Supplementary

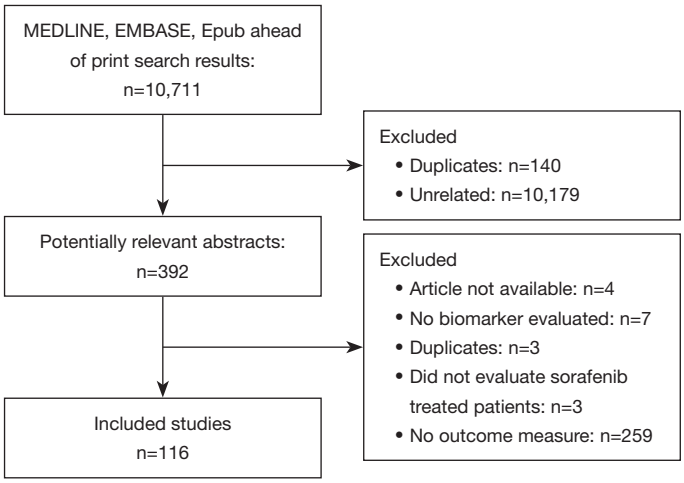

Figure S1 Consort diagram of literature review. Breakdown of the numbers of study results from initial literature search as well as number of studies excluded after reviewing abstracts and full papers.

https://dx.doi.org/10.21037/tgh-23-22

Table S1 Studies of biomarkers prognostic or predictive of sorafenib in advanced HCC

Article	Study description	N	Tested biomarker(s)	Results	Comparator non-sorafenib cohort?
Next generation sequencing					
(38)	Cohort study	13	FGFR ¾ amplification	FGFR3/4 amplification predicts for response	N
(39)	Cohort study	127	341 cancer associated genes	PI3K-mTOR pathway alterations were associated with reduced DCR, PFS, OS	Y-Immune CPI
(40)	Cohort study	46	40 genes for DNA and RNA sequencing	Average number of oncogene mutations predicts disease control, RNA expression of *TGFa*, *PECAM1*, and *NRG1* predicts PFS	N
(41)	Gene database analysis		1,319 differentially expressed genes	8 hub genes for sorafenib resistant phenotype kininogen 1, vascular cell adhesion molecule 1, apolipoprotein C3, alpha 2-HS glycoprotein, erb-b2 receptor tyrosine kinase 2, secreted protein acidic and cysteine rich, vitronectin and vimentin	N
(42)	Cohort study	45	FGFR genetic alterations	FGF19 copy number gain predicts CR	N
(43)	Cohort study	42	Genomic profiling of 381 cancer associated genes	Cell cycle gene aberrations predicts lack of response	N
(44)	Cohort study	47	mRNA expression of the CSC genes *EpCAM*, *CD13*, *CK8*, *CD24*, *CD44*, *CD90*, *CD133*, *SALL4*, *ALDH1A1*, *ALB*, and *AFP*	High CD133/CD90 expression predicts OS (HR 2.97)	N
(45)	Cohort study	151	Plasma ctDNA, genome wide CNA, VEGFA amplification	ctDNA level predicts OS (HR 2.5), CNA predicts OS (HR 1.85)	N
(46)	Case report	1	Tumor neoantigens were identified using whole exome sequencing	mutated IL-1β^{D159E} peptide and two additional neoepitopes from HELZ2^{G515A} and MLL2^{A3498V}	N
Tissue IHC					
(47)	Cohort study	39	IHC for p-Jun, p-JNK, CD133	High levels of p-Jun, p-JNK, CD133 associated with worse response	N
(48)	Cohort study	93. 65 received sorafenib	VEGFR-2, PDGFR-β, and c-Met	Low PDGFR-B associated with improved OS, high c-MET associated with improved PFS	N
(49)	Phase 2 trial	137	Tumor IHC pERK, blood cell-RNA microarray analysis	Higher pERK associated with longer TTP. No HR given. 18 genes in blood predicted 'progressors'	N
(50)	Cohort study	73	Ki67, CK19, glutamine synthetase, VEGF, VCP, pERK	Ki67 >20, CK19, VCP associated with OS	N
(51)	Cohort study	54	pERK, S6K, VEGFR2, PTEN	pERKs3 predicts OS (HR 1.504)	N
(52)	Cohort study	50	p-c-Jun	p-c-Jun high predicts OS (HR 2.3)	N
(53)	Cohort study	39	OCT-1	Tumor cell IHC staining for OCT-1 predicts improved OS. No effect measure reported	N
(54)	Phase 3 trials	77	β-catenin glutamine synthetase (GS), phosphorylated extracellular signal regulated kinase (pERK), phosphorylated v-akt murine thymoma viral oncogene homolog (pAKT) and FLK-1/KDR/VEGFR-2	pERK predicts OS (HR 2.09), VEGFR-2 predicts OS (HR 2.28)	N
(55)	Cohort study	35	VEGFR1, 2 expression	Lack of VEGFR1,2 predicts poor OS	N
(56)	Cohort study	44	Mcl-1, activated/phosphorylated extracellular signal-regulated kinase (pERK) 1/2, and activated/phosphorylated AKT (pAKT MYC and MET by FISH	pERK predicts OS (HR 1.013), MCL-1 predicts OS (HR 1.016)	N
(57)	Cohort study	83	HTATIP2, microvessel density	High HTATIP2 and low microvessel density predicts poor OS	N
(58)	Cohort study	41	CXCR4 expression	High CXCR4 expression predicts better OS	N
(59)	Cohort study	94	EDN1 expression	High EDN1 predicts OS (HR 2.374)	N
Circulating tumor cells					
(60)	Cohort study	59	Circulating tumor IHC p-ERK, p-AKT	Patients with pERK$^+$/pAkt$^-$ CTC Had improved DCR and PFS (HR 9.4)	N
Blood counts					
(61)	Phase 3 trial	170	Platelet count	Platelet count >150 predicts worse TTP HR 1.56	N
(62)	Cohort study	145	Baseline neutrophil lymphocyte ratio	NLRp4 HR 1.73 for OS	N
(63)	Cohort study	43	PBMC ROS and pERK	PBMC ROS and pERK predicts response	N- patients also received octreotide LAR
(64)	Cohort study	56	Systemic immune-inflammation index, NLR, PLR	SIIs360 HR 2.99 for OS, NLRs3 HR 2.36 for OS	N
(65)	Cohort study	161	neutrophil-to lymphocyte ratio (NLR), the derived NLR, the platelet-to-lymphocyte ratio (PLR), the monocyte-to-lymphocyte ratio (MLR), the prognostic nutritional index (PNI) and the systemic-immune inflammation index (SII	systemic immune-inflammation index (SII) ≥600×10^9 was independent predictor of OS (HR 1.72)	N
(66)	Cohort study	105	NLR	NLR >3.5 predictive of OS (HR 0.5), AFP <1030 ng/mL predictive of OS (HR 1.93)	N
(67)	Cohort study	82	NLR	NLR decline predicts PFS and OS (HR 0.479)	N
(68)	Cohort study	442	NLR, RDW	NLR predicts OS (HR 1.218), and RDW predicts OS (HR 1.234)	N
(69)	Cohort study	19	PD-1 Tcells, Tregs, MDSCs, cytokines	OS predicted by decrease in CD4/CD8+ PD-1+ Tcells and Foxp3+ Tregs	N
(70)	Cohort study	154	NLR	NLR >2.3 predicts OS (HR 1.72)	N
(71)	Phase 2	40	CEPs, CEC's	CEP predicts OS (HR 2.512) and PFS	N- Sorafenib+ metronomic chemo
(72)	Cohort study	142	MLR	MLR >0.35 predicts OS (HR 0.445), AFP predicts OS (HR 0.445)	
Alpha-fetoprotein					
(73)	Phase 2 trial	544	AFP	AFP <200 had HR 0.679 for OS on multivariate testing	N
(74)	Cohort study	214	AFP, NLR	AFPp7 ng/mL HR for OS 1.64	N
(75)	Phase 2 trial	1130	AFP	Log AFP ng/mL HR 1.087 for OS	N
(76)	Cohort study	320	AFP	AFP reduction of >20% at 3 months predictive of OS HR 0.38	N
(77)	Cohort study	225	AFP	AFP >456 predicts OS (HR 1.76)	N
(78)	Cohort study	254	AFP	AFP >200 ng/mL predicts OS (HR 1.45)	N
Circulating protein					
(79)	Phase 2 trial of sorafenib plus Trebananib	60	Ang-2	Ang-2 >5,700 ng/mL had HR 2.43 for OS	N
(80)	Cohort study	101	IGF-1	Addition of IGF-1 to CP scoring system improved prediction of OS and PFS	N
(81)	Cohort study	23	Chromogranin-A, VEGF	chromogranin A and VEGF were inversely correlated with response. No effect measure given	N
(82)	Analysis of Sharp & AP trials	827	Clinical variables, albumin, AFP, ALP	HCV, Low NLR showed significant interaction with treatment	Y-placebo
(83)	Cohort study	62	VEGF-A, b FGF, sVEGFR2, Ang2, SDF1, VEGF-C, IL-6, IL-8, AFP, HGF, TSP1, BMP9	Ang2, sVEGFR2, IL-6, IL-8, AFP associated with OS	N
(84)	Cohort study	30	IGF-1	Baseline IGF-1 level predictive of TTP in sorafenib treated patients, but also in those receiving TACE	Y- TACE, BSC
(85)	Phase 3 trial	954	VEGF, ANG2, FGF 19, 21,23	VEGF, ANG2, FGF21 predictive of OS. FGF21 predictive of differential OS between sorafenib and lenvatinib	Y-lenvatinib
(86)	Cohort study	78	IGF-1	Adding IGF-1 levels to CP calculation increased prediction of OS	N
(87)	Cohort study	48	18 cytokines	Increase in IL-8 and TNF-a predicts progression	N
(88)	Phase 3 trial-SHARP	602	Ang2, EGF, bFGF, VEGF, sVEGFR-2, sVEGFR-3, HGF, and s-c-KIT IGF-2 circulating Ras	None. High s-c-KIT or low HGF (P of interaction =0.081 and 0.073, respectively)	Y-placebo
(89)	Cohort study	91	TGF-B1	High baseline TGFB predicts poor OS and PFS. Not significant on multivariate analysis	N- receive sorafenib alone or with tegafur/ uracil
(90)	Phase 2	83	IGF-1, IGF-2, IGFBP3	IGF-1	N-Combined two trials. One of sorafenib + tegafur, One Bev+cape
(91)	Phase 2	128	IL-6	IL-6 >4.28 pg/mL predicts OS (HR 2.594)	N-Sorafenib +metronomic chemo
(92)	Cohort study	80	VEGF, HIF-1a	Higher VEGF, and HIF-1a predicts poor OS	N
(93)	Cohort study	124	Ang-2, VEGF, PDGFRb, HGF, CD117, LOXL2, bFGF, PIVKA-II	Predictive model including BCLC stage, bFGF, log PIVKA-II, log HGF, etiology, C-index of 0.884 of tumor response	N
(94)	Cohort study	133	CRP	CRP >1 mg/dL predicts OS (HR 3.31), AFP >400 mg/mL predicts OS (HR 2.76)	N
(95)	Cohort study	165	CRP, AFP	CRP <1mg/dl predicts OS (HR 0.51), AFP <200 ng/mL predicts OS (HR 0.45)	N
(96)	Cohort study	39	EGF, bFGF, HGF, IFN-γ, IL-10, IL-12, IL-2, IL-4, IL-5, IL-6, IL-8, IP-10, MIG, PDGF-BB, SCF, SDF1, TGF-β, TGF-α, TNF-α, and VEGF-A	Elevated IL-5, IL-8, CXCL9, PDGF-BB, TGF-α, and VEGF-A were associated with improved OS in sorafenib but not in hepatic artery infusional chemotherapy	Y- hepatic artery infusional chemotherapy
(97)	Cohort study	97	LDH	Decrease in LDH predicts OS, TTP	N
(98)	Cohort study	44	Lipidomic analysis	phosphatidylcholine (PC)[34:2], PC[34:3]a, PC[35:2], PC[36:4]a, PC[34:3e], acylcarnitine (Car)[18:0], cholesterol ester[20:2], and diacylglycerol (DG)[34:2] predicts response	N
(99)	Cohort study	34	EGF, FGF-2, G-CSF, IFN-γ, IL-12p70, IL-8, IL-17A, IP-10, MCP-1, TNF-α, and VEGF	IL-17A >1.94pg/mL was predictive of PFS (HR 19.96), FGF-2 <20.57pg/mL was predictive of OS (HR 3.24)	N
(100)	Cohort study	115	124 proteins	CDSL, IGJ, LGALS3BP were predictive of sorafenib response (c-index >0.95) and not predictive of TACE response	Y-TACE
(101)	Cohort study	55	VEGF, amphiregulin	Decrease in amphiregulin level was associated with improved OS (HR 0.208)	N
(102)	Cohort study	120	Ang-2, FST, G-CSF, HGF, Leptin, PDGF-BB, PECAM-1, and VEGF (s)-c-KIT	Ang-2 predicts OS (HR 1.95) and PFS, more than 3 cytokines elevated predicts OS	N
(103)	Cohort study	90	FST, G-CSF, HGF, Leptin, PDGF-BB, PECAM-1, Ang-2, VEGF	High Ang2 HR 2.06, and high HGF HR 2.08 were associated with poor OS	N
(104)	Cohort study	63	VEGF levels	VEGF decrease >5% at 8 weeks predicts OS (OR 10 for 1 year survival)	N
(105)	Phase 3 trial	494	VEGFC, heregulin, soluble KIT EPGN and IGF2, VEGFA, HGF, amphiregulin, betacellulin, EGF, epiregulin, hbEGF, TGFα, BFGF, and PDGF-BB	HGF (HR 1.7), VEGFA (HR 1.4), KIT (HR 0.75) predict OS, and VEGFC (HR 0.6) EPGN	N- half of patients received additional erlotinib
(106)	Metanalysis	1202	VEGF	High VEGF HR 1.85 for OS. VEGF SNP associated with OS	N
miRNA					
(107)	Cohort study	20	miR-17-5p, miR-18a, miR-21, miR-34a, miR-122, miR-195, miR-210, miR-214, miR-221, miR-222, miR-223, miR-224, miR-140, miR-328	miR-224 predictive of PFS and OS	N
(108)	Cohort study	93	miR-221	Lower baseline miR-221 predicts response	N
(109)	Cohort study	16	5 miRNAs	miR-181a-5p predicts OS (HR 0.267)	N
(110)	Cohort study	64	522 miRNA from tissue	miR-425-3p predicts PFS	N
(111)	Cohort study	24	miR-18a, miR-21, miR-139-5p, miR-221, miR-224, and miR-10b-3p	High baseline miR-10b-3p Predicts OS (HR 0.522) Not significant on multivariate testing	N
SNPs					
(24)	Cohort study	148	VEGF-A, VEGF-C and VEGFR-1,2,3	*SNPs VEGF-A* rs2010963 and *VEGF-C* rs4604006 predicts OS (HR 0.28, 0.25 respectively) and PFS on multivariate analysis	N
(25)	Cohort study	78	VEGFR2 (KDR) 18 SNPs	*VEGFR2* rs1870377-AA (HR: 0.35) and rs2071559-CC (HR: 2.25) predict OS on multivariate analysis	N
(22)	Cohort study	128	eNOS polymorphisms	eNOS haplotype HT1: T-4b at eNOS -786/eNOS VNTR predicts OS on multivariate analysis (HR 7.03)	N
(23)	Cohort study	135	Ang-2, NOS3 SNPs	*ANGPT2* (Ang2 gene) rs55633437 predicts OS (HR 5.48), NOS3 rs2070744 predicts OS (HR 0.67) on multivariate analysis	N
(112)	Cohort study	210	HIF-1α SNPs	*HIF-1α* rs12434438 no effect measure reported	N
(113)	Cohort study	47	ABCB1 (rs2032582; rs1045642) and ABCG2 (rs2231137; rs2231142; rs2622604	ABCB1 3435C>T, ABCG2 34G>A, ABCG2 1143C>T and ABCG2 421C>A. Trend towards prediction of progression. Not significant	N
(114)	Cohort study	174	whole-genome analysis	SLC15A2 rs2257212 Predicts PFS (HR 2.18)	N

Studies identified by literature review assessing the prognostic ability of biomarkers in patients with advanced HCC treated with sorafenib against clinically relevant endpoints (either overall response rate, disease control rate, PFS or OS) with a statistically significant result. CPI, checkpoint inhibitor; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; miRNA, micro RNA; DCR, disease control rate; PFS, progression-free survival; OS, overall survival; CSC, cancer stem cell; ctDNA, circulating free DNA; CAN, copy number alteration; TTP, time to progression; HR, hazard ratio; IHC, immunohistochemistry; CTC, circulating tumor cell; PBMC, peripheral blood mononuclear cells; ROS, reactive oxygen species; SII, systemic immune inflammation index; NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio; AFP, alpha-fetoprotein; MDSC, myeloid derived suppressor cell; CEC, circulating endothelial cell; CEP, circulating endothelial progenitor; RDW, red cell distribution width; MLR, mixed lymphocyte reaction; CP, Child Pugh; SNP, single nucleotide polymorphism; TACE, trans arterial chemoembolization; CRP, C reactive protein; HCV, hepatitis C virus; HCC, hepatocellular carcinoma.

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Table S2 Literature search results of candidate SNPS

Gene	SNP	Reference
ICAM1	rs1799969 (G241R), rs5498 (K469E)	(115-117)
IL1B	rs1143627 (IL1b- 31 T/C), rs16944 (-511T)	(118-120)
ILA	rs17561, rs143634, rs1800587, rs1143627	(121,122)
IL2	rs2069762 (-330A>C)	(123,124)
IL4	rs2243250, rs2070874	(125,126)
IL 6	rs1800795	(127,128)
IL 8	rs4073 (-251), rs2227306	(129,130)
IL 10	rs3024505, rs1800896, rs3024505, rs1800872 (IL -59)	(131,132)
IL12	rs3212227	(133,134)
IL13	rs20541	(135,136)
IL 17	rs2275913	(137,138)
Mcp-1	rs1024611 (A2518G)	(139)
STAT3	rs3816769	(140,141)
nfkb	rs28362491	(142,143)
TNFa	rs1800629 (-308 G->A)	(144,145)
TGFB	rs1800469	(146,147)
CCL22	rs4359426	(148,149)
iNOS	rs2297518	(150,151)
MMP 1	rs1799750	(152-158)
MMP 7	rs11568818	
MMP 9	rs17576	
MMP 12	rs2276109	
PDL1/PD1	rs11568821, rs11568821 (pd1.3), rs10204525 (pd1.6)	(159,160)
CTLA4 (CD80)	rs231775	(161)
TIM3	rs1036199	(162)
Foxp3	rs3761548, rs2232365	(19,163,164)

No results were found for the following genes: *VCAM1*, *EDNRA/B*, *EMAP2*, *Ang2*, *Tie2*, *IL-5*, *IL18*, *M-CSF (csf1)*, *CSFR1*, *Sdf-1*, *Sema3a*, *NRP1*, *GCSF*, *GM-CSF*, *IFNa*, *OncostatinM*, *CCL2-5*, *CCR2*, *CXCL1-5*, *CXCL8-10*, *CXCL12*, *CXCL17*, *CCL11*, *CCL15*, *CCL28*, *CXCR3*, *CXCR4*, *Bv8*, *ARG1*, *IRF8*, *LAG3*, *ICOS*, *GITR*, *Galectin9*, *CD25.* Candidate SNPs with functional activity identified from literature review of the immune signaling pathways of the HCC tumor immune microenvironment. SNP, single nucleotide polymorphism; HCC, hepatocellular carcinoma.

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https://dx.doi.org/10.21037/tgh-23-22

Table S3 Chromosomal locations, positions and biological effects of investigated SNPs

SNP ID	Gene	Chr.	Gene position/ effect	Codon exchange	Aa. exchange
rs4604006	*VEGFC*	4	Intron variant		
rs2010963	*VEGFA*	6	5 prime UTR		
rs2070744	*NOS3*	7	Upstream transcript variant		
rs1799983	*NOS3*	7	Missense variant	GAT>GAA	D>E
rs55633437	*ANGPT2*	8	Synonymous variant		
rs1870377	*VEGFR2*	4	Missense Variant	CAA>CAT	Q>H
rs2071559	*VEGFR2*	4	Upstream Variant		
rs10204525	*PDCD1*	2	3 Prime UTR Variant		
rs1024611	*CCL2*	17	5 prime UTR		
rs1036199	*TIM-3*	5	Missense	CGG>CTG	R>L
rs1143627	*IL1B*	2	5 Prime UTR Variant		
rs1143634	*IL1B*	2	Synonymous Variant		
rs11568818	*MMP7*	11	Upstream variant		
rs11568821	*PDCD1*	2	Intron variant		
rs16944	*IL1B*	2	Upstream variant		
rs17561	*IL1A*	2	Missense variant	GCA>TCA	A>S
rs17576	*MMP9*	20	Missense variant	CAG>CCG	Q>L
rs1799750	*MMP1*	11	Upstream variant		
rs1799969	*ICAM1*	19	Missense variant	GGG>AGG	G>R
rs1800469	*TGFB1*	19	Upstream variant		
rs1800587	*IL1A*	2	Upstream variant		
rs1800629	*TNF*	6	Upstream variant		
rs1800795	*IL6*	7	Intron variant		
rs1800872	*IL19*	1	Intron variant		
rs1800896	*IL19*	1	Intron variant		
rs20541	*IL13*	5	Missense variant	CAG>CCG	Q>P
rs2069762	*IL2*	4	Upstream variant		
rs2070874	*IL4*	5	5 Prime UTR Variant		
rs2227306	*CXCL8*	4	Intron variant		
rs2232365	*FOXP3*	X	Intron variant		
rs2243250	*IL4*	5	Upstream variant		
rs2275913	*IL17A*	6	Upstream variant		
rs2276109	*MMP12*	11	Upstream variant		
rs2297518	*NOS2*	17	Missense variant	TCG>TTG	S>L
rs231775	*CTLA4*	2	Missense variant	ACC>GCC	T>A
rs28362491	*NFKB1*	4	Upstream variant		
rs3024505	*IL10*	1	Downstream variant		
rs3212227	*IL12B*	5	3 Prime UTR Variant		
rs3761548	*FOXP3*	X	Intron variant		
rs3816769	*STAT3*	17	Intron variant		
rs4073	*CXCL8*	4	Upstream variant		
rs4359426	*CCL22*	16	Missense variant	GAT>GCT	D>A
rs5498	*ICAM1*	19	Missense variant	AAG>GAG	K>E

UTR, untranslated region; SNP, single nucleotide polymorphism.

https://dx.doi.org/10.21037/tgh-23-22

Table S4 Genotype frequencies in study population and general population as well as deviation from Hardy Weinberg Equilibrium

SNP	Genotypes (%)	MAF	HWE
Validation SNPs			
rs4604006	CC/CT/TT (43/44/13)	General population T=0.46 Study population T=0.35	P=0.397840
rs2010963	CC/CG/GG (12/47/41)	General population C=0.37 Study population C=0.37	P=0.635833
rs2070744	CC/CT/TT (11/36/52)	General population C=0.35 Study population C=0.29	P=0.033202
rs1799983	GG/GT/TT (62/31/7)	General population T=0.31 Study population T=0.22	P=0.119858
rs55633437	CC/CA/AA (89/10/1)	General population A=0.04 Study population A=0.07	P=0.250854
rs1870377	AA/AT/TT (11/38/51)	General population A=0.24 Study population A=0.30	P=0.145978
rs2071559	GG/GA/AA (27/41/31)	General population G=0.5 Study population G=0.46	P=0.005423
Exploratory SNPs			
rs10204525	TT/TC/CC (13/31/54)	General population T=0.16 Study population T=0.30	P=0.000274
rs1024611	AA/AG/GG (49/35/16)	General population G=0.28 Study population G=0.33	P=0.000283
rs1036199	CC/CA/AA (2/18/80)	General population C=0.17 Study population C=0.11	P=0.522681
rs1143634	GG/GA/AA (74/22/4)	General population A=0.23 Study population A=0.15	P=0.014409
rs11568818	CC/CT/TT (16/37/47)	General population C=0.44 Study population C=0.33	P=0.004298
rs11568821	CC/CT/TT (87/12/1)	General population T=0.07 Study population T=0.07	P=0.148283
rs16944	GG/GA/AA (36/48/16)	General population A=0.36 Study population A=0.40	P=0.999253
rs17561	CC/CA/AA (65/28/7)	General population A=0.29 Study population A=0.21	P=0.018087
rs17576	AA/AG/GG (34/36/31)	General population G=0.36 Study population G=0.48	P=0.000002
rs1799750	CC/C.DEL/DEL.DEL (32/49/19)	General population DEL=0.49 Study population DEL=0.44	P=0.915367
rs1799969	AA/AG/GG (1/11/88)	General population A=0.1 Study population A=0.07	P=0.340442
rs1800469	GG/GA/AA (36/46/18)	General population A=0.23 Study population A=0.41	P=0.341747
rs1800587	AA/AG/GG (7/29/64)	General population A=0.28 Study population A=0.22	P=0.036665
rs1800629	AA/AG/GG (1/21/77)	General population A=0.15 Study population A=0.12	P=0.644290
rs1800795	CC/CG/GG (8/33/59)	General population C=0.36 Study population C=0.25	P=0.090537
rs1800872	GG/GT/TT (42/41/17)	General population T=0.29 Study population T=0.37	P=0.032667
rs1800896	CC/CT/TT (12/39/49)	General population C=0.45 Study population C=0.32	P=0.107408
rs20541	AA/AG/GG (10/40/50)	General population A=0.21 Study population A=0.3	P=0.447726
rs2069762	CC/CA/AA (15/43/41)	General population C=0.29 Study population C=0.36	P=0.257217
rs2070874	CC/CT/TT (51/26/23)	General population T=0.17 Study population T=0.36	P=0.000000
rs2227306	CC/CT/TT (45/44/11)	General population T=0.36 Study population T=0.33	P=0.784191
rs2232365	CC/CT/TT (51/8/41)	General population T=0.39 Study population T=0.45	P=0.000000
rs2243250	CC/CT/TT (50/25/24)	General population T=0.19 Study population T=0.37	P=0.000000
rs2275913	GG/GA/AA (39/44/17)	General population A=0.33 Study population A=0.39	P=0.169334
rs2276109	TT/TC/CC (86/12/2)	General population C=0.07 Study population C=0.08	P=0.000623
rs2297518	GG/GA/AA (74/24/2)	General population A=0.19 Study population A=0.14	P=0.971112
rs231775	AA/AG/GG (31/49/20)	General population G=0.37 Study population G=0.44	P=0.763588
rs28362491	ATTG.ATTG/ATTG.DEL/DEL.DEL (40/46/15)	General population DEL=0.42 Study population DEL=0.37	P=0.632494
rs3024505	GG/GA/AA (79/21/0)	General population A=0.14 Study population A=0.11	P=0.164468
rs3212227	GG/GT/TT (15/35/50)	General population G=0.22 Study population G=0.32	P=0.000718
rs3761548	GG/GT/TT (58/7/35)	General population T=0.25 Study population T=0.39	P=0.000000
rs3816769	CC/CT/TT (17/44/39)	General population C=0.33 Study population C=0.39	P=0.242653
rs4073	TT/TA/AA (37/44/19)	General population T=0.49 Study population T=0.59	P=0.191191
rs4359426	CC/CA/AA (85/14/1)	General population A=0.05 Study population A=0.08	P=0.755725
rs5498	GG/GA/AA (16/44/40)	General population G=0.43 Study population G=0.38	P=0.247505

SNP, single nucleotide polymorphism.

https://dx.doi.org/10.21037/tgh-23-22

Table S5 Univariable analysis and statistical significance of clinical variables against PFS and OS in sorafenib treated patients

Variable	Categories	PFS			OS		
		Median (months)	Hazard ratio (95% CI)	P value*	Median (months)	Hazard ratio (95% CI)	P value*
Age	≥66.5	5.5	0.83 (0.61-1.14)	0.25	18.5	0.77 (0.52-1.1)	0.19
	<66.5	4.0			12.6		
Sex	Male	5.3	0.85 (0.56-1.29)	0.45	16.1	0.88 (0.55-1.4)	0.59
	Female	4.1			12.8		
Hepatitis status	HBV positive	4.0	1.25 (0.7-1.79)	0.23	18	0.94 (0.6-1.5)	0.81
	HBV negative	5.5			13.7		
	HCV positive	5.45	0.84 (0.60-1.18)	0.32	15.4	0.94 (0.6-1.4)	0.75
	HCV negative	4.8			14.9		
Child-Pugh	5	5.13	Reference	P=0.88	19.2	Reference	0.02
	6	5.06	1.11 (0.76-1.61)		9.6	1.96 (1.27-3.04)	
	7	5.32	1.27 (0.64-2.51)		11.9	1.74 (0.84-3.6)	
	8		n/a			n/a	
ECOG	0	5.32	Reference	0.24	17.6	Reference	0.26
	1	4.0	1.21 (0.88-1.67)		12.6	1.3 (0.9-1.9)	
	2		n/a			n/a	
BCLC	A	17.5	Reference	0.58	18.0	Reference	0.33
	B	5.3	1.98 (0.56-6.96)		13.4	4 (0.5-31)	
	C	5.1	1.86 (0.57-6.10)		14.9	3.8 (0.5-27)	
T stage	0	4.6	Reference	0.75	43.3	Reference	0.08
	1	3.5	2.27 (0.55-9.27)		23.2	3.00 (0.60-14.93)	
	2	5.3	1.46 (0.61-3.49)		18.1	1.98 (0.60-6.56)	
	3	5.2	1.46 (0.63-3.37)		12.9	3.12 (0.98-10.00)	
	4	2.8	2.06 (0.61-6.83)		11.9	4.55 (1.00-20.64)	
PVT	Yes	5.3	0.90 (0.65-1.23)	0.49	13	1.4 (0.9-2)	0.11
	No	4.8			18		
Extrahepatic disease	Yes	3.9	1.26 (0.92-1.74)	0.14	13.3	1.06 (0.7-1.6)	0.74
	No	5.5			18		
AFP	≥200	3.7	1.11 (0.80-1.54)	0.54	13.0	1.09 (0.7-1.6)	0.67
	<200	5.7			15.4		
NLR	≥3	4.8	1.13 (0.80-1.61)	0.48	12.8	1.3 (0.95-1.6)	0.06
	<3	5.3			18.9		

*, P values for cox proportional hazards model testing. HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; NLR, neutrophil-lymphocyte ratio; ECOG, eastern cooperative oncology group; PVT, portal vein thrombosis; PFS, progression-free survival; OS, overall survival.

https://dx.doi.org/10.21037/tgh-23-22

Table S6 Multivariable analysis of clinical variables, validation SNPs and exploratory SNPs for Sorafenib treated patients

Variable	PFS, hazard ratio (P value)	OS, hazard ratio (P value)
Gender (male)	0.66 (0.10)	0.58 (0.06)
Child-Pugh Score		
6 *vs.* 5	0.88 (0.57)	1.48 (0.15)
7 *vs.* 5	1.18 (0.66)	2.07 (0.08)
T stage		
2 *vs.* 1	1.35 (0.58)	3.9 (0.08)
3 *vs.* 1	2.21 (0.14)	8.8 (0.007)
4 *vs.* 1	2.90 (0.12)	12.3 (0.008)
Portal vein thrombus	0.63 (0.09)	0.77 (0.42)
AFP	1.04 (0.24)	1.04 (0.23)
NLR	1.10 (0.47)	1.24 (0.19)
SNPs		
rs1870377	1.27 (0.12)	1.02 (0.93)
rs1024611	0.86 (0.28)	0.67 (0.02)
rs1800896	0.76 (0.05)	0.66 (0.02)
rs231775	1.22 (0.20)	1.13 (0.51)
rs28362491	1.33 (0.045)	1.34 (0.11)

PFS, progression-free survival; OS, overall survival; AFP, alpha-fetoprotein; NLR, neutrophil lymphocyte ratio; SNP, single nucleotide polymorphism.

https://dx.doi.org/10.21037/tgh-23-22

Table S7 Demographic and clinical characteristics of patients treated with TACE

Characteristic	Categories	Number (total N=147)
Gender, n (%)	Male	121 (82)
	Female	26 (18)
Age, year, median (range)		67.4 (34.6–86.0)
Ethnicity, n (%)	Asian/pacific islander	47 (32)
	Caucasian	79 (54)
	Black	1 (1)
	Latino	4 (2)
	Aboriginal	0
	Other	3 (2)
	Mixed	0
	Missing	13 (9)
Etiology, n (%)	HBV	32 (22)
	HCV	50 (34)
	Alcohol	36 (24)
	NAFLD	24 (16)
	Other	4 (4)
BCLC, n (%)	A	28 (19)
	B	110 (75)
	C	9 (6)
	Missing	0
Serum AFP, n (%)	≥200	41
	<200	105
	Missing	1
Prior therapy, n (%)	Surgical resection	23 (16)
	RFA	65 (44)
	TACE	0
	Radiation	29 (20)
	Transplant	0
Multifocal, n (%)	Yes	123 (84)
	No	24 (16)
PVT, n (%)	Yes	8 (5)
	No	137 (93)
	Missing	2 (2)
Child-Pugh score, n (%)	A5	113 (77)
	A6	31 (21)
	B7	2 (2)
	≥B8	0
Extrahepatic disease, n (%)	Yes	3 (2)
	No	144 (98)
NLR, n (%)	≥3	53
	<3	93
	Missing	1

HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; AFP, alpha-fetoprotein; RFA, radiofrequency ablation; TACE, trans arterial chemoembolization; NLR, neutrophil-lymphocyte ratio; PVT, portal vein thrombosis; PFS, progression-free survival; OS, overall survival.

https://dx.doi.org/10.21037/tgh-23-22

Table S8 Univariable analysis and statistical significance of clinical variables against PFS and OS in TACE treated patients

Variable	Value	Median PFS (months)	Hazard ratio (95% CI)	P value	Median OS (months)	Hazard ratio (95% CI)	P value
Age	≥67.4	5.5	0.98 (0.96–0.99)	0.03	28.8	0.99 (0.97–1.03)	1
	<67.4	3.9 (P=0.62)			26.2		
Sex	Male	4.5	1.096 (0.71–1.69)	0.7	25.8	1.06 (0.59–1.88)	0.9
	Female	4.5			30.7		
Etiology	HBV positive	4.3	1.01 (0.67–1.53)	1	46.2	0.6 (0.3–1.05)	0.06
	HBV negative	4.7			25.5		
	HCV positive	4.4	0.99 (0.7–1.4)	0.9	25.5	1.2 (0.7–1.9)	0.5
	HCV negative	4.7			29		
Child-Pugh	5	4.7	Reference	0.7	30.3	Reference	0.1
	6	4.2	1.2 (0.78–1.8)		21.0	1.7 (1.009–2.8)	
	7	5.1	1.4 (0.34–5.6)		3.8	1.8 (0.24–12.9)	
ECOG	0	5	Reference	0.4	46.2	Reference	0.1
	1	4.4	0.82 (0.58–1.2)		23.9	1.5 (0.98–2.4)	
	2	3.5	1.2 (0.49–3)		5.5	2.3 (0.32–17.3)	
BCLC	A	6	Reference	0.03	30.7	Reference	0.3
	B	4.1	1.68 (1.08–2.6)		24.3	1.4 (0.84–2.59)	
	C	6.1	0.99 (0.46–2.2)		27.5	1.7 (0.7–4.1)	
PVT	Yes	2.4	1.2 (0.58–2.5)	0.6	21.6	1.8 (0.77–4.1)	0.2
	No	4.7			27.5		
AFP	≥200	4	1.05 (0.98–1.1)	0.2	21.6	1.09 (1.009–1.18)	0.03
	<200	4.7 (P=0.1)			29		
NLR	≥3	3.8	0.8 (0.59–1.2)	0.4	23.5	1.3 (0.82–2.06)	0.3
	<3	4.6 (P=0.03)			30.3		

HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; AFP, alpha-fetoprotein; TACE, trans arterial chemoembolization; NLR, neutrophil-lymphocyte ratio; ECOG, Eastern Cooperative Oncology Group; PVT, portal vein thrombosis; PFS, progression-free survival; OS, overall survival.

Table S9 Univariable analysis validation and exploratory SNPs- TACE treated patients

Gene	Genotypes (%)	Median PFS (months)				Median OS (months)			
		Wt.	Het.	Homo.	P value	Wt.	Het.	Homo.	P value
NOS3 rs2070744	CC/CT/TT (15/54/76)	4	4.4	9	0.06, 0.039	28.8	25.8	27.5	0.9, 0.84
TNF rs1800629	AA/AG/GG (2/32/111)	4.5	4.4	4	*0.4, $^{+}$0.48	23.9	45	41	*0.01, $^{+}$0.007
IL-13 rs20541	AA/AG/GG (13/62/70)	5	4.1	3.9	0.4, 0.48	25.5	30.7	45	0.2, 0.048
NFKB rs28362491	ATTG.ATTG/ATTG.DEL/DEL.DEL (60/66/19)	4.1	5.1	4.8	1, 0.78	25.8	24.3	28.8	0.1, 0.044

*, logrank statistical test, $^{+}$, nested LR statistical test. SNP, single nucleotide polymorphism; TACE, trans arterial chemoembolization; PFS, progression-free survival; OS, overall survival; wt, wild type; Het, heterozygous; Homo, homozygous; LR, likelihood ratio.

https://dx.doi.org/10.21037/tgh-23-22

Appendix 1 REMARK Checklist for scoring the quality of the study: Marisi G, Petracci E, Raimondi F, *et al. ANGPT2* and *NOS3* Polymorphisms and Clinical Outcome in Advanced Hepatocellular Carcinoma Patients Receiving Sorafenib (23)

Item to be reported		Page no.	Comment
INTRODUCTION			
1	State the marker examined, the study objectives, and any pre-specified hypotheses.	✓	Discusses aim to determine prognostic value of SNPs within defined genes
MATERIALS AND METHODS			
Patients			
2	Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.	✓	Intermediate/advanced HCC treated with sorafenib. Describes recruitment center, eligibility criteria stated
3	Describe treatments received and how chosen (e.g., randomized or rule-based).	✓	Describes all got sorafenib
Specimen characteristics			
4	Describe type of biological material used (including control samples) and methods of preservation and storage.	✓	DNA extracted from whole blood, in EDTA tubes. No description of preservation
Assay methods			
5	Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	✓	DNA extracted using QIAamp DNA Minikit, quality control with nanodrop 1000, genotyping on ABI 3130 Genetic Analyzer. Analysis blinded
Study design			
6	State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	✓	Retrospective, case selection described, 2012-2015, median f/u 8.9mo
7	Precisely define all clinical endpoints examined.	✓	PFS, OS described
8	List all candidate variables initially examined or considered for inclusion in models.	X	No description of candidate variables
9	Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	X	No rationale given
Statistical analysis methods			
10	Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	✓	Describes using log rank test and cox proportional hazards model. Describes model was built using variables significant on univariable analysis
11	Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.	✓	categorical
RESULTS			
Data			
12	Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.	X	
13	Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.	✓	*Table 1* describes basic characteristics including missing data
Analysis and presentation			
14	Show the relation of the marker to standard prognostic variables.	X	Not shown
15	Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.	✓	
16	For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	✓	
17	Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	✓	Includes nonsignificant clinical variables in final model
18	If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	X	Not reported
DISCUSSION			
19	Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.	✓	Describes exisisting basic science research on ANPT2 and NOS3 snps, as well as data on prognostic significance in other cancers, and other snps studied in HCC
20	Discuss implications for future research and clinical value.	X	Does not describe how studies could validate the predictive use of these markers or how could use in clinic

https://dx.doi.org/10.21037/tgh-23-22

Appendix 2 REMARK Checklist for scoring the quality of the study: Casadei Gardini A, Marisi G, Faloppi L, *et al.* eNOS polymorphisms and clinical outcome in advanced HCC patients receiving sorafenib: final results of the ePHAS study (22)

Item to be reported		Page no.	Comment
INTRODUCTION			
1	State the marker examined, the study objectives, and any pre-specified hypotheses.	✓	States SNPs of interest and states the aim of investigating the prognostic value
MATERIALS AND METHODS			
Patients			
2	Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.	✓	Describes included stages and that must be refractory to local treatments, describes recruitment centers, inclusion criteria stated
3	Describe treatments received and how chosen (e.g., randomized or rule-based).	X	Only described that all patients received sorafenib
Specimen characteristics			
4	Describe type of biological material used (including control samples) and methods of preservation and storage.	✓	Used whole blood or FFPE. did not describe storage methods
Assay methods			
5	Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	✓	Describes kits for processing, QIAmp DNA minikit or Recoverall, DNA quality assessed by Nanodrop 1000, sequencing on 7500 realtime PCR system (applied biosystems). Does not describe blinding
Study design			
6	State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	✓	Retrospective, no matching, specifies time period of collection, median follow up 50 months
7	Precisely define all clinical endpoints examined.	✓	PFS, OS described
8	List all candidate variables initially examined or considered for inclusion in models.	✓	age, gender, etiology, Barcelona-Clinic Liver Cancer [BCLC] stage, serum α-FP level and MELD score
9	Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	X	Not mentioned
Statistical analysis methods			
10	Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	X	Does not describe variable selection procedures, just states clinical covariates were included in model. Does not describe assumption verification or missing data
11	Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.	✓	Categorical
RESULTS			
Data			
12	Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.	X	
13	Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.	✓	Includes *Table 1* and mentions missing values
Analysis and presentation			
14	Show the relation of the marker to standard prognostic variables.	X	
15	Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.	✓	
16	For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	✓	Gives HR in multivariate model for snps but not for other variables
17	Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	✓	
18	If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	✓	Includes validation cohort of separate patients. Does not mention sensitivity analysis
DISCUSSION			
19	Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.	✓	. Describes one other study of SNPs as biomarkers for HCC, describes basic science research on eNOS. Describes weakness
20	Discuss implications for future research and clinical value.	X	Discussed results as predictive when they are prognostic

https://dx.doi.org/10.21037/tgh-23-22

Appendix 3 REMARK Checklist for scoring the quality of the study: Scartozzi M, Faloppi L, Svegliati Baroni G, *et al.* VEGF and VEGFR genotyping in the prediction of clinical outcome for HCC patients receiving sorafenib: the ALICE-1 study (24)

Item to be reported		Page no.	Comment
INTRODUCTION			
1	State the marker examined, the study objectives, and any pre-specified hypotheses.	✓	Criteria for selection and SNP list specified. Hypothesis not clearly stated
MATERIALS AND METHODS			
Patients			
2	Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.	✓	Exclusion criteria not stated
3	Describe treatments received and how chosen (e.g., randomized or rule-based).	✓	Described all patients received sorafenib
Specimen characteristics			
4	Describe type of biological material used (including control samples) and methods of preservation and storage.	✓	HCC tissue blocks or whole blood, preservation method not stated
Assay methods			
5	Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	✓	Commercial assay methods specified. Personnel performing tests were blinded
Study design			
6	State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	✓	Stated dates of collection, and that patients with intermediate-advanced HCC were chosen, retrospectively. Follow up time stated
7	Precisely define all clinical endpoints examined.	✓	PFS, OS defined
8	List all candidate variables initially examined or considered for inclusion in models.	✓	Lists examined variables
9	Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	✓	Calculates sample size based on absence of progression at 6months
Statistical analysis methods			
10	Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	X	States model was created using variables significant on univariable testing. Does not comment on missing data handling or verification of model assumptions
11	Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.	✓	Categorical variables (snps)
RESULTS			
Data			
12	Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.	X	No flow diagram or comment on dropout
13	Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.	✓	Prognostic variable distribution listed, does not describe missing values
Analysis and presentation			
14	Show the relation of the marker to standard prognostic variables.	X	No association between SNPs and other prognostic variables
15	Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.	✓	
16	For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	✓	HR given with p value but no CI
17	Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	X	Only included significant prognostic variables in final model
18	If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	X	Not described
DISCUSSION			
19	Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.	X	Limitations discussed. Did not comment on other studies associating VEGF SNPs with clinical outcomes
20	Discuss implications for future research and clinical value.	✓	Discusses finding prognostic and recommends validation

https://dx.doi.org/10.21037/tgh-23-22

Appendix 4 REMARK Checklist for scoring the quality of the study: Zheng YB, Zhan MX, Zhao W, *et al*. The relationship of kinase insert domain receptor gene polymorphisms and clinical outcome in advanced hepatocellular carcinoma patients treated with sorafenib (25)

	Item to be reported	Page no.	Comment
INTRODUCTION			
1	State the marker examined, the study objectives, and any pre-specified hypotheses.	✓	Stated marker (KDR polymorphisms) and objective to determine prognostic effects on TTP, OS
MATERIALS AND METHODS			
Patients			
2	Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.	✓	HCC diagnosed by AASLD guidelines, metastatic/locally advanced not curable and received sorafenib. Excluded if medical comorbidities but did not define
3	Describe treatments received and how chosen (e.g., randomized or rule-based).	✓	All patients received sorafenib, dose described and basis for dose adjustments
Specimen characteristics			
4	Describe type of biological material used (including control samples) and methods of preservation and storage.	✓	Peripheral blood in tube with anticoagulant stored at -80c
Assay methods			
5	Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	✓	DNA isolated using Qiagen DNA Isolation Kit according to manufacturer. Genotyping was carried out using the iPLEX Gold™ assay on the MassARRAY Platform. PCR protocol described. Genotyping blinded
Study design			
6	State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.	✓	Retrospective, patients admitted to local hospital between Jan 2010 and Mar 2013. Median follow up described
7	Precisely define all clinical endpoints examined.	✓	Described method for assessing response (mRECIST) and defines TTP and OS
8	List all candidate variables initially examined or considered for inclusion in models.	X	
9	Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	X	
Statistical analysis methods			
10	Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	X	Described the use of log rank testing and CPH model but did not describe model building or verification, missing data
11	Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.	✓	
RESULTS			
Data			
12	Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.	X	
13	Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.	✓	Included in *Table 2*
Analysis and presentation			
14	Show the relation of the marker to standard prognostic variables.	X	
15	Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.	✓	*Table 4* shows univariate analyses. Kaplan Meier plots presented
16	For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.	✓	*Table 5*
17	Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.	X	Not all prognostic variables included in multivariable model
18	If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.	X	
DISCUSSION			
19	Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.	✓	Provides an overview of literature describing functional effects of KDR SNPs, descripes limitations
20	Discuss implications for future research and clinical value.	✓	Discusses need for validation given small sample size and that results may help tailor treatment with sorafenib

https://dx.doi.org/10.21037/tgh-23-22