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Review

Predictive and prognostic factors in HCC patients treated with Sorafenib

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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, currently 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; predicitve factors.

1. Introduction

Sorafenib (NEXAVAR®) is a small molecule classified as an oral multi-targeted tyrosine kinase inhibitor (TKI) since it inhibits PDGFR, VEGF-R, KIT, and FGFR1. As is known, this TKI impares angiogenesis, cancer proliferation, and cell apoptosis [1].

In 2007 the FDA approved this drug in unresectable and advanced HCC according to the results of 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 a mOS significantly higher 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 performed subsequently and comparing other TKIs compared with sorafenib didn't report an improvement in terms of clinical outcomes [4-6]. In particular, sunitinib [4] and linifanib [5] didn't 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 vs9.5 months p > 0.05) [6].

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

Only lenvatinib showed similar results in terms of survival in a non-inferiority randomized trial, with a 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 to sorafenib according to clinical or biological predictive and/or prognostic markers?

2. Clinical predictive/prognostic markers

Although it is an expected situation, *Barcelona Clinic Liver Cancer (BCLC) staging* and *Child-Pugh (CP)* cirrhosis classifications resemble the most important criteria for HCC patients selection suitable of treatment with sorafenib. In the registrative trials [2-3], almost all enrolled patients were CP-A. Correlation between CP and 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 mOSs of 10.0 months and 3.8 months, respectively (P < 0.001). GIDEON trial [11], an observational registry study evaluated the survival and safety of sorafenib in 1968 and 666 patients with CP-A and CP-B status, respectively. mOSs 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.

Also, BCLC has been evaluated as clinical predictive criteria of 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 with 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, demonstrating that BCLC B had a better survival than those with BCLC C HCC patients (HR = 1.59; P = 0.02), confirming the predictive role of BCLC staging.

Since *HBV* and *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. Interestingly, in a more recent meta-analysis on sorafenib and lenvatinib trials, lenvatinib resembled the best agent for both HBV and HCV infected patients, presenting a more favorable HR versus sorafenib treated HCC (HR 0.83, 95% CI 0.68-1.01 and HR 0.91, 95% CI 0.66-1.25, respectively) [14].

Recently, *diabetes* and *use of oral antidiabetics* have been analyzed in HCC patients. Diabetes mellitus has been acquired as a risk factor for 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 didn't confirm the prognostic role of diabetes with mOSs of 9 months and 10 months in HCC non diabetic and diabetic patients, respectively (P = .535). Furthermore, mTTP was longer in diabetic patients (P = .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 (2-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 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.

Interestingly, among clinical predictive markers, several studies displayed a positive correlation in HCC patients between survival and *adverse events due to sorafenib*. mOS of 634 HCC patients who presented any grade of toxicities related to sorafenib (HFSR, hypertension, diarrhea) mOS was significantly improved compared to patients without adverse events (8.8 months vsvs5.4 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 compared to 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 metanalysis of 12 cohort studies including 1017 patients conformed the significant correlation between HFSR and response to sorafenib (pooled HR for mOS of 0.45; 95% CI 0.36, 0.55; P < 0.00001; I2 = 35%) and TTP of 0.41 (95% CI 0.28, 0.60; P < 0.00001; I2 = 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 the beginning of 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).

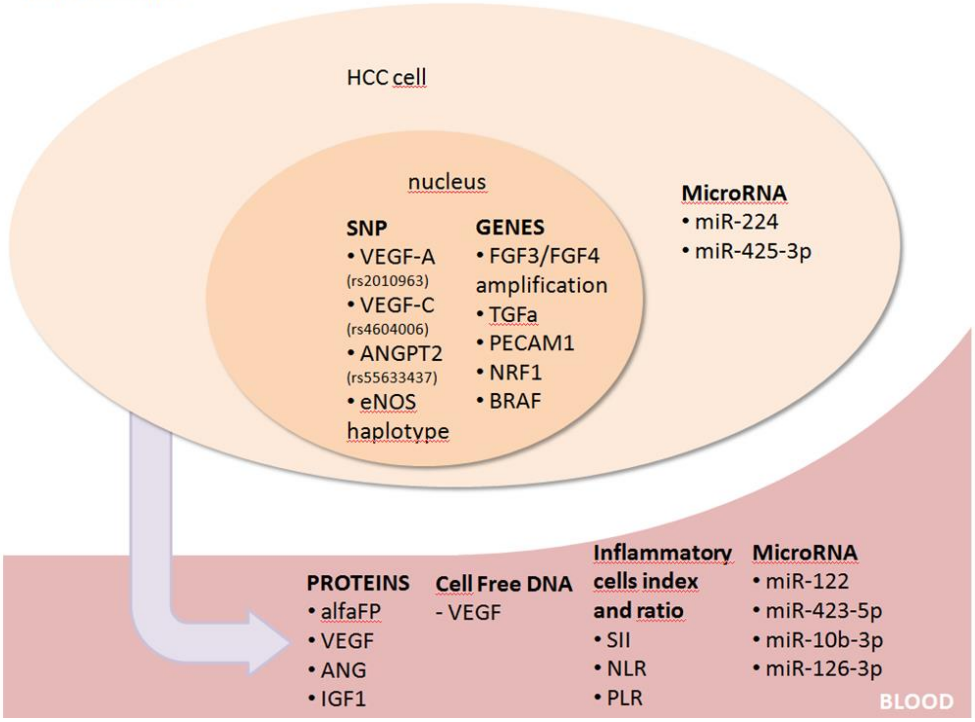
Regarding *diarrhea*, Koschny R et al [25] demonstrated a significant correlation between the grade of this symptom and mOS (grade 2-3 vs 0-1: 11.8 months vs4.2 months - 95% CI 6.9-16.6 vs 95% CI 0.0-9.1, respectively; p = 0.009).

In a large multicentric 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 of 15.1 months (95% CI 12.9–17.3) and 7.5 months (95% CI 5.9–9.2) (both p < 0.001), respectively. mOSs 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 score 0, 1, 2, and 3, respectively (p < 0.001).

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 putative response to target therapy, also in HCC patients treated with sorafenib (Figure 1).

Figure 1. Potential predictive/prognostic molecular markers in HCC patients treated with sorafenib



A graphical representation of biological prognostic/predictive factors analysed in HCC patients treated with sorafenib.

High alpha-fetoprotein (AFP) values are observed in about half of HCC patients, so far it is still the principal serological biomarker used for the management of this malignancy, even if it is elevated in cirrhotic patients as well [28]. In the SHARP trial, 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]. Shaoet al. [29] defined as early AFP responder patients those with a reduction of more than 20% from baseline of serum levels after 2 to 4 weeks of treatment. Responders were compared with non responders with a significantly improved ORR (33% vs 8%;P=.037) and disease control rate (DCR) (83% vs 35%;P=.002), respectively. Moreover, mPFSs were 7.5 months vs 1.9 months (P=.001) and mOSs 15.3 months vs 4.1 months (P=.019) for responder and non responder, respectively. Sanchez et al. [30] demonstrated that a more than 20% decrease of AFP at 6-8 weeks from baseline was 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 responder and non-responders patients, respectively. Furthermore, Nakazawa et al. [31] defined increase in AFP when its serum levels was 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.

Angiogenetic markers have been analyzed in several studies, since angiogenesis represented 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 resulted a predictive biomarker to sorafenib.

Tsuchiya et al. [33] revealed that a decrease of plasma VEGF concentrations with sorafenib treatment after 8 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 a better survival compared to non-amplified tumors [34]. Moreover, they verified the data on HCC serum of patients treated with sorafenib in vivo. So far, the 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).

In a recent study [35], the circulating cell free DNA (cfDNA) concentrations of VEGF were analized in HCC patients treated with sorafenib. Patients who underwent to progression disease with sorafenib had significantly higher cfDNA levels than the others (0.82 ng/μL vs 0.63 ng/μL; 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 the latter.

Also *Single Nucleotide Polymorphisms (SNPs)* of VEGF were analyzed. In a study by Scartozzi et al. [36], at 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. At 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, a Ang2 gene, 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 response to sorafenib should to 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 antiangiogenic biomarker. ALICE-2 study [40] evaluated the role of hypoxia-inducible factor 1-alpha (HIF-1α) and SNPs of HIF-1α, VEGF, and Ang2. The multivariate analysis demonstrated that rs12434438 (SNP of HIF-1α), rs2010963 (SNP of VEGF-A) and rs4604006 (SNP of VEGF-C) were independent factors and were predictive biomarkers of sorafenib response. Currently, a prospective ongoing study (INNOVATE) has the aim to confirm the role of SNPs of VEGF, HIF-1α, Ang-2 and eNOS SNPs in relation to treatment with sorafenib [41].

Also systemic inflammatory microenvironment have a strong correlation with angiogenesis, tumor invasion, and metastatization 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, inflammatory microenvironment and circulating immune cells and cytokines play a determinant role in HCC prognosis [45-47]. So far, Hu B et al. [45] used a systemic immune-inflammation (SII) index with the aim to predict 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. Later, Lue et al. [46] demonstrated that a neutrophil-lymphocyte ratio (NRL) ≥ 2.3 was a negative predictive biomarker of response to sorafenib in both univariate and multivariate (P = 0.005 and HR 1.72, 95% CI: 1.03-2.71, respectively) analyses 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 platelet-lymphocyte ratio (PLR) demonstrating that the increase of PLR predicted an unfavorable outcome in terms of mOS also (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 multicenter case series. They observed that patients treated with sorafenib and with SII ≥ 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 ≥ 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). So far, SII and NLR could represent predictive factors for patients with advanced HCC treated with sorafenib.

Another potential predictive factor is *Insulin-like Growth Factor (IGF)-1*. Eighty-three patients with high (i.e levels ≥ 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) [50]. 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.

Arao et al. analyzed a comparative genomic hybridization in frozen HCC samples of patients responsive to sorafenib [51]. 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 sorafenib response, a DNA and RNA sequencing using fine-needle biopsy was performed in 46 patients [52]. Intriguingly, comparisons of TGFa 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 PECAM1 gene expression levels of these two groups of patients (110.2 vs 13.2 median read number, respectively P = 0.0131) was performed. Both TGFa and PECAM1 gene expression levels were significantly increased in non-PD group. Moreover, mPFS of patients with high and low NRG1 expressions were 80 days and 90 days in sorafenib responder patients, respectively (P = 0.0497). So far, high TGFa and PECAM1 and low NRF1 gene levels should be predictor of response to sorefenib.

Although *B-type Raf kinase (BRAF) mutation* could play a role in the response to sorafenib. BRAF, a protein located downstream of the KRAS pathway, is implicated in the response to anti-EGFR treatment [53]. In a case series of advanced GIST [54], sorafenib was administrated to patients resistant to imatinib, sunitinib, and regorafenib. In these patients, BRAF was tested for mutations. So far, 2 BRAF wild-type patients achieved a long-term disease control (49 months and 19 months, respectively), while sorafenib-resistant patient carried a BRAF V600E mutation. Interestingly, in a report by Casadei Gardini et al. [55], a patient with a 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, shedding light on a possible negative prognostic role of this mutation.

Recently *micro RNAs (miRNAs)* achieved a key role in gastrointestinal cancers [56-58]. In particular, up/down-regulation of several miRNAs has been reported to be able to impair TKI response affecting the expression of genes involved in several pathways [59-60]. For example, miRNA-21 should enhance resistence to sorafenib in vitro through PTEN/Akt pathway through the inhibition of autophagy [61]. MicroRNA-122 get sorafenib resistance to HCC cell lines through RAS/RAF/ERK pathway [62]. Moreover, in an animal HCC model, elevated miR-122 levels were associated with a stem-like phenotype in HCC [63] associated with resistance to sorafenib. So far, an anti-miRNA122 transfection increased cell viability in sorafenib-treated HCC cells, restoring sorafenib activity HCCs. Predictive role of circulating miRNAs have been also investigated. miRNA181a-5p levels resulted the unique independent factor for sorefenib-treated patients achieving a DCR in 53 patients (HR 0.139, 95% CI 0.011-0.658, P = 0.0092) [64]. Furthermore, miR-181a-5p resulted the only independent factor in terms of OS in multivariate analysis (HR 0.267, 95%CI 0.070-

0.818, P = 0.0194) [64]. Interestingly, sorafenib upregulated MiRNA423-5p both in vitro and in vivo and its increase from baseline to evaluation at 6 months correlated with response. In fact, 75% of patients with miR423-5p level increase achieved a disease control [65]. Also MiR-126-3p was down-regulated after sorafenib treatment in HCC cells lines [66]. So far, Faranda et al. determinated 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±0.48 vs RQPT average=5.84±0.51; P-value=0.0074). Moreover, circulating miR-126-3p expression levels were significantly higher in HCC patients compared to control subjects (26.7 vs 26.6 mean expression levels; P-value=0.0002) [66]. In vitro data and in vivo determination lead Authors to hypothesize that a reduction of this miRNA could be predictive of response to sorafenib.

In an exploratory study [67], several miRNAs (miRNA10b-3p, miRNA18a, miRNA139-5p, miRNA21, miRNA224, miRNA221) were evaluated as predictive markers of 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) with a putative prediction of short survival of sorafenib-treated patients.

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 = 0.0.24, 95% CI: 0.07-0.79, P = 0.012) in patients treated with sorafenib [68]. 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) [69].

4. Conclusion

Nowaday, even if sorafenib still remains a gold quality standard in the first line treatment of advanced HCC patients, new targeted therapies and immnotherapies 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 drug. I ant case, we are still far from obtaining a panel useful for clinical practice. The efforts must be to identify a score [27, 70] able to include various variables useful for perfecting the therapeutic choice in HCC.

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Abbreviations: 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;

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