

Review

# Predictive and Prognostic Factors in HCC Patients Treated with Sorafenib

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Received: 5 September 2019; Accepted: 16 October 2019; Published: 21 October 2019

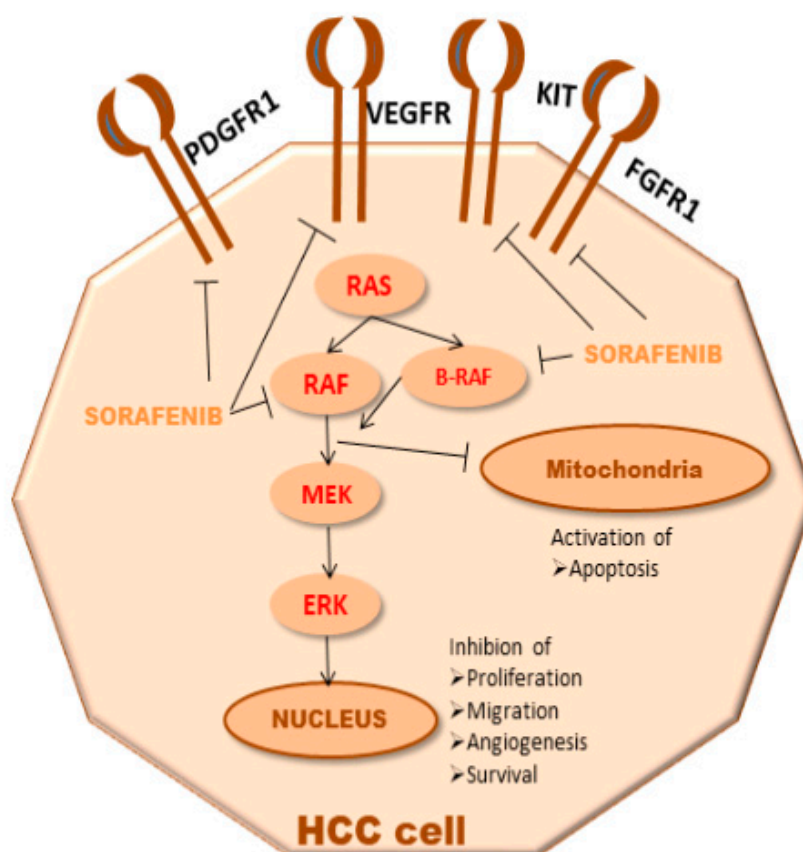


**Abstract:** Sorafenib is an oral kinase inhibitor that enhances survival in patients affected by advanced hepatocellular carcinoma (HCC). According to the results of two registrative trials, this drug represents a gold quality standard in the first line treatment of advanced HCC. Recently, lenvatinib showed similar results in terms of survival in a non-inferiority randomized trial study considering the same subset of patients. Unlike other targeted therapies, predictive and prognostic markers in HCC patients treated with sorafenib are lacking. Their identification could help clinicians in the daily management of these patients, mostly in light of the new therapeutic options available in the first.

**Keywords:** Sorafenib; hepatocellular carcinoma; prognostic factors; predictive factors

## 1. Introduction

Sorafenib (NEXAVAR<sup>®</sup>) is a small molecule classified as an oral multi-targeted tyrosine kinase inhibitor (TKI) since it inhibits platelet-derived growth factor receptors (PDGFR), vascular endothelial growth factor (VEGF-R), tyrosine-protein kinase KIT, and fibroblast growth factor receptors FGFR1. As is known, this TKI impairs angiogenesis, cancer proliferation, and cell apoptosis (Figure 1) [1].



**Figure 1.** Mechanisms of Sorafenib. A graphic representation of sorafenib mechanisms in HCC patients. Abbreviations—ERK: extracellular signal-regulated kinase; FGFR1: fibroblast growth factor receptors; HCC hepatocellular carcinoma; MEK: mitogen-activated protein kinase; DGFR: platelet-derived growth factor receptors; RAF: VEGF-R: vascular endothelial growth factor receptor.

In 2007, the FDA approved this drug in unresectable and advanced HCC, according to the results of Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) [2] and Asia-Pacific [3] randomized phase III studies. In the SHARP trial, median overall survival (mOS) was 10.7 months and 7.9 months for sorafenib and placebo groups, respectively (hazard ratio (HR) 0.69, 95% confidence interval (CI) 0.55 to 0.87,  $p < 0.001$ ) [2]. In the Asia-Pacific trial as well, sorafenib-treated patients showed an mOS significantly higher as a result compared to the placebo group (6.5 months vs. 4.2 months, respectively, HR 0.68, 95% CI 0.50–0.93,  $p = 0.014$ ) [3].

Hand-foot skin reaction (HFSR), fatigue, diarrhea, anorexia, and weight reduction were the most common treatment-related adverse effects (AE) in both studies [2,3].

Several randomized clinical trials were performed subsequently and evaluated other tyrosine kinase inhibitors (TKIs) compared with sorafenib, which did not report an improvement in terms of clinical outcomes [4–6]. In particular, sunitinib [4] and linifanib [5] did not achieve superior mOS compared to sorafenib (8.1 months vs. 10.0 months, respectively,  $p = 0.0019$ –9.1 months vs. 9.8 months, HR 1.046, 95% CI, 0.896 to 1.221, respectively). Furthermore, sorafenib achieved a significant improvement in terms of mOS compared to brivanib (9.9 months vs. 9.5 months  $p > 0.05$ ) [6].

Methodological bias influenced these negative results. In particular, the bias includes the lack of phase II studies evaluating liver toxicity (required in cirrhotic patients with HCC), the need for stronger secondary endpoints (i.e., time to progression, TTP, and objective response rate, ORR), according to the modified RECIST (mRECIST) criteria, and the lack of predictive biomarkers [7].

Only lenvatinib showed similar results in terms of survival in a non-inferiority randomized trial, with an mOS for lenvatinib of 13.6 months (95% CI 12.1–14.9) compared to sorafenib (12.3 months, 95% CI 10.4–13.9, HR 0.92) with different toxicity profiles [8].

In this manuscript, we reviewed the data available in the literature with the aim to try to answer the following question. Is it possible today to select patients as candidates for sorafenib according to clinical or biological predictive and/or prognostic markers?

## 2. Clinical Predictive/Prognostic Markers

### 2.1. Barcelona Clinic Liver Cancer Staging and Child-Pugh Cirrhosis Classifications

Although it is an expected situation, Barcelona Clinic Liver Cancer (BCLC) staging and Child-Pugh (CP) cirrhosis classifications resemble the most important criteria in the selection of HCC patients who are suitable for treatment with sorafenib. In the registrative trials [2,3], almost all enrolled patients were CP-A. Correlation between CP and the response to sorafenib has been confirmed in two prospective studies. The first considered 120 patients [9] with mOS of 13 months and 4.5 months, respectively ( $p = 0.0008$ ). The second evaluated 300 patients [10] with mOS of 10.0 months and 3.8 months, respectively ( $p < 0.001$ ). The GIDEON trial [11], which is an observational registry study, evaluated the survival and safety of sorafenib in 1968 and 666 patients with a CP-A and CP-B status, respectively. The mOS were 13.6 months (95% CI 12.8–14.7) and 5.2 months (95% CI 4.6–6.3) in CP-A and CP-B patients, respectively.

In addition, BCLC has been evaluated as the clinical predictive criteria for the response to sorafenib. In the SHARP trial, BCLC B and BCLC C achieved mOS of 14.5 months and 9.7 months, respectively [2]. Later, the Italian study SOFIA [12] compared mOS of BCLC B and BCLC C patients treated with sorafenib, and found a significant advantage for BCLC B (mOS: 20.6 months and 8.4 months,  $p < 0.0001$ , respectively). More recently, a pooled analysis of SHARP and Asia-Pacific trials [13] endorsed previous results, which demonstrated that BCLC B had a better survival than those with BCLC C HCC patients (HR = 1.59,  $p = 0.02$ ). This confirms the predictive role of BCLC staging.

### 2.2. Viral Status

Since hepatitis B virus (HBV) and hepatitis C virus (HCV) represent the main causes of HCC, the viral status has been analyzed in several studies. Data from the SHARP and the Asia-Pacific trials pooled in the analysis performed by Bruix et al. [13] demonstrated that non-HCV related HCC has a worse OS (HR = 0.7,  $p = 0.02$ ), while HBV infection did not achieve a significant difference in patients treated with sorafenib (HR = 1.128,  $p = 0.4538$ ) compared to HBV-positive HCC patients. In a more recent meta-analysis of sorafenib and lenvatinib trials, lenvatinib was shown to be the best agent for both HBV and HCV infected patients, which presented a more favorable HR versus sorafenib treated with HCC (HR 0.83, 95% CI 0.68–1.01 and HR 0.91, 95% CI 0.66–1.25, respectively) [14].

### 2.3. Diabetes and Use of Oral Antidiabetics

Recently, diabetes and use of oral antidiabetics have been analyzed in HCC patients. Diabetes mellitus is as a risk factor in the development of HCC mostly in patients who are not HBV/HCV positive [15,16]. On the contrary, Di Costanzo et al., in an observational study, did not confirm the prognostic role of diabetes with mOS of 9 months and 10 months in HCC non-diabetic and diabetic patients, respectively ( $p = 0.535$ ). Furthermore, median time to progression (mTTP) was longer in diabetic patients ( $p = 0.038$ ). As for oral antidiabetics, the role of metformin is still uncertain. In a propensity score-matched cohort analysis, the combination of metformin and radiation therapy in unresectable HCC prolonged the OS rate (two-year, 76% vs. 37%,  $p = 0.022$ ) [17]. On the contrary, in a retrospective study [18], metformin reduced sorafenib activity in HCC patients with type II diabetes mellitus with median progression free survival (mPFS) of 2.6 months and 5.0 months and mOS of 10.4 months and 15.1 months for patients chronically-treated with or without metformin, respectively. These data were validated in a case-series of more than 279 HCC patients [19]. There are no clinical trials on the use of metformin in HCC patients. So far, the efficacy of metformin in diabetic and non-diabetic patients is still unknown.

#### 2.4. Adverse Events Due to Sorafenib

Among clinical predictive markers, several studies displayed a positive correlation in HCC patients between survival and adverse events due to sorafenib. The mOS of 634 HCC patients who presented any grade of toxicities related to sorafenib (Hand-Foot Skin Reaction - HFSR, hypertension, diarrhea) and mOS significantly improved when compared to patients without adverse events (8.8 months vs. 5.4 months, respectively, IQR 2.7–8.8, log-rank  $p = 0.004$ ) [20].

Reig et al. assessed 147 patients treated with sorafenib [21]. They observed that HFSR represented an independent predictive factor of better survival, since patients with early HFSR displayed better OS than the patients who did not show this adverse event within the first 60 days of treatment (18.2 months vs. 10.1 months, respectively,  $p = 0.009$ ) [21]. A recent meta-analysis of 12 cohort studies including 1017 patients confirmed the significant correlation between HFSR and the response to sorafenib (pooled HR for mOS of 0.45, 95% CI 0.36, 0.55,  $p < 0.00001$ ,  $I^2 = 35\%$ ) and time to progression (TTP) of 0.41 (95% CI 0.28, 0.60,  $p < 0.00001$ ,  $I^2 = 0\%$ ) [22].

The predictive role of hypertension appears uncertain. A study involving 61 HCC patients demonstrated that those with hypertension who developed this side effect 15 days after beginning sorafenib compared to others who had better mPFS (6.0 months vs. 2.5 months,  $p < 0.001$ ) and mOS (14.6 months vs. 3.9 months;  $p = 0.003$ ). On the contrary, in the studies by Shin SY et al. [23] and Otsuka T et al. [24], hypertension was not related to OS ( $p = 0.262$  and  $p = 0.332$ , respectively).

Regarding diarrhea, Koschny R et al. [25] demonstrated a significant correlation between the grade of this symptom and mOS (grade 2–3 vs. 0–1: 11.8 months vs. 4.2 months—95% CI 6.9–16.6 vs. 95% CI 0.0–9.1, respectively,  $p = 0.009$ ).

In a large, multi-centric retrospective analysis, Di Costanzo et al. [26] demonstrated that mOS was 14.4 months (95% CI 12.0–16.8) and 5.8 months (95% CI 4.6–7.1) in patients with and without HFSR ( $p = 0.005$ ). Furthermore, patients with and without hypertension achieved mOS at 15.1 months (95% CI 12.9–17.3) and 7.5 months (95% CI 5.9–9.2) (both  $p < 0.001$ ), respectively. The mOS were 15.6 months (95% CI 11.1–20.1) and 9.2 months (95% CI 7.6–10.8) in patients with and without diarrhea, respectively (both  $p < 0.001$ ). When a score from 0 to 3 was assigned depending on the number of side effects suffered, a progressive increase in survival was noted. In particular, mOS was 7.9 months, 9.2 months, 15.1 months, and 23.9 months in patients with scores of 0, 1, 2, and 3, respectively ( $p < 0.001$ ).

It would be interesting to discover if the disease background, family history, and treatment history could be considered prognostic biomarkers in the sorafenib response. To date, no data are available in literature concerning these topics.

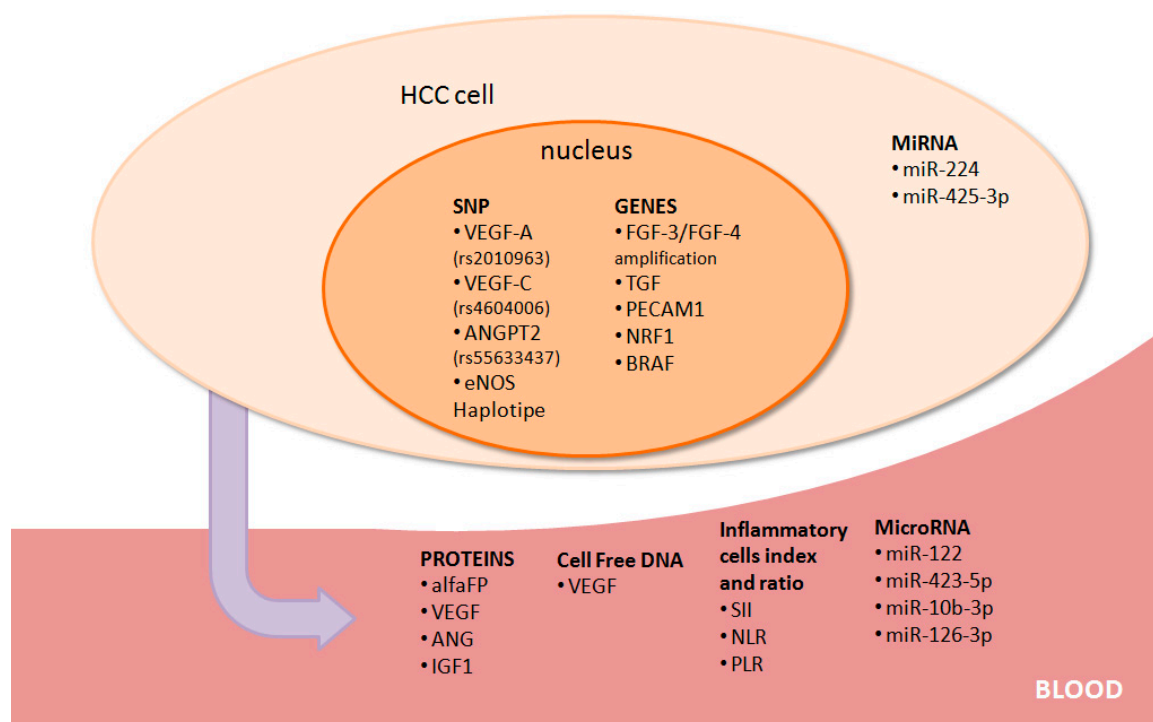
### 3. Biological Predictive Markers

In the era of target therapy and liquid dynamic medicine [27], plasmatic and histological biomarkers have always been used as predictive markers of assumed responses to target therapy. This holds true in HCC patients treated with sorafenib (Figure 2) as well.

#### 3.1. Alpha-Fetoprotein

High alpha-fetoprotein (AFP) values are observed in about half of HCC patients. So far, it is still the principal serological biomarker used for managing this malignancy, even if it is elevated in cirrhotic patients as well [28]. In the SHARP trial, alpha fetoprotein (AFP) plasma levels  $>200$  ng/mL were a negative prognostic marker [2]. These data have been recently confirmed in a pooled analysis of the two registrative trials [13]. Furthermore, an early decrease of AFP seems to be a predictive biomarker [29,30]. Shao et al. [29] defined patients with a reduction of more than 20% from baseline serum levels after two to four weeks of treatment as early AFP responders. Responders were compared with non-responders with a significantly improved overall response rate (ORR) (33% vs. 8%;  $p = 0.037$ ) and disease control rate (DCR) (83% vs. 35%,  $p = 0.002$ ), respectively. Moreover, mPFSs were 7.5 months vs. 1.9 months ( $p = 0.001$ ) and mOS 15.3 months vs. 4.1 months ( $p = 0.019$ ) for responding and non-responding patients,

respectively. Sanchez et al. [30] demonstrated that a decrease of more than 20% AFP at 6–8 weeks from baseline was a positive predictive marker of response to sorafenib. In a multivariate analysis ( $p = 0.002$ ), with mOS of 18 months and 10 months ( $p = 0.004$ ) for responding and non-responding patients, respectively. Furthermore, Nakazawa et al. [31] defined as an increase in AFP when its serum levels were 20% more than the baseline. An early increase of AFP after sorafenib was a significant negative predictive factor, since mOS ( $p < 0.001$ , HR 4.14; 95% CI 1.946–8.811) and mPFS ( $p = 0.001$ , HR 2.852, 95% CI 1.524–5.337) of these patients were worse than the others.



**Figure 2.** Potential predictive/prognostic markers in hepatocellular carcinoma (HCC) patients treated with sorafenib. A graphic representation of biological prognostic/predictive factors analysed in HCC patients treated with sorafenib.

### 3.2. Angiogenetic Markers

Angiogenetic markers were analyzed in several studies, since angiogenesis represents one of the most activated pathways in HCC [32]. Among angiogenetic factors, the most studied have been angiopoietin-2 (Ang-2) and vascular endothelial growth factor-A (VEGF-A). In the SHARP study [2], baseline VEGF and ANG2 plasma levels were prognostic factors in sorafenib and placebo-treated HCC. Anyway, none of them led to a predictive biomarker of sorafenib.

Tsuchiya et al. [33] revealed that a decrease of plasma VEGF concentrations with sorafenib treatment after eight weeks was a predictor of better mOS than others (30.9 months vs. 14.4 months,  $p = 0.038$ ).

In a mouse model of HCC, Horwitz et al. demonstrated that VEGF-A gene amplification was related to better survival when compared to non-amplified tumors [34]. Moreover, they verified the data on HCC serum of patients treated with sorafenib *in vivo*. So far, the mOS were 10 months and not achieved for patients with negative (47 patients) and positive (7 patients) VEGF-A gene amplification, respectively ( $p = 0.029$ ).

In a recent study [35], the circulating cell-free DNA (cfDNA) concentrations of VEGF were analyzed in HCC patients treated with sorafenib. Patients whose disease progressed with sorafenib had significantly higher cfDNA levels than the others (0.82 ng/ $\mu$ L vs. 0.63 ng/ $\mu$ L,  $p = 0.006$ ). Moreover, when patients were classified into cfDNA-high-low groups (above and below the median of cfDNA



concentrations of VEGF, respectively), a significantly worse TTP (2.2 months vs. 4.1 months, respectively, HR = 1.71,  $p = 0.002$ ) and OS (4.1 months vs. 14.8 months, respectively, HR = 3.50,  $p < 0.0001$ ) were achieved in the first group than in the latter.

In addition, Single Nucleotide Polymorphisms (SNPs) of VEGF were analyzed. In a study by Scartozzi et al. [36], univariate analysis of VEGF-A alleles C of rs25648, T of rs833061, C of rs699947, C of rs2010963, VEGF-C alleles T of rs4604006, G of rs664393, VEGFR-2 alleles C of rs2071559, C of rs2305948 showed significant predictive factors of PFS and OS in sorafenib-treated HCC. In the multivariate analysis, VEGF-A rs2010963 and VEGF-C rs4604006 were independent factors influencing PFS (HR = 0.25, 95% CI: 0.19–1.02,  $p = 0.0376$  and HR = 0.22, 95% CI: 0.14–0.81,  $p = 0.004$ , respectively) and OS (HR = 0.28, 95% CI: 0.23–0.96,  $p = 0.02$  and HR = 0.25, 95% CI: 0.17–0.99,  $p = 0.04$ , respectively).

Miyahara et al. [37] described a negative predictive outcome in HCC patients with high Ang-2 serum levels before sorafenib (HR = 2.51, 95% CI: 1.01–6.57,  $p = 0.048$ ). More recently, the expression of a SNP for ANGPT2, an Ang2 gene, and a rs55633437 GG genotype showed a significantly longer PFS ( $p < 0.001$ ) and OS ( $p < 0.001$ ) than those with the other genotypes (GT+TT) [38]. In any case, even if these results describe a potential prognostic role of Ang-2 or its polymorphisms in HCC, its role in predicting a response to sorafenib should be verified.

In another interesting study on the angiogenic gene [39], eNOS polymorphisms were analyzed in relation to PFS and OS. In univariate and multivariate analyses, a training cohort of HCC patients homozygous for endothelial nitric oxide synthase (eNOS) haplotype (HT1:T-4b at eNOS-786/eNOS VNTR) had a worse mPFS (2.6 months vs. 5.8 months, HR = 5.43, 95% CI: 2.46–11.98,  $p < 0.0001$ ) and OS (3.2 months vs. 14.6 months, HR = 2.35, 95% CI: 1.12–4.91,  $p = 0.024$ ) when compared with other haplotypes.

Other studies are evaluating more than one anti-angiogenic biomarker. ALICE-2 study [40] evaluated the role of hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ ) and SNPs of HIF-1 $\alpha$ , VEGF, and Ang2. The multivariate analysis demonstrated that rs12434438 (SNP of HIF-1 $\alpha$ ), rs2010963 (SNP of VEGF-A), and rs4604006 (SNP of VEGF-C) were independent factors and were predictive biomarkers of the sorafenib response. Currently, a prospective ongoing study (INNOVATE) has the aim to confirm the role of SNPs of VEGF, HIF-1 $\alpha$ , Ang-2, and eNOS SNPs in relation to treatment with sorafenib [41].

### 3.3. Inflammatory Cells, Proteins, and Index

It must be said that the systemic inflammatory micro-environment has a strong correlation with angiogenesis, tumor invasion, and metastasis through an upregulation of inflammatory cells and cytokines (i.e., the activation of mechanisms of immune-tolerance in gastrointestinal cancer, including HCC) [42–44]. In particular, an inflammatory micro-environment and circulating immune cells and cytokines play a significant role in HCC prognosis [45–47]. So far, Hu B et al. [45] used a systemic immune-inflammation (SII) index with an aim to predict the prognosis of patients after curative resection. This index was based on lymphocyte, neutrophil, and platelet counts and was able to predict survival and recurrence in HCC. Univariate and multivariate analyses revealed that the SII index was an independent predictive factor for mOS and was a prognostic factor for patients with negative AFP levels and BCLC 0/A. Afterward, Lue et al. [46] demonstrated that a neutrophil-lymphocyte ratio (NLR)  $\geq 2.3$  was a negative predictive biomarker in the response to sorafenib in both univariate and multivariate analyses ( $p = 0.005$  and HR 1.72, 95% CI: 1.03–2.71, respectively) in HCC European patients. More recently, similar data have been achieved in an Asiatic cohort [47]. A meta-analysis conducted on 6318 patients [48] observed that a high NLR before any treatment was predictive of a short mOS (HR: 1.54, 95% CI: 1.34 to 1.76,  $p < 0.001$ ). In this study, authors analyzed the platelet-lymphocyte ratio (PLR) demonstrating that the increase of PLR predicted an unfavorable outcome in terms of mOS as well (HR: 1.63, 95% CI: 1.34 to 1.98,  $p < 0.001$ ). In any case, these data were not found when they were analyzed in the subgroup of sorafenib-treated patients. Casadei-Gardini et al. [49] considered SII, NLR, and PLR in a retrospective multi-center case series. They observed that patients treated with sorafenib and with SII  $\geq 360$  showed poorer survival outcomes when compared to patients with

SII < 360 in terms of mPFS (2.6 months vs. 3.9 months, respectively,  $p < 0.026$ ) and mOS (5.6 months vs. 13.9 months, respectively,  $p = 0.027$ ). Patients with NLR  $\geq 3$  compared with those with NLR < 3, had a lower mPFS (2.6 months vs. 3.3 months,  $p < 0.049$ ). However, no significant data were reported in terms of mOS (5.6 months vs. 13.9 months,  $p = 0.062$ ). So far, SII and NLR could represent predictive factors for patients with advanced HCC treated with sorafenib.

Oxidative stress is a key pathogenic event in the development and progression of HCC [50]. Nuclear erythroid 2-related factor 2 (Nrf2) is a cytosolic transcription factor regulating the cellular protection by inducing anti-inflammatory, antioxidant, and cyto-protective gene expression [51]. In particular, its dysregulation enhances the resistance of cancer cells against drugs. So far, the identification of molecules targeting Nrf2 might open a new scenario for preventing and treating HCC. Preliminary data from in vitro studies showed the ability of Sorafenib to reverse 5-fluorouracil (5-FU) resistance likely through the suppression of Nrf2 expression induced by 5-FU [52], with a putative role in the Sorafenib-response prediction.

Another potential predictive factor is Insulin-like Growth Factor (IGF)-1. Eighty-three patients with high (i.e., levels  $\geq$  the median level) baseline IGF-1 levels achieved a significantly higher disease control rate (DCR) when treated with antiangiogenic therapies (including sorafenib) than those with low levels (71% vs. 39%, respectively— $p = 0.003$ ) [53]. Moreover, patients with high IGF-1 levels, when compared with those with low levels, showed longer mPFS (4.3 months vs. 1.9 months, respectively— $p = 0.014$ ) and mOS (10.7 months vs. 3.9 months, respectively— $p = 0.009$ ). Multivariate analysis demonstrated that high baseline IGF-1 levels were an independent predictive factor of anti-angiogenetic drugs in terms of PFS and OS.

#### 3.4. Growth Factors and Other Targets

Arao et al. analyzed a comparative genomic hybridization in frozen HCC samples from patients responsive to sorafenib [54]. Fibroblast growth factor (FGF) 3/FGF4 amplification was observed in 30% of HCC samples while it was not seen in 38 non-responsive patients ( $p = 0.006$ ). These data were confirmed in vitro with a growth inhibitory assay, since FGF3/FGF4-amplified HCC cell lines exhibited hypersensitivity to sorafenib. To assess a complete panel of genes predictive of the sorafenib response, DNA and RNA sequencing using a fine-needle biopsy was performed in 46 patients [55]. Comparisons were conducted between the transforming growth factor (TGF) gene expression levels of progressive disease (PD)-patients and non PD-patients (74.1 vs. 20.3 median read number, respectively  $p = 0.0180$ ) and the Platelet And Endothelial Cell Adhesion Molecule 1 (PECAM1) gene expression levels of these two groups of patients (110.2 vs. 13.2 median read number, respectively  $p = 0.0131$ ). Both TGF $\alpha$  and PECAM1 gene expression levels were significantly increased in the non-PD group. Moreover, mPFS of patients with high and low neuregulin 1 (NRG1) expressions were 80 days and 90 days in sorafenib responding patients, respectively ( $p = 0.0497$ ). So far, high TGF $\alpha$  and PECAM1 and low NRF1 gene levels should be predictors of response to sorafenib.

Although the B-type Raf kinase (BRAF) mutation could play a role in the response to sorafenib. BRAF, which is a protein located downstream of the Kirsten Ras Sarcoma virus (KRAS) pathway, is implicated in the response to anti-EGFR treatment [56]. In a case series of advanced gastrointestinal stromal tumor (GIST) [57], sorafenib was administered to patients resistant to imatinib, sunitinib, and regorafenib. In these patients, BRAF was tested for mutations. So far, two BRAF wild-type patients achieved long-term disease control (49 months and 19 months, respectively), while sorafenib-resistant patients carried a BRAF V600E mutation. In a report by Casadei-Gardini et al. [58], a patient with synchronous lung cancer (LC) and HCC, treated with sorafenib, achieved a response in LC but not in liver cancer. The mutational analysis revealed a BRAF exon11 mutation (G469V) only in LC. Authors hypothesized that this mutation could be responsible for HCC resistant to sorafenib, which sheds light on a possible negative prognostic role of this mutation.

### 3.5. MiRNAs

Recently, micro RNAs (miRNAs) achieved a key role in gastrointestinal cancers [59–61]. In particular, up/down-regulation of several miRNAs has been reported to be able to impair the TKI response, which affects the expression of genes involved in several pathways [61–63]. For example, miRNA-21 could enhance resistance to sorafenib *in vitro* through the PTEN/Akt pathway by inhibiting autophagy [62]. MicroRNA-122 obtained sorafenib resistance to HCC cell lines through the RAS/RAF/ERK pathway [63]. Moreover, in an animal HCC model, elevated miR-122 levels were associated with a stem-like phenotype in HCC [64] associated with resistance to sorafenib. So far, an anti-miRNA122 transfection increased cell viability in sorafenib-treated HCC cells, which restored sorafenib activity HCCs. The predictive role of circulating miRNAs has also been investigated. The miRNA181a-5p levels resulted in the unique independent factor for sorafenib-treated patients achieving a DCR in 53 patients (HR 0.139, 95% CI 0.011–0.658,  $p = 0.0092$ ) [65]. Furthermore, miR-181a-5p resulted in the only independent factor in terms of OS in multivariate analysis (HR 0.267, 95% CI 0.070–0.818,  $p = 0.0194$ ) [65]. Sorafenib upregulated MiRNA423-5p both *in vitro* and *in vivo* and its increase from baseline to evaluation at six months correlated with the response. In fact, 75% of patients with an miR423-5p level increase achieved disease control [66]. In addition, MiR-126-3p was down-regulated after sorafenib treatment in HCC cells lines [67]. So far, Faranda et al. determined the expression levels of miR-126-3p in HCC tissues and plasma. This miRNA was down-regulated in HCC tissues compared to levels of peritumoral tissues (HCC average =  $3.91 \pm 0.48$  vs. RQPT average =  $5.84 \pm 0.51$ ,  $p$ -value = 0.0074). Moreover, circulating miR-126-3p expression levels were significantly higher in HCC patients when compared to control subjects (26.7 vs. 26.6 mean expression levels,  $p$ -value = 0.0002) [67]. *In vitro* data and *in vivo* determination led authors to hypothesize that a reduction of this miRNA could be predictive of a response to sorafenib.

In an exploratory study [68], several miRNAs (miRNA10b-3p, miRNA18a, miRNA139-5p, miRNA21, miRNA224, miRNA221) were evaluated as predictive markers for the response to sorafenib. Only miRNA10b-3p expression levels were significantly higher (fold increase = 5.8) in the subgroup of HCC patients with worse OS ( $p = 0.008$ ) and with a putative prediction of short survival of sorafenib-treated patients.

The predictive role of miRNA has been evaluated in HCC tissue in clinical studies. In particular, high levels of miRNA-224 in HCC samples were correlated with an increase of PFS (HR = 0.28, 95% CI: 0.09–0.92,  $p = 0.029$ ) and OS (HR = 0.024, 95% CI: 0.07–0.79,  $p = 0.012$ ) in patients treated with sorafenib [69]. In another study, patients with high levels of miR-425-3p in HCC tissue treated with sorafenib achieved a better PFS (HR = 0.5, 95% CI: 0.3–0.9,  $p = 0.007$ ) and TTP (HR = 0.4, 95% CI: 0.2–0.7,  $p = 0.0008$ ) [70].

## 4. Conclusions

Currently, even if sorafenib still remains a gold quality standard in the first line treatment of advanced HCC patients, new targeted therapies and immunotherapies have been approved and will be approved soon. So far, several clinical and biological biomarkers have been evaluated with the aim to improve the choice of patients suitable for treatment with these drugs (Table A1). Nonetheless, we are still far from obtaining a panel useful for clinical practice. The efforts must be to identify a score [27,71], which is able to include various variables useful for perfecting the therapeutic choice in HCC.

Furthermore, *in vitro* and *in vivo* preclinical models evaluated the combination of Sorafenib with the drugs that are able to overcome its acquired resistance mechanisms [72]. Contradictory results from clinical trials considering the combination of Sorafenib with small molecules in the first line setting were reported [73,74].

**Author Contributions:** Conceptualization, O.B., N.S.; methodology, O.B., V.L., A.A.; A.G.; writing—original draft preparation, all authors; supervision, O.B., N.S.

**Acknowledgments:** The Apulian Regional Project “Medicina di Precisione” partially supported this work.



**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations/Nomenclature**

alfaFP alpha feto protein; ANG angiopoietin; ANGPT ANG gene; BRAF B-type Raf kinase eNOS endothelial nitric oxide synthase; FGF fibroblast growth factor; HCC hepatocellular carcinoma; IGF1 insulin growth factor1; miR miRNA; NLR neutrophil lymphocyte ratio; NRF1 Nuclear respiratory factor 1; PECAM1 Platelet And Endothelial Cell Adhesion Molecule 1; SII systemic immune-inflammation; SNP Single Nucleotide Polymorphisms; PLR platelet lymphocyte ratio; TGFA Transforming growth factor alpha; VEGF vascular endothelial growth factor.

**Appendix A**

**Table A1.** Summary table of major studies described in the review.

Clinical/Biological Biomarker	Study Model	Mechanisms/Results	Reference
CLINICAL BIOMARKERS			
Child-Pugh	Observational registry study	CP-A patients mOS > CP-A patients mOS (13.6 months and 5.2 months, respectively)	[11]
BCLC stadiation	Pooled analysis and observational trials	BCLC B achieved a better response compared to BCLC C (mOS of 14.5 months and 9.7 months, respectively) (mOS: 20.6 months and 8.4 months, $p < 0.0001$ , respectively). BCLC B had a better survival than those with BCLC C HCC patients (HR = 1.59; $p = 0.02$ )	[2,12,13]
Viral status	Pooled analysis	Non-HCV related HCC had a worse OS (HR = 0.7, $p = 0.02$ ), while HBV infection did not achieve a significant difference in patients treated with sorafenib (HR = 1.128, $p = 0.4538$ )	[13]
Diabetes	Retrospective study	Metformin reduced sorafenib activity in HCC patients with type II diabetes mellitus with mPFS of 2.6 months and 5.0 months and mOS of 10.4 months and 15.1 months for patients chronically treated with or without metformin, respectively.	[18]
Adverse events due to sorafenib.	Observational study	mOS of HCC patients with any grade of toxicities related to sorafenib (HFSR, hypertension, diarrhea) was significantly improved compared to patients without adverse events (8.8 months vs. 5.4 months, respectively, IQR 2.7–8.8, log-rank $p = 0.004$ )	[20]
HFSR—sorafenib related	Observational study	Early HFSR displayed better OS compared to patients who did not show this adverse event (18.2 months vs. 10.1 months, respectively, $p = 0.009$ )	[21]
HFSR—sorafenib related	Metanalysis of 12 cohort studies	Early HFSR displayed better OS compared to patients who did not show this adverse event (pooled HR for mOS of 0.45, 95% CI 0.36, 0.55, $p < 0.00001$ , $I^2 = 35\%$ ) and TTP of 0.41 (95% CI 0.28, 0.60, $p < 0.00001$ ; $I^2 = 0\%$ )	[22]
Hypertension—sorafenib related	Observational study	Patients who developed this side effect 15 days after beginning sorafenib compared to others who had better mPFS (6.0 months vs. 2.5 months, $p < 0.001$ ) and mOS (14.6 months vs. 3.9 months, $p = 0.003$ ).	[23]

Table A1. Cont.

Clinical/Biological Biomarker	Study Model	Mechanisms/Results	Reference
Diarrhea—sorafenib related	Observational study	Significant correlation between the grade of this symptom and mOS (grade 2–3 vs. 0–1: 11.8 months vs. 4.2 months—95% CI 6.9–16.6 vs. 95% CI 0.0–9.1, respectively, $p = 0.009$ )	[25]
BIOLOGICAL BIOMARKERS			
Alpha-fetoprotein	Retrospective analysis	Early AFP responding patients with a reduction of more than 20% from baseline of serum levels after two to four weeks of treatment. Responders were compared with non-responders with a significantly improved ORR (33% vs. 8%, $p = 0.037$ ) and disease control rate (DCR) (83% vs. 35%, $p = 0.002$ ), respectively.	[29]
Alpha-fetoprotein	Pooled analysis	AFP is a positive predictive marker of response to sorafenib in a multivariate analysis ( $p = 0.002$ ), with mOSs of 18 months and 10 months ( $p = 0.004$ ) for responding and non-responding patients, respectively.	[30]
VEGF concentrations	Observational analysis	A decrease of plasma VEGF concentrations with sorafenib treatment after eight weeks was a predictor of better mOS than others (30.9 months vs. 14.4 months, $p = 0.038$ ).	[33]
VEGF-A gene amplification	Observational analysis	mOSs were 10 months and not achieved for patients with negative (47 patients) and positive (7 patients) VEGF-A gene amplification, respectively ( $p = 0.029$ ).	[34]
cfDNA concentrations of VEGF	Observational analysis	Patients whose disease progressed with sorafenib had significantly higher cfDNA levels than the others (0.82 ng/ $\mu$ L vs. 0.63 ng/ $\mu$ L, $p = 0.006$ )	[35]
SNPs of VEGF	Observational analysis	Univariate analysis VEGF-A alleles C of rs25648, T of rs833061, C of rs699947, C of rs2010963, VEGF-C alleles T of rs4604006, G of rs664393, VEGFR-2 alleles C of rs2071559, C of rs2305948 were significant predictive factors of PFS and OS in sorafenib-treated HCC. In a multivariate analysis, VEGF-A rs2010963 and VEGF-C rs4604006 were independent factors influencing PFS (HR = 0.25, 95% CI: 0.19–1.02, $p = 0.0376$ and HR = 0.22, 95% CI: 0.14–0.81, $p = 0.004$ , respectively) and OS (HR = 0.28, 95% CI: 0.23–0.96, $p = 0.02$ and HR = 0.25, 95% CI: 0.17–0.99, $p = 0.04$ , respectively).	[36]
Ang-2	Pooled analysis	Negative predictive outcome in HCC patients with high Ang-2 serum levels before sorafenib (HR = 2.51, 95% CI: 1.01–6.57, $p = 0.048$ )	[37]
SNP for ANGPT2	Observational analysis	rs55633437 GG genotype showed a significantly longer PFS ( $p < 0.001$ ) and OS ( $p < 0.001$ ) than those with the other genotypes (GT+TT).	[38]
eNOS polymorphisms	Observational analysis	In univariate and multivariate analyses, a training cohort of HCC patients homozygous for endothelial nitric oxide synthase (eNOS) haplotype (HT1:T-4b at eNOS-786/eNOS VNTR) had a worse mPFS (2.6 months vs. 5.8 months, HR = 5.43, 95% CI: 2.46–11.98, $p < 0.0001$ ) and OS (3.2 months vs. 14.6 months, HR = 2.35, 95% CI: 1.12–4.91, $p = 0.024$ ) when compared with other haplotypes.	[39]

Table A1. Cont.

Clinical/Biological Biomarker	Study Model	Mechanisms/Results	Reference
HIF-1 $\alpha$ /SNPs of HIF-1 $\alpha$ , VEGF, and Ang2	Observational analysis	The multivariate analysis demonstrated that rs12434438 (SNP of HIF-1 $\alpha$ ), rs2010963 (SNP of VEGF-A), and rs4604006 (SNP of VEGF-C) were independent factors and were predictive biomarkers of the sorafenib response.	[40]
NRL	Observational analysis	NRL $\geq$ 2.3 was a negative predictive biomarker of the sorafenib response in both univariate and multivariate environments ( $p = 0.005$ and HR 1.72, 95% CI: 1.03–2.71, respectively)	[46]
NLR	Meta-analysis	High NLR before any treatment was predictive of a short mOS (HR: 1.54, 95% CI: 1.34 to 1.76, $p < 0.001$ )	[48]
PLR	Meta-analysis	Increase of PLR predicted an unfavorable outcome in terms of mOS (HR: 1.63, 95% CI: 1.34 to 1.98, $p < 0.001$ ).	[48]
SII, NLR, and PLR	Multicenter case series	Patients treated with sorafenib and with SII $\geq$ 360 showed poorer survival outcomes compared to patients with SII $<$ 360 in terms of mPFS (2.6 months vs. 3.9 months, respectively, $p < 0.026$ ) and mOS (5.6 months vs. 13.9 months, respectively, $p = 0.027$ ). Patients with NLR $\geq$ 3 compared with those with NLR $<$ 3, had a lower mPFS (2.6 months vs. 3.3 months, $p < 0.049$ ) but no significant data were reported in terms of mOS (5.6 months vs. 13.9 months, $p = 0.062$ )	[49]
IGF-1	Observational analysis	Patients with high (i.e., levels $\geq$ the median level) baseline IGF-1 levels achieved a significantly higher disease control rate (DCR) when treated with anti-angiogenic therapies (including sorafenib) than those with low levels (71% vs. 39%, respectively— $p = 0.003$ ). Patients with high IGF-1 levels, when compared with those with low levels showed longer mPFS (4.3 months vs. 1.9 months, respectively— $p = 0.014$ ) and mOS (10.7 months vs. 3.9 months, respectively— $p = 0.009$ ).	[53]
FGF3/FGF4 amplification	Observational analysis	FGF3/FGF4 amplification was observed in 30% of HCC samples while it was not seen in 38 non-responsive patients ( $p = 0.006$ ).	[54]
TGF $\alpha$ /PECAM1 and NRG1 gene	Observational analysis	TGF $\alpha$ and PECAM1 gene expression levels were significantly increased in non-PD patients. Moreover, mPFS of patients with high and low NRG1 expressions were 80 days and 90 days in sorafenib responding patients, respectively ( $p = 0.0497$ ). So far, high TGF $\alpha$ and PECAM1 and low NRF1 gene levels could be predictors of response to sorafenib.	[55]
miRNA181a-5p	Observational analysis	The miRNA181a-5p levels resulted in the unique independent factor for sorafenib-treated patients achieving a DCR in 53 patients (HR 0.139, 95% CI 0.011–0.658, $p = 0.0092$ )	[65]
miRNA423-5p	In vivo/in vitro study	Sorafenib upregulated both in vitro and in vivo and its increase from baseline to evaluation at 6 months correlated with the response. In fact, 75% of patients with the miR423-5p level increase achieved a disease control.	[66]

Table A1. Cont.

Clinical/Biological Biomarker	Study Model	Mechanisms/Results	Reference
miRNA-126-3p	In vivo/in vitro study	MiR-126-3p was down-regulated after sorafenib treatment in HCC celllines. Circulating miR-126-3p expression levels were significantly higher in HCC patients when compared with control subjects (26.7 vs. 26.6 mean expression levels; $p$ -value=0.0002).	[67]
miRNA10b-3p	Exploratory study	MiRNA10b-3p expression levels were significantly higher (fold increase = 5.8) in the subgroup of HCC patients with worse OS ( $p = 0.008$ ) and with a putative prediction of short survival of sorafenib-treated patients	[68]
miRNA-224	Exploratory study	High levels of HCC samples were correlated with an increase of PFS (HR = 0.28, 95% CI: 0.09–0.92, $p = 0.029$ ) and OS (HR = 0.024, 95% CI: 0.07–0.79, $p = 0.012$ ) in patients treated with sorafenib [69].	[69]
miR-425-3p	Exploratory study	Patients with high levels of miR-425-3p in HCC tissue treated with sorafenib achieved a better PFS (HR = 0.5, 95% CI: 0.3–0.9, $p = 0.007$ ) and TTP (HR = 0.4, 95% CI: 0.2–0.7, $p = 0.0008$ )	[70]

Abbreviations: Ang: angiopoietin; AFP: alpha fetoprotein; BCLC: Barcelona Clinic Liver Cancer; CI: confidence interval; CP: Child-Pugh; DCR: disease control rate; eNOS: endothelial nitric oxide synthase; ERK: extracellular signal-regulated kinase; FGFR1: fibroblast growth factor receptors1; FGF: fibroblast growth factor; HBC: hepatitis B virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma; HIF: hypoxia-inducible factor; HFSR: Hand-foot skin reaction; IGF: insulin growth factor; MEK: mitogen-activated protein kinase; microRNA: miR-; mOS: median Overall Survival; mPFS: median Progression free Survival; NRG1: neuregulin 1; NLR: neutrophils/lymphocytes ratio; ORR: overall response rate; SNP: single nucleotide polymorphism; PECAM1: Platelet And Endothelial Cell Adhesion Molecule 1; PGFR: platelet-derived growth factor receptors; PLR: platelet/lymphocytes ratio; SII: systemic immune-inflammation; TGF: transforming growth factor ; VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor;.

## References

- Chen, C.; Wang, G. Mechanisms of hepatocellular carcinoma and challenges and opportunities for molecular targeted therapy. *World J. Hepatol.* **2015**, *7*, 1964–1970. [[CrossRef](#)] [[PubMed](#)]
- Llovet, J.M.; Ricci, S.; Mazzaferro, V.; Hilgard, P.; Gane, E.; Blanc, J.F.; de Oliveira, A.C.; Santoro, A.; Raoul, J.L.; Forner, A.; et al. Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* **2008**, *359*, 378–390. [[CrossRef](#)] [[PubMed](#)]
- Cheng, A.L.; Kang, Y.K.; Chen, Z.; Tsao, C.J.; Qin, S.; Kim, J.S.; Luo, R.; Feng, J.; Ye, S.; Yang, T.S.; et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* **2009**, *10*, 25–34. [[CrossRef](#)]
- Faivre, S.; Raymond, E.; Boucher, E.; Douillard, J.; Lim, H.Y.; Kim, J.S.; Zappa, M.; Lanzalone, S.; Lin, X.; Deprimo, S.; et al. Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: An open-label, multicentre, phase II study. *Lancet Oncol.* **2009**, *10*, 794–800. [[CrossRef](#)]
- Cainap, C.; Qin, S.; Huang, W.T.; Chung, I.J.; Pan, H.; Cheng, Y.; Kudo, M.; Kang, Y.K.; Chen, P.J.; Toh, H.C.; et al. Lenvatinib versus Sorafenib in patients with advanced hepatocellular carcinoma: Results of a randomized phase III trial. *J. Clin. Oncol.* **2015**, *33*, 172–179. [[CrossRef](#)] [[PubMed](#)]
- Johnson, P.J.; Qin, S.; Park, J.W.; Poon, R.T.; Raoul, J.L.; Philip, P.A.; Hsu, C.H.; Hu, T.H.; Heo, J.; Xu, J.; et al. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: Results from the randomized phase III BRISK-FL study. *J. Clin. Oncol.* **2013**, *31*, 3517–3524. [[CrossRef](#)]
- Llovet, J.M.; Hernandez-Gea, V. Hepatocellular carcinoma: Reasons for phase III failure and novel perspectives on trial design. *Clin. Cancer Res.* **2014**, *20*, 2072–2079. [[CrossRef](#)]
- Kudo, M.; Finn, R.S.; Qin, S.; Han, K.H.; Ikeda, K.; Piscaglia, F.; Baron, A.; Park, J.W.; Han, G.; Jassem, J.; et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: A randomised phase 3 non-inferiority trial. *Lancet* **2018**, *391*, 1163–1173. [[CrossRef](#)]

9. Hollebecque, A.; Cattani, S.; Romano, O.; Sergent, G.; Mourad, A.; Louvet, A.; Dharancy, S.; Boleslawski, E.; Truant, S.; Pruvot, F.R.; et al. Safety and efficacy of sorafenib in hepatocellular carcinoma: The impact of the Child-Pugh score. *Aliment. Pharmacol. Ther.* **2011**, *34*, 1193–1201. [[CrossRef](#)]
10. Pressiani, T.; Boni, C.; Rimassa, L.; Labianca, R.; Fagioli, S.; Salvagni, S.; Ferrari, D.; Cortesi, E.; Porta, C.; Mucciari, C.; et al. Sorafenib in patients with Child-Pugh class A and B advanced hepatocellular carcinoma: A prospective feasibility analysis. *Ann. Oncol.* **2013**, *24*, 406–411. [[CrossRef](#)]
11. Marrero, J.A.; Kudo, M.; Venook, A.P.; Ye, S.L.; Bronowicki, J.P.; Chen, X.P.; Dagher, L.; Furuse, J.; Geschwind, J.H.; de Guevara, L.L.; et al. Observational registry of sorafenib use in clinical practice across Child-Pugh subgroups: The GIDEON study. *J. Hepatol.* **2016**, *65*, 1140–1147. [[CrossRef](#)] [[PubMed](#)]
12. Iavarone, M.; Cabibbo, G.; Piscaglia, F.; Zavaglia, C.; Grieco, A.; Villa, E.; Cammà, C.; Colombo, M.; SOFIA (SOraFenib Italian Assessment) study group. Field-practice study of sorafenib therapy for hepatocellular carcinoma: A prospective multicenter study in Italy. *Hepatology* **2011**, *54*, 2055–2063. [[CrossRef](#)] [[PubMed](#)]
13. Bruix, J.; Cheng, A.L.; Meinhardt, G.; Nakajima, K.; De Sanctis, Y.; Llovet, J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: Analysis of two phase III studies. *J. Hepatol.* **2017**, *67*, 999–1008. [[CrossRef](#)]
14. Park, J.; Cho, J.; Lim, J.H.; Lee, M.H.; Kim, J. Relative Efficacy of Systemic Treatments for Patients with Advanced Hepatocellular Carcinoma According to Viral Status: A Systematic Review and Network Meta-Analysis. *Target. Oncol.* **2019**, *14*, 395–403. [[CrossRef](#)] [[PubMed](#)]
15. El-Serag, H.B.; Hampel, H.; Javadi, F. The association between diabetes and hepatocellular carcinoma: A systematic review of epidemiologic evidence. *Clin. Gastroenterol. Hepatol.* **2006**, *4*, 369–380. [[CrossRef](#)]
16. Gandini, S.; Puntoni, M.; Heckman-Stoddard, B.M.; Dunn, B.K.; Ford, L.; De Censi, A.; Szabo, E. Metformin and cancer risk and mortality: A systematic review and meta-analysis taking into account biases and confounders. *Cancer Prev. Res.* **2014**, *7*, 867–885. [[CrossRef](#)] [[PubMed](#)]
17. Jang, W.; Kim, M.S.; Lim, J.S.; Yoo, H.J.; Seo, Y.S.; Han, C.J.; Park, S.C.; Kay, C.S.; Kim, M.; Jang, H.S.; et al. Survival Advantage Associated with Metformin Usage in Hepatocellular Carcinoma Patients Receiving Radiotherapy: A Propensity Score Matching Analysis. *Anticancer Res.* **2015**, *35*, 5047–5054.
18. Casadei-Gardini, A.; Marisi, G.; Scarpi, E.; Scartozzi, M.; Faloppi, L.; Silvestris, N.; Masi, G.; Vivaldi, C.; Brunetti, O.; Tamberi, S.; et al. Effects of metformin on clinical outcome in diabetic patients with advanced HCC receiving sorafenib. *Expert Opin. Pharmacother.* **2015**, *16*, 2719–2725. [[CrossRef](#)]
19. Casadei-Gardini, A.; Faloppi, L.; De Matteis, S.; Foschi, F.G.; Silvestris, N.; Tovoli, F.; Palmieri, V.; Marisi, G.; Brunetti, O.; Vespasiani-Gentilucci, U.; et al. Metformin and insulin impact on clinical outcome in patients with advanced hepatocellular carcinoma receiving sorafenib: Validation study and biological rationale. *Eur. J. Cancer* **2017**, *86*, 106–114. [[CrossRef](#)]
20. Howell, J.; Pinato, D.J.; Ramaswami, R.; Bettinger, D.; Arizumi, T.; Ferrari, C.; Yen, C.; Gibbin, A.; Burlone, M.E.; Guaschino, G.; et al. On-target sorafenib toxicity predicts improved survival in hepatocellular carcinoma: A multi-centre, prospective study. *Aliment. Pharmacol. Ther.* **2017**, *45*, 1146–1155. [[CrossRef](#)]
21. Reig, M.; Torres, F.; Rodriguez-Lopez, C.; Forner, A.; Llovet, J.; Rimola, J.; Darnell, A.; Ríos, J.; Ayuso, C.; Bruix, J. Early dermatologic adverse events predict better outcome in HCC patients treated with sorafenib. *J. Hepatol.* **2014**, *61*, 318–324. [[CrossRef](#)] [[PubMed](#)]
22. Wang, P.; Tan, G.; Zhu, M.; Li, W.; Zhai, B.; Sun, X. Hand-foot skin reaction is a beneficial indicator of sorafenib therapy for patients with hepatocellular carcinoma: A systemic review and metaanalysis. *Expert Rev. Gastroenterol. Hepatol.* **2018**, *12*, 1–8. [[CrossRef](#)] [[PubMed](#)]
23. Shin, S.Y.; Lee, Y.J. Correlation of skin toxicity and hypertension with clinical benefit in advanced hepatocellular carcinoma patients treated with sorafenib. *Int. J. Clin. Pharmacol. Ther.* **2013**, *51*, 837–846. [[CrossRef](#)] [[PubMed](#)]
24. Otsuka, T.; Eguchi, Y.; Kawazoe, S.; Yanagita, K.; Ario, K.; Kitahara, K.; Kawasoe, H.; Kato, H.; Mizuta, T.; Saga Liver Cancer Study Group; et al. Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib. *Hepatol. Res.* **2012**, *42*, 879–886. [[CrossRef](#)] [[PubMed](#)]
25. Koschny, R.; Gotthardt, D.; Koehler, C.; Jaeger, D.; Stremmel, W.; Ganten, T.M. Diarrhea is a positive outcome predictor for sorafenib treatment of advanced hepatocellular carcinoma. *Oncology* **2013**, *84*, 6–13. [[CrossRef](#)]
26. Di Costanzo, G.G.; CasadeiGardini, A.; Marisi, G.; Foschi, F.G.; Scartozzi, M.; Granata, R.; Faloppi, L.; Cascinu, S.; Silvestris, N.; Brunetti, O.; et al. Validation of a Simple Scoring System to Predict Sorafenib Effectiveness in Patients with Hepatocellular Carcinoma. *Target. Oncol.* **2017**, *12*, 795–803. [[CrossRef](#)]



27. Silvestris, N.; Ciliberto, G.; De Paoli, P.; Apolone, G.; Lavitrano, M.L.; Pierotti, M.A.; Stanta, G.; On the behalf of the “dynamic medicine OECI group”. On the behalf of the “dynamic medicine OECI group”. Liquid dynamic medicine and N-of-1 clinical trials: A change of perspective in oncology research. *J. Exp. Clin. Cancer Res.* **2017**, *36*, 128. [[CrossRef](#)]
28. Forner, A.; Llovet, J.M.; Bruix, J. Hepatocellular carcinoma. *Lancet* **2012**, *379*, 1245–1255. [[CrossRef](#)]
29. Shao, Y.Y.; Lin, Z.Z.; Hsu, C.; Shen, Y.C.; Hsu, C.H.; Cheng, A.L. Early alpha-fetoprotein response predicts treatment efficacy of antiangiogenic systemic therapy in patients with advanced hepatocellular carcinoma. *Cancer* **2010**, *116*, 4590–4596. [[CrossRef](#)]
30. Sánchez, A.I.P.; Roces, L.V.; García, I.Z.; López, E.L.; Hernandez, M.A.C.; Parejo, M.I.B.; Peña-Díaz, J. Value of  $\alpha$ -fetoprotein as an early biomarker for treatment response to sorafenib therapy in advanced hepatocellular carcinoma. *Oncol. Lett.* **2018**, *15*, 8863–8870.
31. Nakazawa, T.; Hidaka, H.; Takada, J.; Okuwaki, Y.; Tanaka, Y.; Watanabe, M.; Shibuya, A.; Minamino, T.; Kokubu, S.; Koizumi, W. Early increase in  $\alpha$ -fetoprotein for predicting unfavorable clinical outcomes in patients with advanced hepatocellular carcinoma treated with sorafenib. *Eur. J. Gastroenterol Hepatol.* **2013**, *25*, 683–689. [[CrossRef](#)] [[PubMed](#)]
32. Longo, V.; Brunetti, O.; Gnoni, A.; Cascinu, S.; Gasparini, G.; Lorusso, V.; Ribatti, D.; Silvestris, N. Angiogenesis in pancreatic ductal adenocarcinoma: A controversial issue. *Oncotarget* **2016**, *7*, 58649–58658. [[CrossRef](#)] [[PubMed](#)]
33. Tsuchiya, K.; Asahina, Y.; Matsuda, S.; Muraoka, M.; Nakata, T.; Suzuki, Y.; Tamaki, N.; Yasui, Y.; Suzuki, S.; Hosokawa, T.; et al. Changes in plasma vascular endothelial growth factor at 8 weeks after sorafenib administration as predictors of survival for advanced hepatocellular carcinoma. *Cancer* **2014**, *120*, 229–237. [[CrossRef](#)]
34. Horwitz, E.; Stein, I.; Andreozzi, M.; Nemeth, J.; Shoham, A.; Pappo, O.; Schweitzer, N.; Tornillo, L.; Kanarek, N.; Quagliata, L.; et al. Human and mouse VEGFA-amplified hepatocellular carcinomas are highly sensitive to sorafenib treatment. *Cancer Discov.* **2014**, *4*, 730–743. [[CrossRef](#)] [[PubMed](#)]
35. Oh, C.R.; Kong, S.Y.; Im, H.S.; Kim, H.J.; Kim, M.K.; Yoon, K.A.; Cho, E.H.; Jang, J.H.; Lee, J.; Kang, J.; et al. Genome-wide copy number alteration and VEGFA amplification of circulating cell-free DNA as a biomarker in advanced hepatocellular carcinoma patients treated with Sorafenib. *BMC Cancer* **2019**, *19*, 292. [[CrossRef](#)]
36. Scartozzi, M.; Faloppi, L.; Svegliati Baroni, G.; Loretelli, C.; Piscaglia, F.; Iavarone, M.; Toniutto, P.; Fava, G.; De Minicis, S.; Mandolesi, A.; et al. VEGF and VEGFR genotyping in the prediction of clinical outcome for HCC patients receiving sorafenib: The ALICE-1 study. *Int. J. Cancer* **2014**, *135*, 1247–1256. [[CrossRef](#)]
37. Miyahara, K.; Nouse, K.; Tomoda, T.; Kobayashi, S.; Hagihara, H.; Kuwaki, K.; Toshimori, J.; Onishi, H.; Ikeda, F.; Miyake, Y.; et al. Predicting the treatment effect of sorafenib using serum angiogenesis markers in patients with hepatocellular carcinoma. *J. Gastroenterol. Hepatol.* **2011**, *26*, 1604–1611. [[CrossRef](#)]
38. Marisi, G.; Petracci, E.; Raimondi, F.; Faloppi, L.; Foschi, F.G.; Lauletta, G.; Iavarone, M.; Canale, M.; Valgiusti, M.; Neri, L.M.; et al. ANGPT2 and NOS3 Polymorphisms and Clinical Outcome in Advanced Hepatocellular Carcinoma Patients Receiving Sorafenib. *Cancers* **2019**, *11*, 1023. [[CrossRef](#)]
39. Casadei-Gardini, A.; Marisi, G.; Faloppi, L.; Scarpi, E.; Foschi, F.G.; Iavarone, M.; Lauletta, G.; Corbelli, J.; Valgiusti, M.; Facchetti, F.; et al. eNOS polymorphisms and clinical outcome in advanced HCC patients receiving sorafenib: Final results of the ePHAS study. *Oncotarget* **2016**, *7*, 27988–27999.
40. Faloppi, L.; Casadei Gardini, A.; Masi, G.; Silvestris, N.; Loretelli, C.; Ulivi, P.; Bianconi, M.; Gianpieri, R.; Bittoni, A.; Andrikou, K.; et al. Angiogenesis polymorphisms profile in the prediction of clinical outcome of advanced HCC patients receiving sorafenib: Combined analysis of VEGF and HIF-1 $\alpha$ . Final results of the ALICE-2 study. *J. Clin. Oncol.* **2016**, *34* (Suppl. 4), 280. [[CrossRef](#)]
41. Casadei-Gardini, A.; Faloppi, L.; Aprile, G.; Brunetti, O.; Caparello, C.; Corbelli, J.; Chessa, L.; Bruno, D.; Ercolani, G.; Leonetti, A.; et al. Multicenter Prospective Study of Angiogenesis Polymorphism Validation in HCC Patients Treated with Sorafenib. An INNOVATE Study Protocol. *Tumori. J.* **2017**, *104*, 476–479. [[CrossRef](#)] [[PubMed](#)]
42. Longo, V.; Gnoni, A.; Casadei-Gardini, A.; Pisconti, S.; Licchetta, A.; Scartozzi, M.; Memeo, R.; Palmieri, V.; Aprile, G.; Santini, D.; et al. Immunotherapeutic approaches for hepatocellular carcinoma. *Oncotarget* **2017**, *8*, 33897–33910. [[CrossRef](#)] [[PubMed](#)]

43. Basile, D.; Garattini, S.K.; Bonotto, M.; Ongaro, E.; Casagrande, M.; Cattaneo, M.; Fanotto, V.; De Carlo, E.; Loupakis, F.; Urbano, F.; et al. Immunotherapy for colorectal cancer: Where are we heading? *Expert Opin. Biol. Ther.* **2017**, *17*, 709–721. [[CrossRef](#)] [[PubMed](#)]
44. FarajzadehValilou, S.; Keshavarz-Fathi, M.; Silvestris, N.; Argentiero, A.; Rezaei, N. The role of inflammatory cytokines and tumor associated macrophages (TAMs) in microenvironment of pancreatic cancer. *Cytokine Growth Factor Rev.* **2018**, *39*, 46–61. [[CrossRef](#)] [[PubMed](#)]
45. Hu, B.; Yang, X.R.; Xu, Y.; Sun, Y.F.; Sun, C.; Guo, W.; Zhang, X.; Wang, W.M.; Qiu, S.J.; Zhou, J.; et al. Systemic immune-inflammationindex predicts prognosis of patients after curative resection for hepatocellular carcinoma. *Clin. Cancer Res.* **2014**, *20*, 6212–6222. [[CrossRef](#)]
46. Lué, A.; Serrano, M.T.; Bustamante, F.J.; Iñarrairaegui, M.; Arenas, J.I.; Testillano, M.; Lorente, S.; Gil, C.; de la Torre, M.; Gomez, A.; et al. Neutrophil-to-lymphocyte ratio predicts survival in European patients with hepatocellular carcinoma administered sorafenib. *Oncotarget* **2017**, *8*, 103077–103086. [[CrossRef](#)]
47. Hong, Y.M.; Yoon, K.T.; Hwang, T.H.; Heo, J.; Woo, H.Y.; Cho, M. Changes in the neutrophil-to-lymphocyte ratio predict the prognosis of patients with advanced hepatocellular carcinoma treated with sorafenib. *Eur. J. Gastroenterol. Hepatol.* **2019**, *31*, 1250–1255. [[CrossRef](#)]
48. Zheng, J.; Cai, J.; Li, H.; Zeng, K.; He, L.; Fu, H.; Zhang, J.; Chen, L.; Yao, J.; Zhang, Y.; et al. Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio as Prognostic Predictors for Hepatocellular Carcinoma Patients with Various Treatments: A Meta-Analysis and Systematic Review. *Cell Physiol. Biochem.* **2017**, *44*, 967–981. [[CrossRef](#)]
49. Casadei-Gardini, A.; Scarpi, E.; Faloppi, L.; Scartozzi, M.; Silvestris, N.; Santini, D.; de Stefano, G.; Marisi, G.; Negri, F.V.; Foschi, F.G.; et al. Immune inflammation indicators and implication for immune modulation strategies in advanced hepatocellular carcinoma patients receiving sorafenib. *Oncotarget* **2016**, *7*, 67142–67149.
50. Xu, D.; Xu, M.; Jeong, S.; Qian, Y.; Wu, H.; Xia, Q.; Kong, X. The Role of Nrf2 in liver disease: Novel molecular mechanisms and therapeutic approaches. *Front. Pharmacol.* **2019**, *9*, 1428. [[CrossRef](#)]
51. Raghunath, A.; Sundarraj, K.; Arfuso, F.; Sethi, G.; Perumal, E. Dysregulation of Nrf2 in hepatocellular carcinoma: Role in cancer progression and chemoresistance. *Cancers* **2018**, *10*, 481. [[CrossRef](#)] [[PubMed](#)]
52. Zhou, S.; Ye, W.; Duan, X.; Zhang, M.; Wang, J. The noncytotoxic dose of sorafenib sensitizes Bel-7402/5-FU cells to 5-FU by down-regulating 5-FU-induced Nrf2 expression. *Dig. Dis. Sci.* **2013**, *58*, 1615–1626. [[CrossRef](#)] [[PubMed](#)]
53. Shao, Y.Y.; Huang, C.C.; Lin, S.D.; Hsu, C.H.; Cheng, A.L. Serum insulinlike growth factor-1 levels predict outcomes of patients with advanced hepatocellular carcinoma receiving antiangiogenic therapy. *Clin. Cancer Res.* **2012**, *18*, 3992–3997. [[CrossRef](#)] [[PubMed](#)]
54. Arao, T.; Ueshima, K.; Matsumoto, K.; Nagai, T.; Kimura, H.; Hagiwara, S.; Sakurai, T.; Haji, S.; Kanazawa, A.; Hidaka, H.; et al. FGF3/FGF4 amplification and multiple lung metastases in responders to sorafenib in hepatocellular carcinoma. *Hepatology* **2013**, *57*, 1407–1415. [[CrossRef](#)] [[PubMed](#)]
55. Sakai, K.; Takeda, H.; Nishijima, N.; Orito, E.; Joko, K.; Uchida, Y.; Izumi, N.; Nishio, K.; Osaki, Y. Targeted DNA and RNA sequencing of fine-needle biopsy FFPE specimens in patients with unresectable hepatocellular carcinoma treated with sorafenib. *Oncotarget* **2015**, *6*, 21636–21644. [[CrossRef](#)] [[PubMed](#)]
56. Santini, D.; Spoto, C.; Loupakis, F.; Vincenzi, B.; Silvestris, N.; Cremolini, C.; Canestrari, E.; Graziano, F.; Galluccio, N.; Salvatore, L.; et al. High concordance of BRAF status between primary colorectal tumours and related metastatic sites: Implications for clinical practice. *Ann. Oncol.* **2010**, *21*, 1565. [[CrossRef](#)] [[PubMed](#)]
57. Franck, C.; Rosania, R.; Franke, S.; Haybaeck, J.; Canbay, A.; Venerito, M. The BRAF Status May Predict Response to Sorafenib in Gastrointestinal Stromal Tumors Resistant to Imatinib, Sunitinib, and Regorafenib: Case Series and Review of the Literature. *Digestion* **2019**, *99*, 179–184. [[CrossRef](#)]
58. Casadei-Gardini, A.; Chiadini, E.; Faloppi, L.; Marisi, G.; Delmonte, A.; Scartozzi, M.; Loretelli, C.; Lucchesi, A.; Oboldi, D.; Dubini, A.; et al. Efficacy of sorafenib in BRAF-mutated non-small-cell lung cancer (NSCLC) and no response in synchronous BRAF wild type-hepatocellular carcinoma: A case report. *BMC Cancer* **2016**, *16*, 429. [[CrossRef](#)]
59. Nedaenia, R.; Manian, M.; Jazayeri, M.H.; Ranjbar, M.; Salehi, R.; Sharifi, M.; Mohaghegh, F.; Goli, M.; Jahednia, S.H.; Avan, A.; et al. Circulating exosomes and exosomal microRNAs as biomarkers in gastrointestinal cancer. *Cancer Gene Ther.* **2017**, *24*, 48–56. [[CrossRef](#)]

60. Brunetti, O.; Russo, A.; Scarpa, A.; Santini, D.; Reni, M.; Bittoni, A.; Azzariti, A.; Aprile, G.; Delcuratolo, S.; Signorile, M.; et al. MicroRNA in pancreatic adenocarcinoma: Predictive/prognostic biomarkers or therapeutic targets? *Oncotarget* **2015**, *6*, 23323–23341. [[CrossRef](#)]
61. Gnoni, A.; Santini, D.; Scartozzi, M.; Russo, A.; Licchetta, A.; Palmieri, V.; Lupo, L.; Faloppi, L.; Palasciano, G.; Memeo, V.; et al. Hepatocellular carcinoma treatment over sorafenib: Epigenetics, microRNAs and microenvironment. Is there a light at the end of the tunnel? *Expert Opin. Ther. Targets* **2015**, *19*, 1623–1635. [[CrossRef](#)] [[PubMed](#)]
62. He, C.; Dong, X.; Zhai, B.; Jiang, X.; Dong, D.; Li, B.; Jiang, H.; Xu, S.; Sun, X. MiR-21 mediates sorafenib resistance of hepatocellular carcinoma cells by inhibiting autophagy via the PTEN/Akt pathway. *Oncotarget* **2015**, *6*, 28867–28881. [[CrossRef](#)]
63. Xu, Y.; Huang, J.; Ma, L.; Shan, J.; Shen, J.; Yang, Z.; Liu, L.; Luo, Y.; Yao, C.; Qian, C. MicroRNA-122 confers sorafenib resistance to hepatocellular carcinoma cells by targeting IGF-1R to regulate RAS/RAF/ERK signaling pathways. *Cancer Lett.* **2016**, *371*, 171–181. [[CrossRef](#)] [[PubMed](#)]
64. Turato, C.; Fornari, F.; Pollutri, D.; Fassan, M.; Quarta, S.; Villano, G.; Ruvoletto, M.; Bolondi, L.; Gramantieri, L.; Pontisso, P. MiR-122 Targets SerpinB3 and Is Involved in Sorafenib Resistance in Hepatocellular Carcinoma. *J. Clin. Med.* **2019**, *8*, 171. [[CrossRef](#)] [[PubMed](#)]
65. Nishida, N.; Arizumi, T.; Hagiwara, S.; Ida, H.; Sakurai, T.; Kudo, M. MicroRNAs for the Prediction of Early Response to Sorafenib Treatment in Human Hepatocellular Carcinoma. *Liver Cancer* **2017**, *6*, 113–125. [[CrossRef](#)] [[PubMed](#)]
66. Stiuso, P.; Potenza, N.; Lombardi, A.; Ferrandino, I.; Monaco, A.; Zappavigna, S.; Vanacore, D.; Mosca, N.; Castiello, F.; Porto, S.; et al. MicroRNA-423-5p Promotes Autophagy in Cancer Cells and Is Increased in Serum From Hepatocarcinoma Patients Treated with Sorafenib. *Mol. Ther. Nucleic Acids* **2015**, *4*, e233. [[CrossRef](#)] [[PubMed](#)]
67. Faranda, T.; Grossi, I.; Manganelli, M.; Marchina, E.; Baiocchi, G.; Portolani, N.; Crosatti, M.; De Petro, G.; Salvi, A. Differential expression profiling of long non-coding RNA GAS5 and miR-126-3p in human cancer cells in response to sorafenib. *Sci. Rep.* **2019**, *9*, 9118. [[CrossRef](#)]
68. Yoon, E.L.; Yeon, J.E.; Ko, E.; Lee, H.J.; Je, J.H.; Yoo, Y.J.; Kang, S.H.; Suh, S.J.; Kim, J.H.; Seo, Y.S.; et al. An Explorative Analysis for the Role of Serum miR-10b-3p Levels in Predicting Response to Sorafenib in Patients with Advanced Hepatocellular Carcinoma. *J. Korean Med. Sci.* **2017**, *32*, 212–220. [[CrossRef](#)]
69. Gyöngyösi, B.; Végh, É.; Járay, B.; Székely, E.; Fassan, M.; Bodoky, G.; Schaff, Z.; Kiss, A. Pretreatment MicroRNA Level and Outcome in Sorafenib-treated Hepatocellular Carcinoma. *J. Histochem. Cytochem.* **2014**, *62*, 547–555. [[CrossRef](#)]
70. Vaira, V.; Roncalli, M.; Carnaghi, C.; Favarsani, A.; Maggioni, M.; Augello, C.; Rimassa, L.; Pressiani, T.; Spagnuolo, G.; Di Tommaso, L.; et al. MicroRNA-425-3p predicts response to sorafenib therapy in patients with hepatocellular carcinoma. *Liver Int.* **2015**, *35*, 1077–1086. [[CrossRef](#)]
71. Kim, H.Y.; Lee, D.H.; Lee, J.H.; Cho, Y.Y.; Cho, E.J.; Yu, S.J.; Kim, Y.J.; Yoon, J.H. Novel biomarker-based model for the prediction of sorafenib response and overall survival in advanced hepatocellular carcinoma: A prospective cohort study. *BMC Cancer* **2018**, *18*, 307. [[CrossRef](#)] [[PubMed](#)]
72. Gavini, J.; Dommann, N.; Jakob, M.O.; Keogh, A.; Bouchez, L.C.; Karkampouna, S.; Julio, M.K.; Medova, M.; Zimmer, Y.; Schläfli, A.M.; et al. Verteporfin-induced lysosomal compartment dysregulation potentiates the effect of sorafenib in hepatocellular carcinoma. *Cell Death Dis.* **2019**, *10*, 749. [[CrossRef](#)] [[PubMed](#)]
73. Kelley, R.K.; Gane, E.; Assenat, E.; Siebler, J.; Galle, P.R.; Merle, P.; Hourmand, I.O.; Cleverly, A.; Zhao, Y.; Gueorguieva, I.; et al. A phase 2 study of Galunisertib (TGF- $\beta$ 1 Receptor Type I Inhibitor) and Sorafenib in patients with advanced hepatocellular carcinoma. *Clin. Transl. Gastroenterol.* **2019**, *7*, e00056. [[CrossRef](#)] [[PubMed](#)]
74. Abou-Alfa, G.K.; Miksad, R.A.; Tejani, M.A.; Williamson, S.; Gutierrez, M.E.; Olowokure, O.O.; Sharma, M.R.; El Dika, I.; Sherman, M.L.; Pandya, S.S. A phase Ib, open-label study of Dalantercept, an Activin Receptor-Like Kinase 1 Ligand Trap, plus Sorafenib in advanced hepatocellular carcinoma. *Oncologist* **2019**, *24*, 161.e70. [[CrossRef](#)]

