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PIK3R5 genetic predictors of hypertension induced by VEGF-pathway inhibitors

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Abstract

No biomarkers are available to predict patients at risk of developing hypertension induced by VEGF-pathway inhibitors. This study aimed to identify predictive biomarkers of hypertension induced by these drugs using a discovery-replication approach. The discovery set included 140 sorafenib-treated patients (TARGET study) genotyped for 973 SNPs in 56 genes. The most statistically significant SNPs associated with grade ≥2 hypertension were tested for association with grade ≥2 hypertension in the replication set of a GWAS of 1,039 bevacizumab-treated patients from four clinical trials (CALGB/Alliance). In the discovery set, rs444904 (G>A) in *PIK3R5* was associated with an increased risk of sorafenib-induced hypertension (p=0.006,

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Author contributions

JCFQ contributed to formal analysis, investigation and writing – original draft, review and editing. AR contribute to formal analysis and investigation. JW contributed to data curation, methodology and formal analysis. ASE contributed to formal analysis, investigation and writing – review and editing. SD contributed to formal analysis and investigation. CEP was responsible for resources. ADS contributed to data curation, formal analysis and methodology. DJC contributed to formal analysis and writing – review and editing. DL contributed to data curation, methodology and supervision. FI was responsible for conceptualization and funding acquisition, and contributed to formal analysis, investigation, methodology, supervision and writing – original draft, review and editing.

ClinicalTrials.gov Identifier: NCT00073307 (TARGET).

Competing interests

JCFQ, JW, DL, and FI are coinventors of a patent application, serial number 16/932,002. FI is an advisor for Emerald Lake Safety. These relationships have been disclosed to and are under management by UNC-Chapel Hill. CEP was employed by Bayer Health Care Pharmaceuticals at the time this work was conceived.

OR=3.88 95% CI 1.54-9.81). In the replication set, rs427554 (G>A) in *PIK3R5* (in complete linkage disequilibrium with rs444904) was associated with an increased risk of bevacizumab-induced hypertension (p=0.008, OR=1.39, 95% CI 1.09-1.78). This study identified a predictive marker of drug-induced hypertension that should be evaluated for other VEGF-pathway inhibitors.

Keywords

Sorafenib; bevacizumab; hypertension; PIK3R5; PI3Kγ

Introduction

Sorafenib is an orally administered multikinase inhibitor that targets multiple tyrosine kinases involved in angiogenesis, apoptosis, and tumor cell proliferation, including the vascular endothelial growth factor receptor 2 (VEGFR2) [1]. It is approved by the U.S. Food and Drug Administration (FDA) for the treatment of several tumors, including metastatic renal cell carcinoma (mRCC), advanced hepatocellular carcinoma, and differentiated thyroid cancer [2]. The clear-cell histology of RCC is characterized by overexpression of VEGF [3], which interacts with VEGFR2 to stimulate tumor angiogenesis. In addition to sorafenib, sunitinib, pazopanib, axitinib, lenvatinib, and cabozantinib are also approved for mRCC, sharing the same mechanisms of VEGF-pathway inhibition.

Hypertension is a common toxicity associated with VEGF-pathway inhibitors, can lead to treatment delays or discontinuations, and represents an obstacle to full delivery of effective therapy. Hypertension is one of the most frequent toxicities induced by sorafenib. A meta-analysis showed a prevalence of sorafenib-induced all-grade and grade ≥3 hypertension of 19.1% and 4.3%, respectively. Patients with mRCC experience higher rates of hypertension when compared to other tumor types (all-grade: 24.9% vs 15.7%, high-grade: 8.6% versus 1.8%) [4]. Similar to sorafenib, other VEGF-pathway inhibitors can also induce hypertension. Bevacizumab, a monoclonal antibody that binds directly to VEGF, induces hypertension with a prevalence reported up to 42.9% [5]. Moreover, patients treated with other tyrosine kinase inhibitors, including sunitinib and pazopanib, also experience hypertension as a common side effect [6,7].

The pathophysiology of hypertension induced by sorafenib, bevacizumab and other VEGF-pathway inhibitors is not well understood, although hypotheses about mechanisms have been proposed. One compelling mechanism involves the blockage of the nitric oxide (NO) pathway [8]. NO expression is modulated via the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, which is induced by VEGF signaling, and plays an essential role in angiogenesis [9,10]. The blockage of NO production by VEGF inhibitors leads to vasoconstriction, and hence hypertension [11]. Inhibition of VEGF-pathway signaling also induces endothelial cell apoptosis by disturbing cell-to-cell contact and, associated with NO blockage, leads to vessel rarefaction in the periphery [4,12].

Currently, no biomarkers are validated to predict hypertension induced by any of the VEGF-pathway inhibitors. Although a few studies have reported some associations of genetic variants with hypertension induced by sorafenib [13] and other VEGF-pathway

inhibitors [14], these reported associations have not been validated across studies or within independent cohorts of patients. Therefore, the objective of this study was to identify and replicate genetic biomarkers that are predictive of VEGF-pathway inhibitor-induced hypertension using a two-step, discovery-replication approach. Sorafenib-treated patients from the phase 3 Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET) were used as the discovery set, while a genome-wide association study (GWAS) of bevacizumab-treated patients from four Cancer and Leukemia Group B (CALGB, now Alliance for Clinical Trials in Oncology, Alliance) studies was used as a replication set.

Material and methods

The study was conducted in accordance with Good Clinical Practice Guidelines and the ethical principles that have their origin in the Declaration of Helsinki. All participants provided written informed consent for sample collection and pharmacogenetic analysis.

Discovery set: TARGET study

TARGET was a double-blind, randomized, placebo-controlled phase III trial of patients with mRCC treated with 400 mg sorafenib orally twice daily or placebo [15]. Hypertension was recorded according to Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0. This analysis was conducted in patients who received at least one cycle (28 days) of sorafenib treatment. DNA was obtained from the peripheral blood of 140 patients treated with sorafenib. A total of 1,536 single-nucleotide polymorphisms (SNPs) in 56 candidate genes were genotyped using the Illumina GoldenGate assay as previously described [16]. Genes were selected based upon biological function, including genes involved in VEGF-pathway signaling, genes from additional signaling pathways targeted by sorafenib, genes associated with perycite survival, sorafenib disposition and toxicity, and genes associated with RCC prognosis/pathogenesis or general cancer prognosis (Supplementary Table S1) [16]. SNPs were excluded if the genotype call rate was <97.5% and were selected based upon a minor allele frequency (MAF) >0.05 in Europeans from the 1,000 Genomes Project, and other criteria [16].

After quality control (Figure 1), a total of 973 of those SNPs were used in this study. Tests for association between SNPs and grade ≥2 hypertension were performed in the sorafenib arm by calculating the odds ratio (OR) from a logistic regression analysis, where grade ≥2 hypertension was the binary outcome and SNPs were the independent variables, under an additive model. Age and gender were used as covariates. A p-value <0.01 was used as a feature selection to identify SNPs for replication in the replication set. Codes used for analyses will be provided upon request.

Replication set: GWAS in CALGB 80303, 40503, 90401, and 40502 studies

For the replication cohort, the association between SNPs and hypertension was tested in genetic European patients treated with bevacizumab. A GWAS of four randomized phase III clinical trials from the CALGB/Alliance (CALGB 80303, 40503, 90401, and 40502) was used as a replication set [17]. The details of the individual studies are described in Supplementary Table S2. Patient eligibility, characteristics, stratifications and treatments

can be found in the individual publications for all four trials [18–21]. Blood pressure was measured on day 1 of each cycle in all four trials (and on day 15 in CALGB 80303), and hypertension was recorded according to CTCAE version 3.0. Bevacizumab-induced hypertension was defined as the occurrence of grade ≥2 hypertension after the start of treatment. DNA was obtained from peripheral blood. The genotyping platforms used in each study are described in Supplementary Table S2. Details of the GWAS of the individual studies and the four studies combined have been reported previously [17,22–24]. A cause-specific Cox model, where the outcome was defined as the pair of time event and the censoring indicator (defined as the time from the first administration of bevacizumab to the first incidence of the toxicity of interest, or other treatment-terminating events, whichever occurred first, under a competing risk model), was fitted to obtain the estimate of the SNP effect on hypertension in each individual study. The analyses were powered against an additive genetic model and were adjusted for age and sex. The inverse variance formula was used to combine the SNP effect in each study to obtain the estimate (β) of the SNP-hypertension association. The exponential function of β was used to calculate OR.

SNP replication

The SNPs from the TARGET study (discovery set) associated with grade ≥2 hypertension (p-value <0.01) were tested in the CALGB GWAS (replication set). If the variant was not genotyped in the replication set, a SNP in high linkage disequilibrium (LD, R^2>0.8, Europeans in the 1,000 Genomes Project) according to LDlink [25], was selected for testing. In the replication set, SNPs with a p-value <0.05 for association and the same direction of effect (increased or reduced risk) were considered as replicated.

Functional annotation of SNPs

Functional annotation of SNPs was performed using the SCAN database [26]. LDlink [25] was used for analyses of LD. RegulomeDB [27] and SNPnexus [28] were used for functional inference. The Genotype-Tissue Expression project (GTEx v7) [29] was used for expression quantitative trait loci (eQTL) analysis. atSNP was also used to quantify impact of SNPs on transcription factor binding [30].

Results

SNPs associated with sorafenib-induced hypertension in the TARGET study (discovery set)

A total of 140 mRCC patients treated with sorafenib in TARGET were included in this study. The characteristics of the patients, and the prevalence of grade ≥2 hypertension are shown in Table 1. Variants rs444904 (G>A, MAF 0.14) and rs1346563 (C>T, MAF 0.30) were both selected for testing in the replication set based on p-value <0.01 (Table 2). Both variants were associated with an increased risk of grade ≥2 hypertension (OR=3.88, 95%, CI: 1.54-9.81, p=0.0057 and OR=3.50, 95%, CI: 1.48-8.24, p=0.0064, respectively) (Table 2). Results for all associations with p-value <0.05 are shown in Supplementary Table S3.

Testing of rs444904 and rs1346563 in the GWAS of CALGB 80303, 40503, 90401, and 40502 (replication set)

A total of 1,039 patients treated with bevacizumab in the CALGB trials were included in this study (Table 1). The characteristics of patients from each study and the prevalence of hypertension are shown in Supplementary Table S2. Because neither rs444904 nor rs1346563 were genotyped on the GWAS platforms, rs427554 (G>A, MAF 0.10-0.11 in complete LD R^2=1.0 with rs444904) and rs4888628 (A>G, MAF 0.26-0.29, LD R^2=0.96 with rs1346563) were used as proxies. Variant rs427554 was associated with an increased risk of grade ≥2 hypertension (p=0.008, β=0.33, OR=1.39, 95% CI: 1.09-1.78) (Figure 2, Supplementary Figure S1). This result is consistent with the discovery set, where 50% of patients with AA genotype of rs444904 developed grade ≥2 hypertension, versus 7% of patients with GA genotype and 6% with GG genotype of rs444904. Variant rs4888628 was not associated with hypertension in the replication set (p=0.928, β=0.01, OR=1.01, 95% CI: 0.80-1.28).

Bioinformatic analysis of SNP function

Table 3 provides bioinformatic analyses for both rs444904 and rs427554. There is evidence in GTEx that rs444904 and rs427554 are both eQTLs for *PIK3R5* in whole blood, with the A allele of both variants associated with decreased gene expression (Supplementary Figure S2). DNase 1 hypersensitivity peaks indicate that both rs444904 and rs427554 are located in areas of open chromatin, thus permitting selective binding of transcription factors, while ChIP-seq revealed that the two SNPs are potential loci for binding with putative transcription factors. RegulomeDB, utilizing data from the ENCODE-motif database, indicates that rs427554 is located in the binding motif for the SP1 transcription factor. Additional data from the JASPAR database (using atSNP) predicts preferential binding of SP1 to the G allele of rs444904 rather than the A allele (p=0.012, log-likelihood=−2.15 for the G allele, and p=0.304, log-likelihood=−9.92 for the A allele) (Supplementary Figure S3). The non-coding variation scores provided by SNPnexus show that both rs444904 and rs427554 are at the high end of the range of predicted functionality according to EIGEN PC and DeepSEA scores. High EIGEN PC and low DeepSEA scores reflect that the SNP is predicted to be located in regions of open chromatin, accessible to transcription factors. Both rs444904 and rs427554 are at the low end of the range of predicted functionality according to the other scores (CADD, FitCons, FATHMM, GWAVA, ReMM, and FunSeq2) (Table 3).

Discussion

We have identified a common intronic SNP (rs444904) located in *PIK3R5* that increased the risk of grade ≥2 hypertension in patients treated with the VEGF-pathway inhibitors sorafenib and bevacizumab. To our knowledge, this is the first demonstration of a genetic variant associated with increased risk of toxicity of different VEGF-pathway inhibitors, which suggests a potential class effect.

PI3K enzymes are grouped into three classes (I, II, and III) and various subclasses. *PIK3R5* encodes the p101 regulatory subunit of the class IB of phosphatidylinositol-3-kinase gamma (PI3Kγ), which is required for the recruitment of the p110 catalytic subunit to the plasma

membrane. PI3Ks generate the second messenger phosphatidylinositol 3,4,5-triphosphate (PIP3), which activates AKT. Activated AKT then stimulates endothelial NO synthase, increasing NO production, via mTOR phosphorylation. This PI3K pathway is activated by binding of VEGF to its receptors, mediating, in part, the effects of VEGF on endothelial cells [9]. PI3K also contributes to increased levels of hypoxia-inducible factor 1 (HIF1-1)α [31], which heterodimerize with HIF1-1β for binding to the promoter of numerous genes, including *VEGF*, leading to its increased transcription [9,32].

PIK3R5 plays crucial roles in cell growth, motility, and intracellular trafficking [33], and the abnormal expression of *PIK3R5* has been associated with some types of cancers, mainly ovarian cancer [34]. However, the role of *PIK3R5* in hypertension, either drug-induced hypertension and essential hypertension, is still unknown, and no SNPs in *PIK3R5* have been associated with blood pressure in previous genomic studies [35]. PI3Kγ is largely distributed in blood, mainly within immune cells of hematopoietic origin, but is also distributed in other tissues including the heart [36]. In endothelial cells, the activation of PI3K is required for angiogenesis and vascular permeability, and the pharmacological inhibition of PI3Kγ suppresses VEGF-mediated vascular permeability *in vivo* [37].

Bioinformatic analyses of rs444904 and rs427554 in *PIK3R5* show that both SNPs are located in a region that binds numerous transcription factors, indicating a potential regulatory role for each SNP in gene expression (Table 3). Both SNPs also have high EIGEN PC and low DeepSEA scores, indicating that they are predicted to be located in regions of open chromatin that are accessible to transcription factors. Specifically, we show that rs427554 is located within a binding motif for SP1. Moreover, rs444904 is likely to alter an SP1 binding motif, with the G allele increasing the likelihood of SP1 binding compared to the A allele (Supplementary Figure S3). Considering that both SNPs are in complete LD with each other, it is plausible to suggest that the G allele of both variants is likely to increase binding of the SP1 transcription factor to DNA. The binding site for SP1 is a CpG unmethylated motif. SP1 binding inhibits the action of the DNA methyl transferase (DNMT), and protects DNA from methylation and hence activating gene transcription [38]. Conversely, SP1 binds inefficiently to the A allele, which allows DNA methylation by DNMT and repression of transcription of *PIK3R5.* The effect of this SNP on *PIK3R5* expression is also confirmed by this SNP acting as an eQTL in whole blood, reducing *PIK3R5* expression (Table 3).

Based upon the provided clinical, bioinformatic, and biological evidence, this study proposes a novel mechanism through which rs444904 and rs427554 regulate the expression of *PIK3R5* and mediate hypertension induced by sorafenib and bevacizumab, that should be validated experimentally in further studies. The G allele of both SNPs allows the binding of SP1, which, by inhibiting the action of DNMT, activates *PIK3R5* transcription. This would result into an increased activation of the PI3Kγ p110 catalytic subunit and the resulting activation of the AKT/NO/mTOR pathway. The end result would be an increased vascular permeability, angiogenesis and vasodilation (Figure 3 A). Conversely, the A allele decreases the binding of the SP1, which allows DNA methylation by DNMT, downregulating *PIK3R5* transcription and mediating vasoconstriction. In patients who are carriers of the A allele, the inhibition of VEGF signaling induced by treatment with sorafenib and bevacizumab

might lead to further vasoconstriction, which would explain the higher risk of developing hypertension (Figure 3 B).

In the discovery set, 50% of patients with the AA genotype of rs444904 developed grade ≥2 hypertension, versus only 7% of patients carrying the G allele of rs444904. In the replication set, 43% of patients with the AA genotype of rs427554 developed grade ≥2 hypertension, versus 26% of patients carrying the G allele of rs427554. These common SNPs are present in about 10% of individuals of European ancestry and in up to 18% of individuals of African ancestry [39] and can impact a considerable number of patients treated with VEGF-pathway inhibitors. If validated in additional cohorts and prospective studies, rs444904/rs427554 in *PIK3R5* can be genotyped in cancer patients before receiving VEGF-pathway inhibitors in order to identify patients at higher risk of developing hypertension, providing a better risk assessment of the use of these drugs.

Limitations of our study include the analysis of different clinical trials including patients with different types of cancers and treatment regimens, which may interfere in association and replication analysis. However, the replication of rs444904 in an external dataset utilizing a different VEGF-pathway inhibitor (such as bevacizumab) provides additional evidence of clinical applicability of biomarkers of hypertension induced by different anti-angiogenic drugs. We do not have access to data on baseline blood pressure of patients included in the studies, although all patients had blood pressure below 160/90 mm Hg before treatment with sorafenib or bevacizumab. We also do not have access to data on other possible covariates associated with hypertension besides age and gender. Potential additional confounders might also be considered, such as concomitant anti-hypertensive use, body mass index, and pre-existing conditions. The impact of these confounding variables thus remains a question for future studies. Moreover, the proposed model of the genetic basis of risk of drug-induced hypertension (Figure 3) should be supported by experimental evidence in future studies in model systems.

In conclusion, we provide evidence for rs444904 in *PIK3R5* as a biomarker of hypertension induced by sorafenib and bevacizumab. This study, for the first time, provides evidence for new predictive genetic markers of hypertension induced by two different VEGF-pathway inhibitors that should be further evaluated for other anti-angiogenic drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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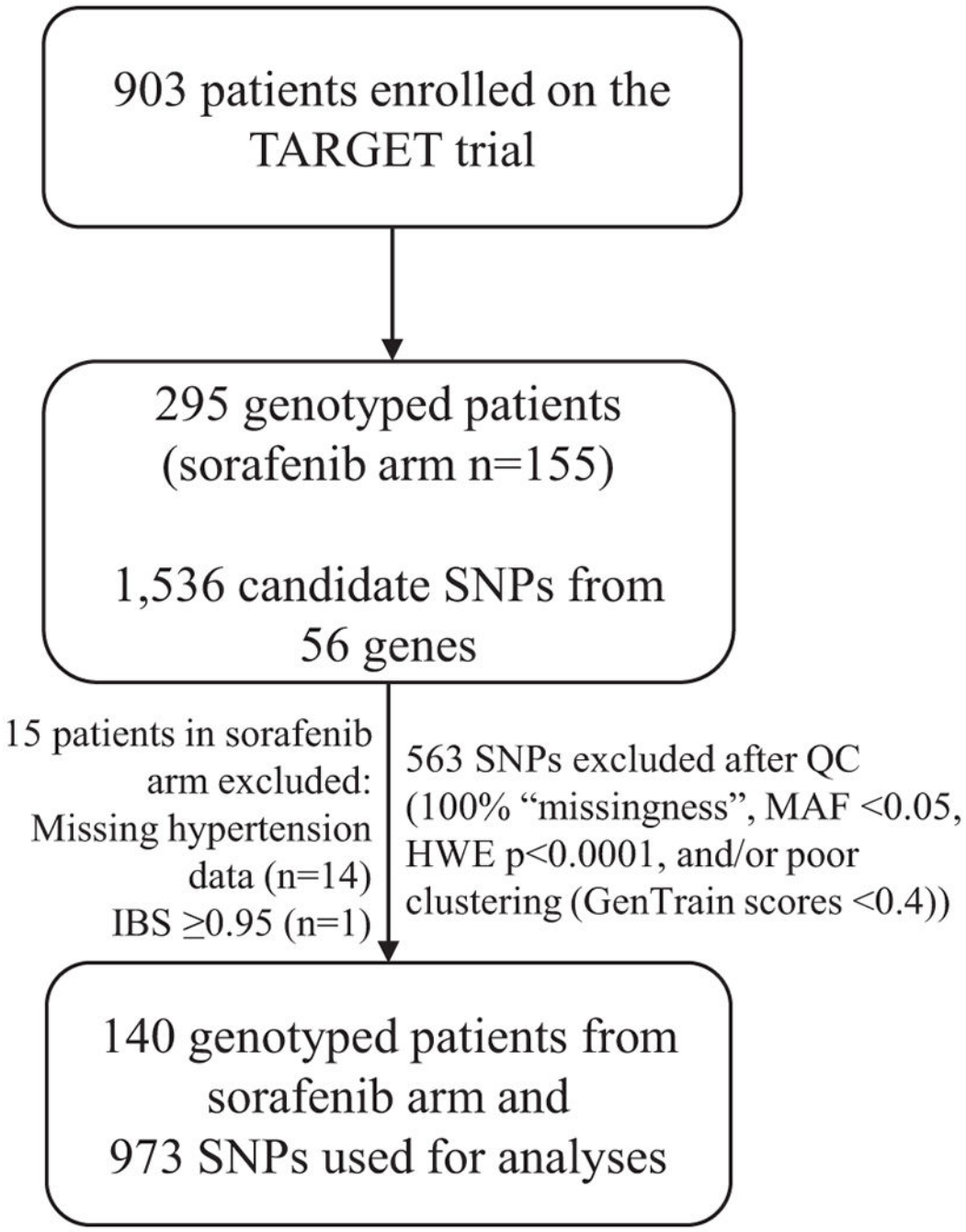

Figure 1. CONSORT and quality control flowchart for the TARGET study.

IBS: identical by state, MAF: minor allele frequency, HWE: Hardy-Weinberg Equilibrium, QC: quality control, SNP: single nucleotide polymorphism.

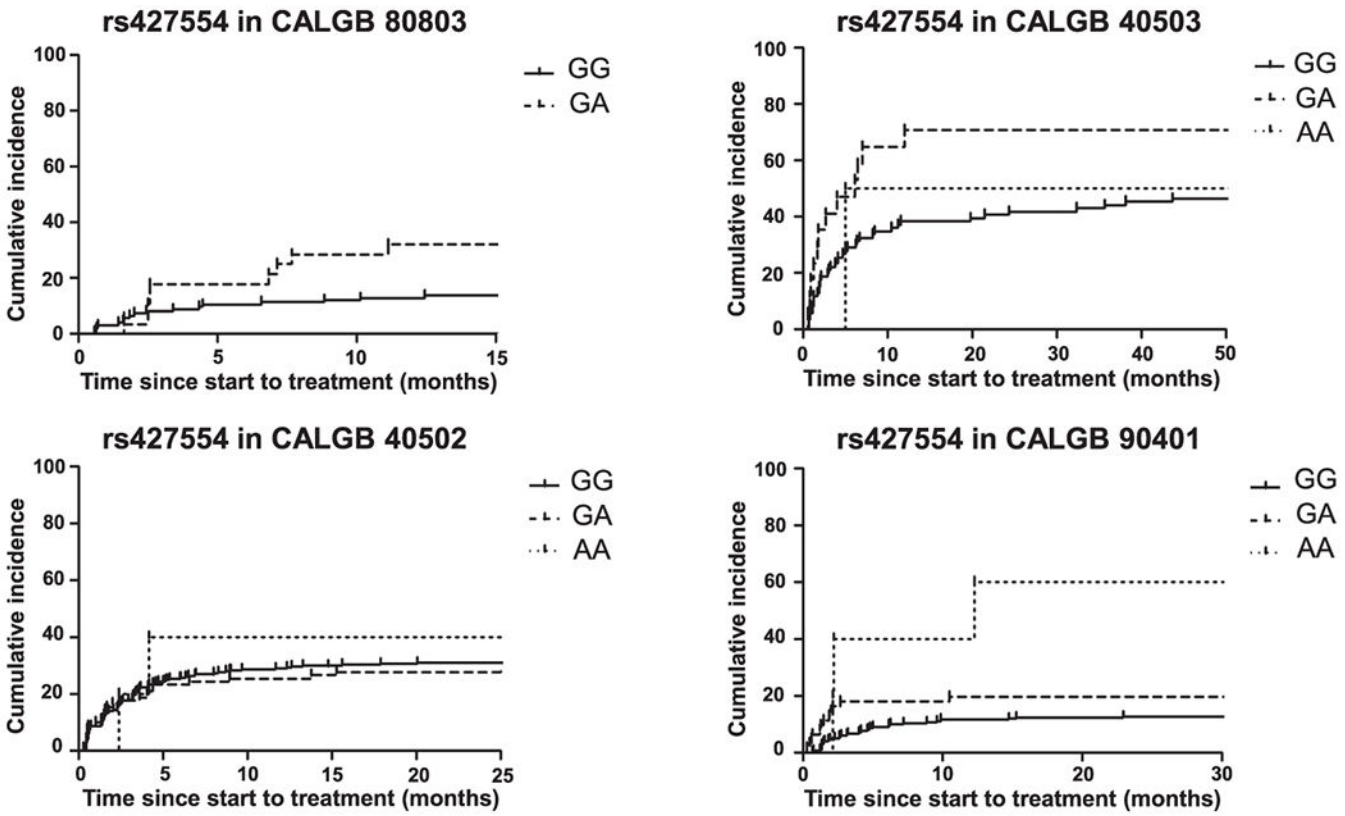

Figure 2. Effect of genotypes of rs427554 on bevacizumab-induced hypertension (replication set).
CALGB: Cancer and Leukemia Group B.

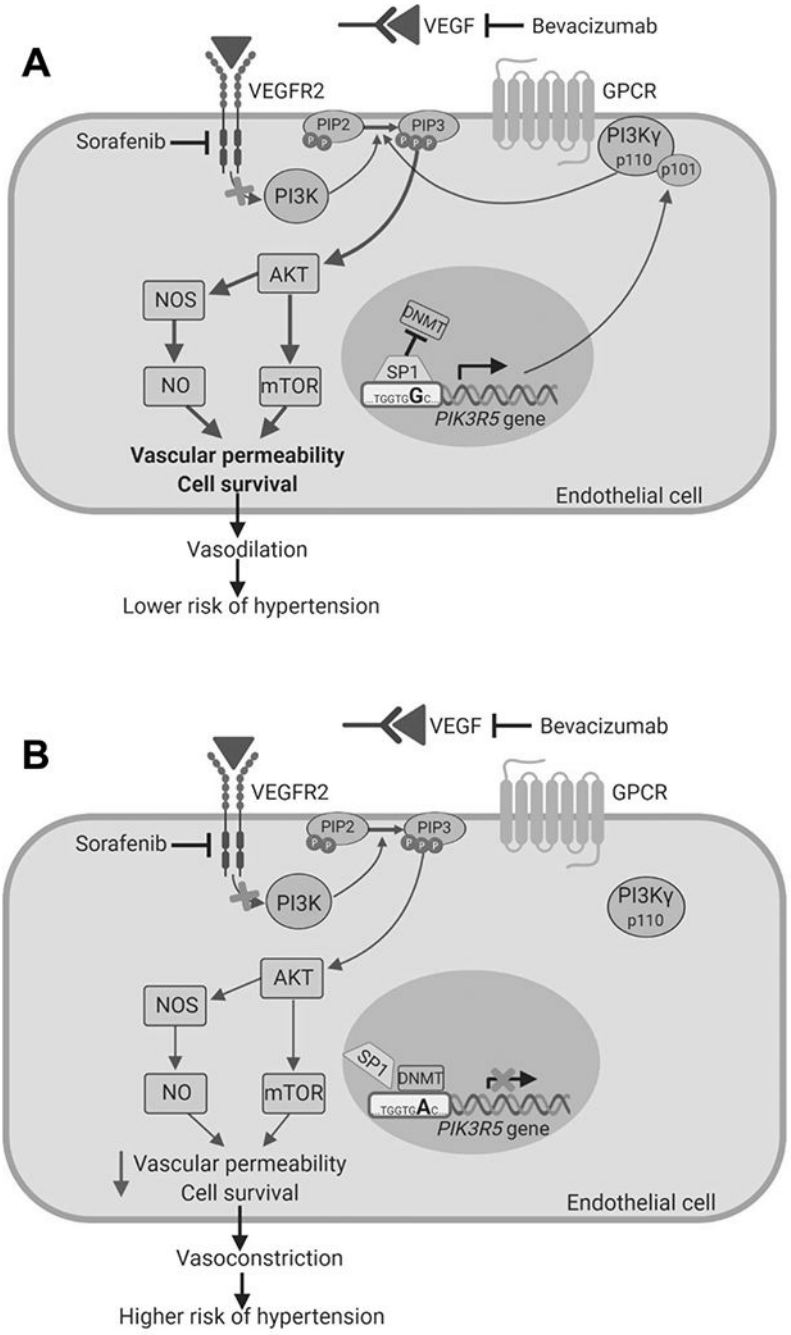

Figure 3. Proposed model for lower and higher risk of developing hypertension induced by VEGF-pathway inhibitors in patients with the G and A allele of rs444904 and rs427554 (Created with BioRender.com.).

A: The G allele of rs444904 and rs427554 increases the binding of the SP1 transcription factor, which inhibits the action of DNMT, activating *PIK3R5* transcription and leading to a higher activation of AKT/NO/mTOR signaling pathway. Higher activation of AKT/NO/mTOR leads to vasodilation and lower risk of developing hypertension. **B:** SP1 binds inefficiently to the A allele of rs444904 and rs427554, which allows DNA methylation by DNMT, repressing *PIK3R5* transcription and leading to a lower activation

of AKT/NO/mTOR signaling pathway. Lower activation of AKT/NO/mTOR leads to vasoconstriction and higher risk of developing hypertension. VEGF: Vascular endothelial growth factor, VEGFR2: VEGF receptor 2, GPCR: G-protein-coupled receptor, DNMT: DNA methyl transferase, PI3K: Phosphatidylinositol-3-kinase, PIP2/3 phosphatidylinositol 3,4,5-bi/triphosphate, NO: nitric oxide, NOS: NO synthase, mTOR: mammalian target of rapamycin.

Table 1.

Patients treated with sorafenib (discovery set) and with bevacizumab (replication set) [17].

CALGB: Cancer and Leukemia Group B, SD: standard deviation.

Trial		Discovery set TARGET n=140	Replication Set CALGB studies n=1,039
Hypertension grade ≥2 * (n, %)		12 (8.6)	269 (25.9%)
Age – Mean (SD)		59.6 (9.8)	61.7 (11.3)
Gender	**Male**	105	404
	Female	35	635
Cancer type		Advanced and metastatic renal cell carcinoma	Advanced or metastatic pancreatic, breast, or prostate cancer

* CTCAE v3.0.

Table 2.

SNPs associated with sorafenib-induced hypertension in the TARGET study (discovery set) and tested for replication in the replication set.

Results are adjusted for age and gender. CI: confidence interval, MAF: minor allele frequency, OR: odds ratio, SNP: single nucleotide polymorphism.

SNP (base change)	Gene	Feature	MAF	OR (95% CI)	p-value
rs444904 (G>A)	*PIK3R5*	Intron	0.14	3.88 (1.54-9.81)	0.0057
rs1346563 (C>T)	*ADAMTS18*	Intron	0.30	3.50 (1.48-8.24)	0.0064

Table 3.

Bioinformatic analysis of rs444904 and rs427554.

The Regulome DB score represents the evidence that a SNP has a regulatory function (1-strong evidence, 6-weak evidence). SNPnexus non-coding variation scoring predicts the functional impact of non-coding SNPs using 8 non-coding SNP scoring algorithms. Higher scores in CADD (range 0-99), FitCons (range 0-1), EIGEN PC (range −2 to 2), FATHMM (range 0-1), GWAVA (3 scores, range 0-1), ReMM (range 0-1), and FunSeq2 (range 0-5) mean that the SNP is most likely to be functional/deleterious, while a lower score in DeepSEA (range 0-1) means that the SNP is most likely to be functional/deleterious. eQTL: expression quantitative trait loci, NES: normalized effect size.

SNP (Regulome DB score)	eQTL (GTEx)	RegulomeDB/ENCODE			SNPnexus non-coding variation rankings							
		ChIP-seq	DNase I Sensitivity region	Motif	CADD	FitCons	EIGEN PC	FATHMM	GWAVA	ReMM	FunSeq2	DeepSEA
rs444904 (4)	Whole blood ($p=2.7x10^{-12}$, NES=−0.16)	POLR2A, RELA, EP300, TCF12, EBF1, IKZF2, IKZF1, TAF1, MEF2C, TBL1XR1, FOXM1, ZNF687, TARDBP, NFATC1, MTA3, ZEB1, MTA2, YY1, CBFB, RUNX3, GATAD2B, CHD2, ELF1, BCLAF1, ETS1, ARNT, EGR1, IRF4, PBX3, TCF7, NFIC, BHLHE40, POU2F2, CREM, BCL3, MEF2A, ETV6.	Yes	-	0.775	0.102	0.176	0.110	0.38/0.48/0.44	0.019	1.560	0.035
rs427554 (2c)	Whole blood ($p=9.4x10^{-13}$, NES=−0.17)	POLR2A, GATA1, SPI1, IKZF1, IKZF2, ZBTB40, TCF7, MLLT1, EP300, ELF1, LEF1, PAX5, BCLAF1, DPF2, TRIM22, RELB, ARNT, MXI1, FOXM1, NR2F1, SMARCA5, ZMYM3, SP1, MEF2A, RBM25, TARDBP, NBN, TAF1, BHLHE40, BCL3, RUNX3, KLF5, BATF, ETV6, NFATC3, ZFHX2, ZBTB33, ETS1, IRF4, NKRF, VEZF1, ZBED1, SKIL, ZNF207, CREM, ETV1, CBFB, HDGF, TBX21, ARID3A, BCL11A, POU2F2, EGR1, COMMD3-BMI1, BMI1, ATF2, REST, RFX1, MAZ, EBF1, ZNF143, EED.	Yes	SP1	2.641	0.102	0.614	0.141	0.45/0.53/0.41	0.122	1.560	0.118