refractory colorectal cancer (BOND-2 study). Anticancer Res 2010;30(10):4209-17.

14. Argiris A, Karamouzis MV, Gooding WE, Branstetter BF, Zhong S, Raez LE, et al. Phase II trial of pemetrexed and bevacizumab in patients with recurrent or metastatic head and neck cancer. J Clin Oncol 2011;29(9):1140-5.

15. Schultheis AM, Lurje G, Rhodes KE, Zhang W, Yang D, Garcia AA, et al. Polymorphisms and clinical outcome in recurrent ovarian cancer treated with cyclophosphamide and bevacizumab. Clin Cancer Res 2008;14(22):7554-63.

16. Smerdel MP, Steffensen KD, Waldstrom M, Brandslund I, Jakobsen A. The predictive value of serum VEGF in multiresistant ovarian cancer patients treated with bevacizumab. Gynecol Oncol 2010;118(2):167-71.

17. Jain L, Sissung TM, Danesi R, Kohn EC, Dahut WL, Kummar S, et al. Hypertension and hand-foot skin reactions related to VEGFR2 genotype and improved clinical outcome following bevacizumab and sorafenib. J Exp Clin Cancer Res 2010;29:95.

18. Sakaeda T, Yamamori M, Kuwahara A, Hiroe S, Nakamura T, Okumura K, et al. VEGF G-1154A is predictive of severe acute toxicities during chemoradiotherapy for esophageal squamous cell carcinoma in japanese patients. Ther Drug Monit 2008;30(4):497-503.

19. Steeghs N, Mathijssen RH, Wessels JA, de Graan AJ, van der Straaten T, Mariani M, et al. Influence of pharmacogenetic variability on the pharmacokinetics and toxicity of the aurora kinase inhibitor danusertib. Invest New Drugs 2011;29(5):953-62.

20. Steeghs N, Gelderblom H, Wessels J, Eskens FA, de Bont N, Nortier JW, et al. Pharmacogenetics of telatinib, a VEGFR-2 and VEGFR-3 tyrosine kinase inhibitor, used in patients with solid tumors. Invest New Drugs 2011;29(1):137-43.

21. Balasubramanian SP, Cox A, Cross SS, Higham SE, Brown NJ, Reed MW. Influence of VEGF-A gene variation and protein levels in breast cancer susceptibility and severity. Int J Cancer 2007;121(5):1009-16.

22. Kidd LR, Brock GN, VanCleave TT, Benford ML, Lavender NA, Kruer TL, et al. Angiogenesis-associated sequence variants relative to breast cancer recurrence and survival. Cancer Causes Control 2010;21(10):1545-57.

23. Knechtel G, Hofmann G, Gerger A, Renner W, Langsenlehner T, Szkandera J, et al. Analysis of common germline polymorphisms as prognostic factors in patients with lymph node-positive breast cancer. J Cancer Res Clin Oncol 2010;136(12):1813-9.

24. Lu H, Shu XO, Cui Y, Kataoka N, Wen W, Cai Q, et al. Association of genetic polymorphisms in the VEGF gene with breast cancer survival. Cancer Res 2005;65(12):5015-9.

25. Forsti A, Jin Q, Altieri A, Johansson R, Wagner K, Enquist K, et al. Polymorphisms in the KDR and POSTN genes: Association with breast cancer susceptibility and prognosis. Breast Cancer Res Treat 2007;101(1):83-93.

26. Balasubramanian SP, Cross SS, Globe J, Cox A, Brown NJ, Reed MW. Endostatin gene variation and protein levels in breast cancer susceptibility and severity. BMC Cancer 2007;7:107.

27. Dassoulas K, Gazouli M, Rizos S, Theodoropoulos G, Christoni Z, Nikiteas N, et al. Common polymorphisms in the vascular endothelial growth factor gene and colorectal cancer development, prognosis, and survival. Mol Carcinog 2009;48(6):563-9.

28. Vidaurreta M, Sanchez-Munoz R, Veganzones S, Rafael S, Gutierrez M, de-la-Orden V, et al. Vascular endothelial growth factor gene polymorphisms in patients with colorectal cancer. Rev Esp Enferm Dig 2010;102(1):20-31.

29. Lurje G, Zhang W, Schultheis AM, Yang D, Groshen S, Hendifar AE, et al. Polymorphisms in VEGF and IL-8 predict tumor recurrence in stage III colon cancer. Ann Oncol 2008;19(10):1734-41.

30. Hansen TF, Garm Spindler KL, Andersen RF, Lindebjerg J, Brandslund I, Jakobsen A. The predictive value of genetic variations in the vascular endothelial growth factor A gene in metastatic colorectal cancer. Pharmacogenomics J 2011;11(1):53-60.

31. Zhang W, Gordon M, Press OA, Rhodes K, Vallbohmer D, Yang DY, et al. Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with cetuximab. Pharmacogenet Genomics 2006;16(7):475-83.

32. Kim JG, Chae YS, Sohn SK, Cho YY, Moon JH, Park JY, et al. Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with colorectal cancer. Clin Cancer Res 2008;14(1):62-6.

33. Hansen TF, Sorensen FB, Spindler KL, Olsen DA, Andersen RF, Lindebjerg J, et al. Microvessel density and the association with single nucleotide polymorphisms of the vascular endothelial growth factor receptor 2 in patients with colorectal cancer. Virchows Arch 2010;456(3):251-60.

34. Bradbury PA, Zhai R, Ma C, Xu W, Hopkins J, Kulke MJ, et al. Vascular endothelial growth factor polymorphisms and esophageal cancer prognosis. Clin Cancer Res 2009;15(14):4680-5.

35. Lurje G, Leers JM, Pohl A, Oezcelik A, Zhang W, Ayazi S, et al. Genetic variations in angiogenesis pathway genes predict tumor recurrence in localized adenocarcinoma of the esophagus. Ann Surg 2010;251(5):857-64.

36. Lorenzen S, Panzram B, Keller G, Lordick F, Herrmann K, Becker K, et al. Association of the VEGF 936C>T polymorphism with FDG uptake, clinical, histopathological, and metabolic response in patients with adenocarcinomas of the esophagogastric junction. Mol Imaging Biol 2011;13(1):178-86.

37. Stocker G, Ott K, Henningsen N, Becker K, Hapfelmeier A, Lordick F, et al. CyclinD1 and interleukin-1 receptor antagonist polymorphisms are associated with prognosis in neoadjuvant-treated gastric carcinoma. Eur J Cancer 2009;45(18):3326-35.

38. Guan X, Zhao H, Niu J, Tan D, Ajani JA, Wei Q. Polymorphisms of TGFB1 and VEGF genes and survival of patients with gastric cancer. J Exp Clin Cancer Res 2009;28:94.

39. Lurje G, Husain H, Power DG, Yang D, Groshen S, Pohl A, et al. Genetic variations in angiogenesis pathway genes associated with clinical outcome in localized gastric adenocarcinoma. Ann Oncol 2010;21(1):78-86.

40. Tzanakis N, Gazouli M, Rallis G, Giannopoulos G, Papaconstantinou I, Theodoropoulos G, et al. Vascular endothelial growth factor polymorphisms in gastric cancer development, prognosis, and survival. J Surg Oncol 2006;94(7):624-30.

41. Al-Moundhri MS, Al-Nabhani M, Burney IA, Al-Farsi AA, Al-Bahrani B. Gastric cancer risk predisposition and prognostic significance of vascular endothelial growth factor (VEGF) gene polymorphisms--a case-control study in an omani population. Mol Carcinog 2009;48(12):1170-6.

42. Kim JG, Sohn SK, Chae YS, Cho YY, Bae HI, Yan G, et al. Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with gastric cancer. Ann Oncol 2007;18(6):1030-6.

43. Kawai Y, Sakano S, Korenaga Y, Eguchi S, Naito K. Associations of single nucleotide polymorphisms in the vascular endothelial growth factor gene with the characteristics and prognosis of renal cell carcinomas. Eur Urol 2007;52(4):1147-55.

44. Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, et al. Genotypes of TNF-alpha, VEGF, hOGG1, GSTM1, and GSTT1: Useful determinants for clinical outcome of bladder cancer. Urology 2005;65(1):70-5.

45. Mucci LA, Stark JR, Figg WD, Schumacher F, Li H, Abe M, et al. Polymorphism in endostatin, an angiogenesis inhibitor, and prostate cancer risk and survival: A prospective study. Int J Cancer 2009;125(5):1143-6.

46. Li HC, Cai QY, Shinohara ET, Cai H, Cao C, Wang ZF, et al. Endostatin polymorphism 4349G/A(D104N) is not associated with aggressiveness of disease in prostate [corrected] cancer. Dis Markers 2005;21(1):37-41.

47. Hefler LA, Mustea A, Konsgen D, Concin N, Tanner B, Strick R, et al. Vascular endothelial growth factor gene polymorphisms are associated with prognosis in ovarian cancer. Clin Cancer Res 2007;13(3):898-901.

48. Polterauer S, Grimm C, Mustea A, Concin N, Tanner B, Thiel F, et al. Vascular endothelial growth factor gene polymorphisms in ovarian cancer. Gynecol Oncol 2007;105(2):385-9.

49. Lose F, Nagle CM, O'Mara T, Batra J, Bolton KL, Song H, et al. Vascular endothelial growth factor gene polymorphisms and ovarian cancer survival. Gynecol Oncol 2010;119(3):479-83.

50. Smerdel MP, Waldstrom M, Brandslund I, Steffensen KD, Andersen RF, Jakobsen A. Prognostic importance of vascular endothelial growth factor-A expression and vascular endothelial growth factor polymorphisms in epithelial ovarian cancer. Int J Gynecol Cancer 2009;19(4):578-84.

51. Steffensen KD, Waldstrom M, Brandslund I, Jakobsen A. The relationship of VEGF polymorphisms with serum VEGF levels and progression-free survival in patients with epithelial ovarian cancer. Gynecol Oncol 2010;117(1):109-16.

52. Goode EL, Maurer MJ, Sellers TA, Phelan CM, Kalli KR, Fridley BL, et al. Inherited determinants of ovarian cancer survival. Clin Cancer Res 2010;16(3):995-1007.

53. Amano M, Yoshida S, Kennedy S, Takemura N, Deguchi M, Ohara N, et al. Association study of vascular endothelial growth factor gene polymorphisms in endometrial carcinomas in a japanese population. Eur J Gynaecol Oncol 2008;29(4):333-7.

54. Kim YH, Kim MA, Park IA, Park WY, Kim JW, Kim SC, et al. VEGF polymorphisms in early cervical cancer susceptibility, angiogenesis, and survival. Gynecol Oncol 2010;119(2):232-6.

55. Heist RS, Zhai R, Liu G, Zhou W, Lin X, Su L, et al. VEGF polymorphisms and survival in early-stage non-small-cell lung cancer. J Clin Oncol 2008;26(6):856-62.

56. Guan X, Yin M, Wei Q, Zhao H, Liu Z, Wang LE, et al. Genotypes and haplotypes of the VEGF gene and survival in locally advanced non-small cell lung cancer patients treated with chemoradiotherapy. BMC Cancer 2010;10:431.

57. Dong J, Dai J, Shu Y, Pan S, Xu L, Chen W, et al. Polymorphisms in EGFR and VEGF contribute to non-small-cell lung cancer survival in a chinese population. Carcinogenesis 2010;31(6):1080-6.

58. Masago K, Fujita S, Kim YH, Hatachi Y, Fukuhara A, Nagai H, et al. Effect of vascular endothelial growth factor polymorphisms on survival in advanced-stage non-small-cell lung cancer. Cancer Sci 2009;100(10):1917-22.

59. Kong SY, Park JW, Lee JA, Park JE, Park KW, Hong EK, et al. Association between vascular endothelial growth factor gene polymorphisms and survival in hepatocellular carcinoma patients. Hepatology 2007;46(2):446-55.

60. Wu LM, Xie HY, Zhou L, Yang Z, Zhang F, Zheng SS. A single nucleotide polymorphism in the vascular endothelial growth factor gene is associated with recurrence of hepatocellular carcinoma after transplantation. Arch Med Res 2009;40(7):565-70.

61. Streit S, Mestel DS, Schmidt M, Ullrich A, Berking C. FGFR4 Arg388 allele correlates with tumour thickness and FGFR4 protein expression with survival of melanoma patients. Br J Cancer 2006;94(12):1879-86.

62. Monzo M, Brunet S, Urbano-Ispizua A, Navarro A, Perea G, Esteve J, et al. Genomic polymorphisms provide prognostic information in intermediate-risk acute myeloblastic leukemia. Blood 2006;107(12):4871-9.

63. Kim DH, Lee NY, Lee MH, Sohn SK, Do YR, Park JY. Vascular endothelial growth factor (VEGF) gene (VEGFA) polymorphism can predict the prognosis in acute myeloid leukaemia patients. Br J Haematol 2008;140(1):71-9.

64. Kim DH, Xu W, Kamel-Reid S, Liu X, Jung CW, Kim S, et al. Clinical relevance of vascular endothelial growth factor (VEGFA) and VEGF receptor (VEGFR2) gene polymorphism on the treatment outcome following imatinib therapy. Ann Oncol 2010;21(6):1179-88.

65. Kim DH, Kong JH, Byeun JY, Jung CW, Xu W, Liu X, et al. The IFNG (IFN-gamma) genotype predicts cytogenetic and molecular response to imatinib therapy in chronic myeloid leukemia. Clin Cancer Res 2010;16(21):5339-50.

66. Diao LP, Yu XM, Gao YH, Li Y, Liu HS, Liu LH, et al. Association of VEGF genetic polymorphisms with the clinical characteristics of non-hodgkin's lymphoma. J Cancer Res Clin Oncol 2009;135(11):1473-81.

67. Ruiz MT, Biselli PM, Maniglia JV, Pavarino-Bertelli EC, Goloni-Bertollo EM. Genetic variability of vascular endothelial growth factor and prognosis of head and neck cancer in a brazilian population. Braz J Med Biol Res 2010;43(2):127-33.

68. Formento JL, Etienne-Grimaldi MC, Francoual M, Pages G, Onesto C, Formento P, et al. Influence of the VEGF-A 936C>T germinal polymorphism on tumoral VEGF expression in head and neck cancer. Pharmacogenomics 2009;10(8):1277-83.

69. International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437(7063):1299-320.

70. Ocana A, Amir E, Vera F, Eisenhauer EA, Tannock IF. Addition of bevacizumab to chemotherapy for treatment of solid tumors: Similar results but different conclusions. J Clin Oncol 2011;29(3):254-6.

71. Zhai R, Liu G, Zhou W, Su L, Heist RS, Lynch TJ, et al. Vascular endothelial growth factor genotypes, haplotypes, gender, and the risk of non-small cell lung cancer. Clin Cancer Res 2008;14(2):612-7.

72. Hussein A, Askar E, Elsaeid M, Schaefer F. Functional polymorphisms in transforming growth factor-beta-1 (TGFbeta-1) and vascular endothelial growth factor (VEGF) genes modify risk of renal parenchymal scarring following childhood urinary tract infection. Nephrol Dial Transplant 2010;25(3):779-85.

73. Young HS, Summers AM, Bhushan M, Brenchley PE, Griffiths CE. Single-nucleotide polymorphisms of vascular endothelial growth factor in psoriasis of early onset. J Invest Dermatol 2004;122(1):209-15.

74. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: Correlation with variation in VEGF protein production. Cytokine 2000;12(8):1232-5.

Tables:

Please see uploaded files for tables

Table titles and legends:

Table 1: Baseline characteristics of included studies

Table 2: Summary of pharmacogenetic cancer studies involving VEGF pathway (May 1990 – July 2011). (2A) Survival and response (2B) Toxicity

Legend: PFS = progression free survival, OS = overall survival, TTP = time to progression, RR = response rate, NS = Non-significant, CRC = colorectal cancer, SCCHN = squamous cell cancer of the head and neck, HTN = hypertension, 5-FU = 5-flourouracil, FOLFIRI = 5-Fluorouracil, Folinic Acid, Irinotecan; VEGF = vascular endothelial growth factor, KDR = kinase insert domain receptor (also known as VEGFR2 = vascular endothelial growth factor receptor 2), FLT = fms-related tyrosine kinase 1 (also known as VEGFR1 = vascular endothelial growth factor receptor 1), NRP1 = neuropilin 1

Table 3: Meta-analysis results for prognostic significance of specific VEGF polymorphisms

Meta-analysis results for five commonly evaluated polymorphisms from our systematic review. Results from the pooled meta-analysis, where studies were compared regardless of zygosity are shown in Table 3A. Results from our zygosity-segregated analysis are shown in Table 3B. "Subgroup differences" measured whether there was a significant difference in the overall hazard ratios between the two zygosity groups. The "Overall pooled hazard ratios from meta-analysis" and "Overall *P* value" represent results when both homozygous and heterozygous results were pooled together.

Table 4: Forest plots for pooled (4A) and subgroup (4B) analysis of *VEGF* polymorphisms as prognostic biomarkers for *VEGF* +405*G>C*.

The odds ratio for each study is represented by the squares, the size of the squares represents the weight of each study, and the horizontal lines represent their 95% confidence interval. The diamonds represent the estimated pooled effect for the polymorphism. All p-values are two-sided. Test for subgroup differences are described in the text. All p-values are two-sided.

Figures:

Please see uploaded files for figures

Figure Titles and Legends:

Figure 1: Flow chart summarizing the literature search and selection process for inclusion of studies into the systematic review and meta-analysis.

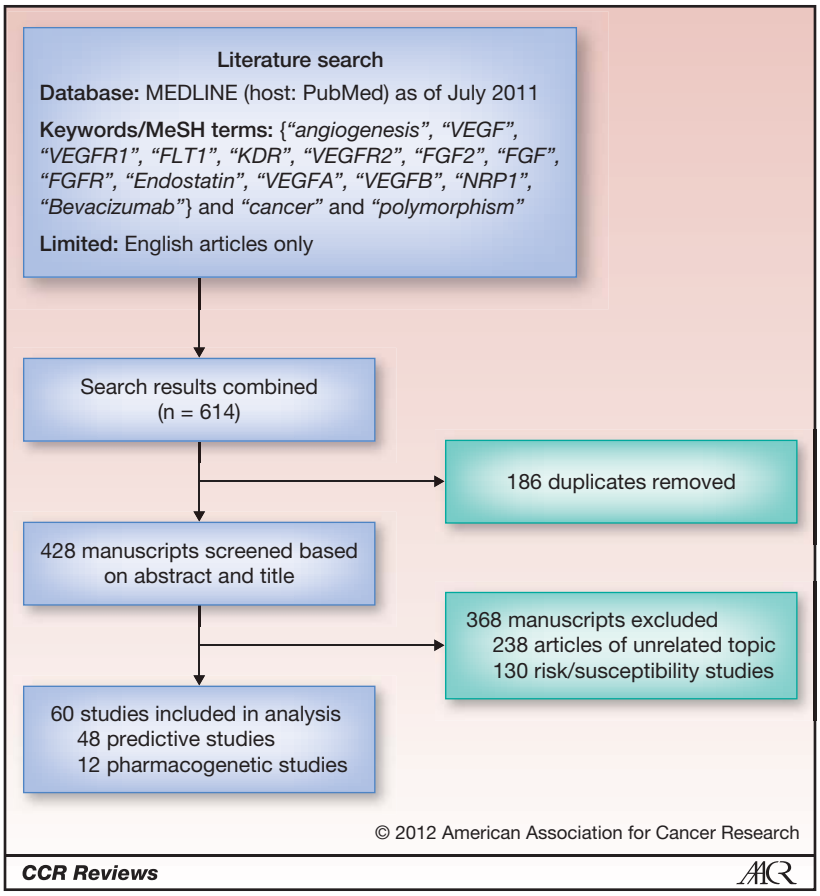

Table 1. Baseline characteristics of included studies

	Prognostic Studies Number of studies (%)	Pharmacogenetic Studies Number of studies (%)
Total number of studies	48 (100%)	12 (100%)
Source of Patients		
Case Series	33 (69)	3 (25)
Cases from Case-Control Risk Studies	11 (23)	0 (0)
Secondary analysis of clinical trial data	4 (9)	9 (75)
Developed cancer after entering cohort study	1 (2)	0 (0)
Type of Patient		
Caucasian Predominant sample	33 (69)	11 (92)
Asian Predominant sample	14 (29)	1 (8)
Location of Study		
North and Central American	14 (29)	5 (42)
South American	1 (2)	0 (0)
European	18 (4)	6 (50)
East Asian	13 (27)	1 (8)
South Asian	1 (2)	0 (0)
Australian	1 (2)	0 (0)
Type of Sample		
Blood	38 (79)	11 (92)
Frozen Normal Tissue	2 (4)	0 (0)
Frozen Tumor Tissue	5 (10)	0 (0)
FFPE Adjacent Tissue	4 (8)	0 (0)
FFPE Tumor Tissue	4 (8)	1 (8)
Disease Site		
Breast	6 (13)	2 (17)
Colorectal (Lower GI)	7 (15)	3 (25)
Gastro-esophageal (Upper GI)	9 (19)	1 (8)
Genitourinary	4 (8)	0 (0)
Gynecologic	8 (17)	2 (17)
Lung	4 (8)	0 (0)
Other (includes: liver, melanoma, AML, CML, NHL, head and neck)	10 (21)	4 (33)
Selection of Polymorphisms		
Any *VEGF*	42 (88)	9 (75)
VEGF -2578C>A	20 (42)	6 (50)
VEGF -1154G>A	13 (27)	5 (42)
VEGF -460C>T	19 (40)	6 (50)
VEGF +405G>C	33 (69)	9 (75)
VEGF +936C>T	31 (65)	9 (75)
Any *KDR/VEGFR2* or *FLT1/VEGFR1*	8 (17)	6 (50)
Any *Endostatin*, *FGF2* or *FGFR*	5 (10)	0 (0)
Type of Analysis		
Univariate Analysis Only	13 (27)	9 (75)
Observational Study	12 (25)	1 (8)
Clinical Trial	1 (2)	8 (67)
Multivariate Analysis Performed	35 (73)	3 (25)
Observational Study	32 (67)	2 (17)
Clinical Trial	3 (6)	1 (8)
Type of Predictive Analysis		
Survival Outcomes	Not Applicable	9 (75)
Toxicity		10 (83)

Table 2: Summary of pharmacogenetic cancer studies involving VEGF pathway (May 1990 – July 2011)

Table 2A: VEGF pathway polymorphic predictive associations of survival and response

Site	Last Name of First Author	Clinical Trial	Drugs	n	Country	Genes assessed	# of polymorphisms assessed	Significant results
Breast	Schneider (9)	Y	Bevacizumab + Paclitaxel vs Paclitaxel	341	USA	*VEGF* *KDR*	7	*VEGF* -2578*C>A*, A associated with improved OS *VEGF* -1154*G>A*, A associated with improved OS
Breast	Eitenne-Grimaldi (10)	Y	Bevacizumab +/- Taxane	138	France	*VEGF*	5	*VEGF* +936*C>T*, T associated with improved TTP
CRC	Loupakis (11)	N	FOLFIRI + Bevacizumab vs FOLFIRI	111	Italy	*VEGF*	4	*VEGF* -460*C>T*, C associated with improved PFS
CRC	Formica (12)	N	FOLFIRI + Bevacizumab	40	Italy	*VEGF*	9	*VEGF* -1154*G>A*, A associated with improved PFS *VEGF* +405*G>C*, G associated with improved RR
CRC	Zhang (13)	Y	Bevacizumab + Cetuximab vs Cetuximab	65	USA	*VEGF* *KDR* *NRP1*	4	
SCCHN	Argiris (14)	Y	Pemetrexed and Bevacizumab	28	USA	*VEGF*	3	*VEGF* +405*G>C*, G associated with improved with improved OS, TTP but only one patient carried G/G
Ovarian	Schulthesis (15)	Y	Bevacizumab + Cyclophosphamide	70	USA	*NRP1* *VEGF* *KDR*	5	*NRP1* 3'UTR C>T, T associated with poorer PFS
Ovarian	Smerdel (16)	Y	Bevacizumab	38	Denmark	*VEGF*	5	
Mixed	Jain (17)	Y	Sorafenib + Bevacizumab	178	USA	*KDR*	2	

Table 2B: VEGF pathway polymorphic predictive associations of toxicity

Site	Last Name of First Author	Clinical Trial	Drugs	n	Country	Genes assessed	# of polymorphisms assessed	Significant results
Breast	Schneider (9)	Y	Bevacizumab + Paclitaxel vs Paclitaxel	341	USA	*VEGF* *KDR*	7	*VEGF* +405*G>C*, G associated with increased HTN *VEGF* -460*C>T*, C associated with increased HTN
Breast	Eitenne-Grimaldi (10)	Y	Bevacizumab +/- Taxane	138	France	*VEGF*	5	*VEGF* +405*G>C*, C associated with increased toxicity
Esophageal	Sakaeda (18)	N	5-FU/Cisplatin/Radiation	49	Japan	*VEGF*	6	*VEGF* -460*C>T*, C associated with increased chelitis *VEGF* -1154*G>A*, A associated with increased chelitis and leukopenia
CRC	Loupakis (11)	N	FOLFIRI + Bevacizumab vs FOLFIRI	111	Italy	*VEGF*	4	
CRC	Formica (12)	N	FOLFIRI + Bevacizumab	40	Italy	*VEGF*	9	
SCCHN	Argiris (14)	Y	Pemetrexed and Bevacizumab	28	USA	*VEGF*	3	
Ovarian	Schulthesis (15)	Y	Bevacizumab + Cyclophosphamide	70	USA	*NRP1* *VEGF* *KDR*	5	
Mixed	Jain (17)	Y	Sorafenib + Bevacizumab	178	USA	*KDR*	2	*KDR* +1719*A>T*, T associated with increased toxicity
Mixed	Steeghs (19)	Y	Danusertib	63	Netherlands	*KDR* *FLT3* *FLT4*	7	
Mixed	Steeghs (20)	Y	Telatinib (Phase 1)	33	Netherlands	*KDR* *FLT4*	5	

Table 3A: Combined heterozygosity and homozygosity results	
***VEGF* SNPs**	**Pooled hazard ratios from meta-analysis, (95% CI); *P* value**
-2578C>A	0.92 (0.65-1.29); p=0.62
-1154G>A	1.07 (0.81-1.41); p=0.63
-460T>C	1.05 (0.78-1.43); p=0.73
+405G>C	**0.74 (0.60-0.91); p=0.004**
+936C>T	1.15 (0.86-1.53); p=0.35

Table 3B: Zygosity-segregated meta-analysis results					
***VEGF* SNPs**	**Comparison**	**Zygosity-specific hazard ratios from meta-analysis, (95% CI); p value**	**Subgroup differences *P* value**	**Overall pooled hazard ratios from meta-analysis (95% CI)**	**Overall *P* value**
-2578C>A	*A/A* vs *C/C*	0.93 (0.55-1.59); p=0.79	0.98	0.91 (0.72-1.14)	0.41
	C/A vs *C/C*	0.93 (0.79-1.08); p=0.33			
-1154G>A	*A/A* vs *G/G*	0.93 (0.55-1.58); p=0.80	0.45	0.98 (0.72-1.33)	0.71
	G/A vs *G/G*	1.18 (0.89-1.56); p=0.26			
-460T>C	*C/C* vs *T/T*	1.10 (0.79-1.53); p=0.56	0.77	1.06 (0.89-1.27)	0.52
	T/C vs *T/T*	1.04 (0.84-1.29); p=0.72			
+405G>C	*C/C* vs *G/G*	0.78 (0.58-1.04); p=0.09	0.72	**0.79 (0.67-0.94)**	**0.007**
	G/C vs *G/G*	0.83 (0.68-1.00); p=0.05			
+936C>T	*T/T* vs *C/C*	1.72 (0.79-3.75); p=0.18	0.35	1.38 (0.96-1.97)	0.08
	C/T vs *C/C*	1.13 (0.78-1.64); p=0.51			

Table 3: Meta-analysis results for prognostic significance of specific *VEGF* polymorphisms

Meta-analysis results for five commonly evaluated polymorphisms from our systematic review. Results from the pooled meta-analysis, where studies were compared regardless of zygosity are shown in Table 3A. Results from our zygosity-segregated analysis are shown in Table 3B. “Subgroup differences” measured whether there was a significant difference in the overall hazard ratios between the two zygosity groups. The “Overall pooled hazard ratios from meta-analysis” and “Overall *P* value” represent results when both homozygous and heterozygous results were pooled together.

Author Manuscript Published OnlineFirst on June 25, 2012; DOI: 10.1158/1078-0432.CCR-12-1315
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Table 4: Forest plots for pooled and subgroup analysis of *VEGF* polymorphisms as prognostic biomarkers for *VEGF* +405*G>C*.

A. Pooled

Study or Subgroup	Disease Site	Study Size	Weight	Hazard Ratio, 95% CI
Bradbury (32)	Gastroesophageal	361	5.8%	0.76 [0.46-1.25]
Dassoulas (25)	Colorectal	312	6.0%	0.27 [0.17-0.43]
Guan (36)	Gastric	167	5.5%	0.79 [0.47-1.34]
Guan (54)	Lung	124	6.4%	1.21 [0.80-1.84]
Heist (53)	Lung	462	7.6%	0.70 [0.54-0.91]
Kawai (41)	Renal	198	6.4%	0.92 [0.60-1.40]
Kidd (20)	Breast	358	5.5%	1.04 [0.61-1.78]
Kim (52)	Cervical	199	1.1%	0.11 [0.02-0.71]
Kim (30)	Colorectal	445	3.9%	0.19 [0.09-0.41]
Kim (40)	Gastric	499	4.0%	0.79 [0.37-1.68]
Kong (57)	Liver	416	6.8%	0.60 [0.41-0.87]
Lose (47)	Ovarian	319	5.3%	1.86 [1.07-3.23]
Lu (22)	Breast	1091	6.0%	0.63 [0.39-1.00]
Lurje (33)	Gastroesophageal	239	5.4%	0.81 [0.47-1.40]
Lurje (37)	Gastric	137	3.6%	1.86 [0.81-4.28]
Masago (56)	Lung	126	7.8%	0.83 [0.66-1.05]
Polerauer (46)	Ovarian	553	6.1%	0.71 [0.46-1.12]
Tzanakis (38)	Gastric	100	6.9%	0.65 [0.46-0.93]
Total (95% CI)			**100.0%**	**0.74 [0.60-0.91]**

Test for overall effect: Z = 2.90 (*P* = .004)

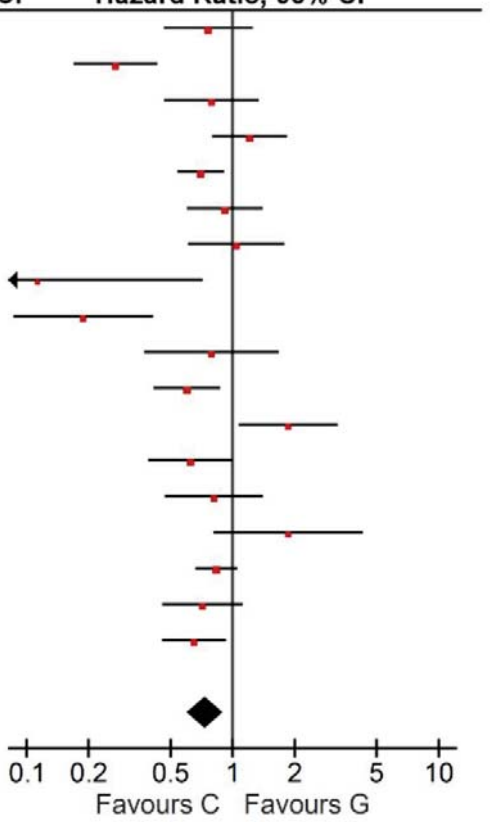

Table 4A

Table 4: Forest plots for pooled and subgroup analysis of *VEGF* polymorphisms...(Cont'd)

B. Subgroup

Study or Subgroup	Disease Site	Study Size	Weight	Hazard Ratio, 95% CI
Homozygous				
Bradbury (32)	Gastroesophageal	361	3.7%	0.76 [0.46 - 1.25]
Dassoulas (25)	Colorectal	312	3.9%	0.27 [0.17 - 0.43]
Guan (36)	Gastric	167	2.8%	1.11 [0.55 - 2.23]
Guan (54)	Lung	124	3.3%	1.28 [0.72 - 2.28]
Heist (53)	Lung	462	4.2%	0.73 [0.49 - 1.09]
Kawai (41)	Renal	198	3.8%	0.94 [0.59 - 1.51]
Kidd (20)	Breast	358	3.5%	1.04 [0.61 - 1.78]
Kim (30)	Colorectal	445	2.5%	0.19 [0.09 - 0.41]
Kim (40)	Gastric	499	2.6%	0.79 [0.37 - 1.68]
Kong (57)	Liver	416	4.3%	0.60 [0.41 - 0.87]
Lose (47)	Ovarian	319	3.4%	1.86 [1.07 - 3.23]
Lu (22)	Breast	1091	3.8%	0.63 [0.39 - 1.00]
Lurje (33)	Gastroesophageal	239	3.5%	0.81 [0.47 - 1.40]
Lurje (37)	Gastric	137	2.3%	1.86 [0.81 - 4.28]
Subtotal (95% CI)			**47.8%**	**0.78 [0.58 - 1.04]**
Test for overall effect: Z = 1.69 (*P* = .09)				
Heterozygous				
Bradbury (32)	Gastroesophageal	361	4.7%	0.93 [0.69 - 1.25]
Dassoulas (25)	Colorectal	312	3.4%	0.76 [0.43 - 1.34]
Guan (36)	Gastric	167	3.4%	0.69 [0.39 - 1.22]
Guan (54)	Lung	124	3.8%	1.17 [0.73 - 1.88]
Heist (53)	Lung	462	4.7%	0.69 [0.52 - 0.92]
Kidd (20)	Breast	358	4.2%	0.88 [0.59 - 1.31]
Kim (30)	Colorectal	445	2.8%	0.16 [0.08 - 0.32]
Kim (40)	Gastric	499	4.1%	0.75 [0.50 - 1.13]
Kong (57)	Liver	416	4.9%	0.93 [0.72 - 1.21]
Lose (47)	Ovarian	319	4.6%	1.11 [0.81 - 1.52]
Lu (22)	Breast	1091	4.2%	0.83 [0.56 - 1.25]
Lurje (33)	Gastroesophageal	239	4.4%	1.30 [0.91 - 1.85]
Lurje (37)	Gastric	137	3.0%	0.83 [0.43 - 1.59]
Subtotal (95% CI)			**52.2%**	**0.83 [0.68 - 1.00]**
Test for overall effect: Z = 1.92 (*P* = .05)				
Total (95% CI)			**100.0%**	**0.79 [0.67 - 0.94]**
Test for overall effect: Z = 2.69 (*P* = .007)				
Test for subgroup differences: Chi^2 = 0.13, df = 1 (*P* = .72), I^2 = 0%				

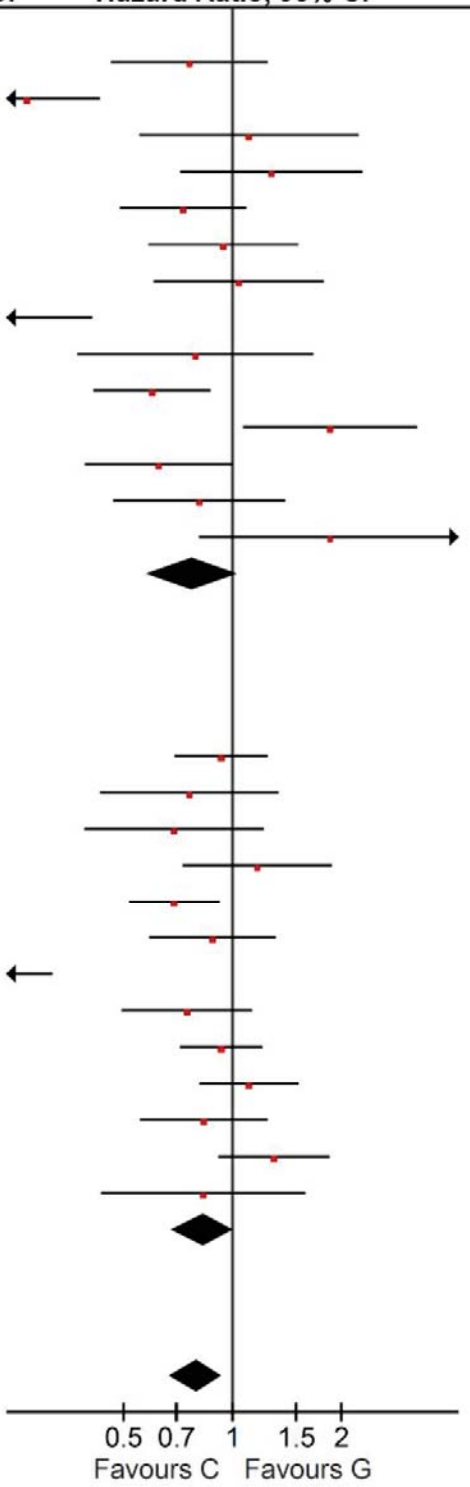

Table 4B

AACR American Association for Cancer Research

Clinical Cancer Research

Vascular Endothelial Growth Factor Pathway Polymorphisms as Prognostic and Pharmacogenetic Factors in Cancer: A Systematic Review and Meta-analysis

Lawson Eng, Abul Kalam Azad, Steven Habbous, et al.

Clin Cancer Res Published OnlineFirst June 25, 2012.