Sedky *et al. Egyptian Liver Journal* (2024) 14:57
https://doi.org/10.1186/s43066-024-00358-z

Egyptian Liver Journal

ORIGINAL RESEARCH ARTICLE

Open Access

Combined neurokinin-1 receptor antagonist and sorafenib is a promising approach for hepatocellular carcinoma therapy

Heba E. Sedky[1*], Yasmine N. Elwany[2], Eman S. El Alfy[2], Mona N. Elwany[3], Yasmin M. Nabil[4], Hazem F. Manna[4], Mohamed A. Abdelaziz[5*] and Wessam F. El Hadidy[1]

Abstract

Background HCC (Hepatocellular carcinoma) is the most common primary malignant cancer in the liver. Treatment options to incurable HCC such as sorafenib, an oral multikinase inhibitor, had numerous side effects and questionable effectiveness. Neurokinin-1 receptor (NK1R) have a major role in inflammation and tumour environment including the resistance to cell death, the induction of angiogenesis and the promotion of cell migration and proliferation. Additionally, NK-1R is over-expressed in human tumour cells including HCC. Moreover, Aprepitant, one of the NK-1R antagonists exerts multiple antitumor activities (antiproliferative, apoptotic, antimigration, and antiangiogenesis) in vivo and in vitro.

Study aim To analyze the effectiveness of combining sorafenib with aprepitant in the management of HCC (experimental).

Patients and methods In this retrospective experimental study, the human HCC cell line, HepG2, cells were exposed to increasing concentrations of sorafenib alone, aprepitant alone and combination of both sorafenib and aprepitant evaluation of cytotoxicity, apoptosis, MMP-9, VEGF, NF-kB p-65, p-AKT and p-ERK were done. Moreover, The extent of the NK-1 receptor expression was assessed by immunocytochemistry on 50 HCC paraffin blocks of Egyptian HCC patients and another 50 paraffin blocks of liver cirrhosis only as a control.

Results Decreased levels of MMP-9, VEGF, NF-kB p-65, p-AKT and p-ERK was more substantial in the combination therapy compared to sorafenib alone and aprepitant alone. Moreover, the rate of apoptosis and cytotoxicity were significantly higher in the combination treatment group than the monotherapy groups with more anti inflammatory, anti angiogentic and anti metastatic effects. Also, among the 50 HCC paraffin blocks, the majority (60%) showed a strong NK-1 expression; which significantly ($p < 0.05$) correlated with the progression free survival (PFS) but not the overall survival (OS) of the patients when applying multivariate analysis.

*Correspondence:
Heba E. Sedky
Heba_sedkey@alexu.edu.eg
Mohamed A. Abdelaziz
dradel2008@windowslive.com
[1] Pharmacology and Experimental Therapeutics Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
[2] Cancer Management and Research Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
[3] Pathology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
[4] Medical Biochemistry Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[5] Tropical Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
Full list of author information is available at the end of the article

Conclusion HCC had strong expression and immunostaining for NK1R.Therefore, combined aprepitant and sorafenib may be a promising approach in HCC treatment compared to each one alone.

Keywords HCC, Liver cirrhosis, NK-1 antagonist, Aprepitant, Sorafenib

Introduction

Liver cancer is estimated to account for 30.5% of cancer mortality globally and rank sixth in terms of incidence, with nearly 906,000 new cases and 830,200 deaths yearly [1]. The World Health Organization predicted that by 2040, liver cancer will claim the lives of more than 1.3 million individuals [2]. Moreover, hepatocellular carcinoma (HCC) is the most prevalent primary malignant liver tumor, accounting for 75–85% of cases [3].

Until now, there are few effective treatment options available for HCC, which is typically identified at an advanced stage [4]. When palliative care and systemic therapy are administered at an advanced stage, the median survival after diagnosis may reach 6 and 12 months [5, 6].

Besides immunotherapy, sorafenib, an oral multikinase inhibitor, is one of the recommended courses of therapy for advanced, incurable HCC [7]. It functions by blocking the activity of multiple tyrosine kinases implicated in the formation and advancement of tumor angiogenesis, such as FMS-like tyrosine kinase 3 (Flt3), c-Kit, platelet-derived growth factor receptor (PDGF-R), vascular endothelial growth factor receptor (VEGFR-2/3), and Raf kinase participating in the mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK) pathway, which plays a part in the proliferation and survival of the tumor [8]. Costly side effects are a common feature of sorafenib therapy. In addition, a percentage of people who receive treatment do not respond to the medication. Hence, a plethora of promising novel systemic options have emerged recently in the treatment of HCC, such as immunotherapy and molecularly targeted treatments [9].

Preclinical research has shown that the substance P (SP) and neurokinin-1 receptor (NK1R) axis have a major role in inflammation and the tumor environment, in addition to the various characteristics of cancer, including the resistance to cell death, the induction of angiogenesis, and the promotion of cell migration and proliferation. Additionally, NK-1R is over-expressed in human tumor cells and may be regarded as a novel tumor marker [10–12]. Moreover, several labs have demonstrated that aprepitant, one of the NK-1R antagonists used as an antiemetic and anxiolytic drug, exerts multiple antitumor activities (antiproliferative, apoptotic, antimigration, and antiangiogenesis) in vivo and in vitro [13], and they have also raised the possibility that NK-1R antagonists could be beneficial in reversing chemoresistance [14, 15].

These findings, taken together, support the necessity of additional research to examine the possible utilization of aprepitant in hepatocellular carcinoma as a novel therapeutic strategy to enhance existing anticancer approaches.

Materials and methods

Patients

Paraffin blocks of 50 Egyptian HCC patients taken from the archives of the Pathology Department at the Medical Research Institute during the period of January 2010 to June 2015 were included in this study. The *inclusion criteria* included the following: (1) patients diagnosed with primary HCC confirmed by pathological reports, clinical criteria, or radiological findings according to the American Association for the Study of the Liver Diseases (AASLD) guidelines, (2) patients aged more than 18 years, (3) the patients' files containing the data about their regular follow-up, and (4) patients had life expectancy more than 6 months. The *exclusion criteria* included the following: (1) any patient who had a history of liver surgery or transplant, (2) those who had any type of hematologic malignancies, (3) patients who were suffering from severe hepatic failure or renal failure, and (4) pregnant or lactating females.

Treatment response and survival

- Treatment response was assessed by computerized tomography (CT), enhancement CT, or magnetic resonance image (MRI).
- Progression-free survival (PFS) and overall survival (OS) were recorded for each patient.

Cell culture

The American Type Culture Collection provides the HepG2 cells (ATCC® HB-8064TM). The cells were kept in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum and 1% streptomycin and penicillin (Gibco, Invitrogen, Carlsbad, CA, USA) in a humidified environment with 5% CO2 and 37 °C. We changed the medium every 48 h. The cells were sown for the tests at a density of 1.3×103 cells/cm^2 and were given 24 h to adhere before the initiation of

treatments. Before starting the experimental incubations, the cultivated cell number was tailored to a density where the cells expanded rapidly [16].

Viability of the cell

Cell viability was determined using the MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). HepG2 (5×103) cells were cultivated for 24 h under usual conditions after being seeded in 96-well plates. Subsequently, the cells were subjected to 1.25–40 μM of sorafenib alone, 10–320 μM of apretinant alone, or a constant drug ratio 1:8 of a combination of sorafenib and aprepitant for 72 h. Cells were treated with MTT solution (0.5 mg/ml in PBS) for 3 h after being washed with PBS. After dissolving the resulting formazan crystals in 0.1-ml dimethyl sulfoxide (DMSO), a Beckman Coulter DTX 880 microplate reader was utilized to determine the absorbance at 560 nm. The extract cytotoxicity was assessed by the absorbance of the treated and control cells utilizing the subsequent equation: [17].

Cytotoxicity(%) = the absorbance at 560 nm. The extract cytotoxicity was assessed by control × 100

The combination impact of sorafenib and aprepitant in a fixed ratio was ascertained using the median effect analysis approach in accordance with their respective IC50 values. The impact on growth inhibition at various drug concentrations and ratios was then entered into the program CompuSyn version 2.1 (Biosoft, Cambridge, UK) to find out the combination index (CI) and dosage reduction index (DRI). The calculation of CI was according to the ChoTalalay formula.

For the calculation of DRI, we used the subsequent equation:

$$\mathrm{DRI} = (\mathrm{D})1/(\mathrm{D})\,\mathrm{com1}$$

wherein (D) com1 represent the IC50 value for drug1 in the combination and (D) 1 represent the IC50 value from individual drug treatment [18].

Cells were cultivated under standard conditions for 24 h in 75 cm^2 flasks. Following this time frame, three 75-cm^2 flasks containing cells were cultured for 72 h with either aprepitant (IC50), sorafenib (IC50), or a combination of the two. After that, the cells were taken out, reconstituted in 500-μl PBS, and refrigerated until the subsequent biomarkers were measured.

Quantitation of VEGF production in culture media

HepG2 cells were cultured for 24 h in a 25 cm^2 culture flask and incubated with sorafenib (IC50), aprepitant (NK-1R antagonist) (IC50), or a combination of the two for 72 h, and culture media were collected, centrifugated at 1000 rpm for 10 min, and then stored at − 80 until used for analysis of VEGF. VEGF in the medium was assessed by a human VEGF ELISA kit provided by MyBioSource (Catalog Number: MBS355343) according to the manufacturer's instructions and using Beckman Coulter DTX 880 microplate reader [19].

Preparation of total cell lysates and quantitation of MMP9, NFkB, AKT, and ERK1

The cells were incubated at 37 °C in a hypotonic buffer containing 1 mmol/L EDTA, 10 mmol/L HEPES, and 50 mmol/L sucrose (pH 7.6) after that they were homogenized. This was followed by a hypertonic solution [1 mmol/L EDTA, 10 mmol/L HEPES, and 450 mmol/L sucrose (pH 7.6)] addition and then centrifugation at 12,000×g at 4 °C for 10 min. Then, the supernatant fraction was gathered, the protein concentration was assessed using a BCA assay, and the mixture was divided and stored at − 80 °C until it was needed for analysis [20, 21].

Then the following parameters were assessed by using a Beckman Coulter DTX 880 microplate reader.

1. Matrix metalloproteinase 9 using human MMP-9 ELISA kit from Elabscience (Catalog No: E-EL-H6075).
2. Nuclear factor Kappa B using NF-κB-(p65) ELISA kit from MyBioSource (Catalog No: MBS450580).
3. Activated kinase (AKT) using AKT [pS473] ELISA kit from Invitrogen (Catalog Number: KHO0111).
4. Extracellular signal-regulated kinase 1 using ERK1/2 [pThr202/Tyr204] ELISA Kit (catalog number: EMS2ERKP). The procedures were done following the manufacturer's protocols [22–25].

Analyzing apoptosis via flow cytometry with annexin V

The (propidium iodide) PI staining was done concurrently using the annexin V assay. In a 25 cm^2 culture flask, HepG2 cells were cultivated for 24 h before being treated for 72 h with either sorafenib (IC50), aprepitant (IC50), or a combination of the two. Following trypsinization and centrifugation for 5 min at 1000 rpm, the cells were extracted. They were then resuspended in 1×binding buffer and stained for 10 min at room temperature using 5 μl of annexin V and 10 μl of propidium iodide solution. Following that, the BD LSRFortessa™ Cell Analyzer (Becton Dickinson, NJ, USA) was utilized to perform FACS analysis in concordance with the manufacturer's instructions. For every analysis, roughly 10,000 counts were recorded [22].

Immunohistochemistry of HCC patient samples

The control samples were patients with liver cirrhosis since cirrhosis is a precursor lesion for HCC. Clinical information of patients, the cause of cirrhosis, Child–Pugh classification, and TNM staging were evaluated. Blocks of HCC and cirrhosis were divided into 4-lm portions, put on slides that were positively charged, and then deparaffinized in xylene. Rehydration using a series of graduated alcohol-to-water ratios came next. The slices were incubated at room temperature in 3% H2O2 in PBS (pH 7.4) to prevent endogenous peroxidase activity. Next, 1-mM Tris–EDTA buffer (pH 9.0) was utilized to retrieve the antigen. An avidin-biotinylated immunoperoxidase technique was used for immunohistochemical staining. The Thermo Scientific, USA's Ultra Vision Detection System, was used to find the bound primary antibody, and DAB chromogen was used to show the immunostaining. The antibody dilutions were as follows: NK1R (1:100) [26].

Without any prior clinical data, a semiquantitative study of the immunostaining degree was carried out. The ratio score of the stained tumor cells and the intensity staining score were combined to calculate the expression level of NK1R using the Allred 8-unit system. The strength of the staining was rated the following way: 0 for no staining, 1 for light yellow staining, 2 for moderate yellow brown staining, and 3 for intense brown staining. The ratio score was based on the proportion of positively stained tumor cells: 0 represented ≤ 5% positive cells, 1 represented 6–25% positive, 2 represented 26–50% positive, and 3 represented ≥ 51% positive. In the end, if the total score was equal to or greater than 4, NK1R was deemed to have high expression.

Statistical analysis

SPSS was utilized for all statistical analyses (Version 13.0; SPSS Inc., Chicago, IL, USA). In vitro tests, Student's *t*-test, and analysis of variance (ANOVA) were utilized to analyze the data, which were given as mean ± standard error of the mean of three separate determinations. Quantitative data for the patient samples were stated as mean, standard deviation, median, and range (minimum and maximum), whereas categorical data were represented as numbers and percentages. Kaplan–Meier survival curve was used for progression-free survival (PFS) and overall survival (OS). At the 5% level, the results' significance was assessed. Univariate Cox proportional hazards regression model analysis was used to identify the elements influencing both OS and PFS; multivariate Cox proportional hazards regression analysis then was used to further examine the same elements. The *p*-value was calculated to determine whether the results were statistically significant.

Results

Cytotoxicity assay

Using HepG2 cell lines, the cytotoxic effects of sorafenib and aprepitant were assessed using the MTT test to determine the concentrations at which cell growth was reduced by 50%. HepG2 cells treated with sorafenib and aprepitant for 72 ho attained IC50 at dosages of 14.02 µM and 60.8 µM, respectively, in comparison to untreated cells (Fig. 1). The synergistic anticancer effects of aprepitant and sorafenib together were then assessed. We investigated the antiproliferative effects of combinations of sorafenib and aprepitant by evaluating fixed ratios of the two medicines' doses (1:8), respectively, and made use of

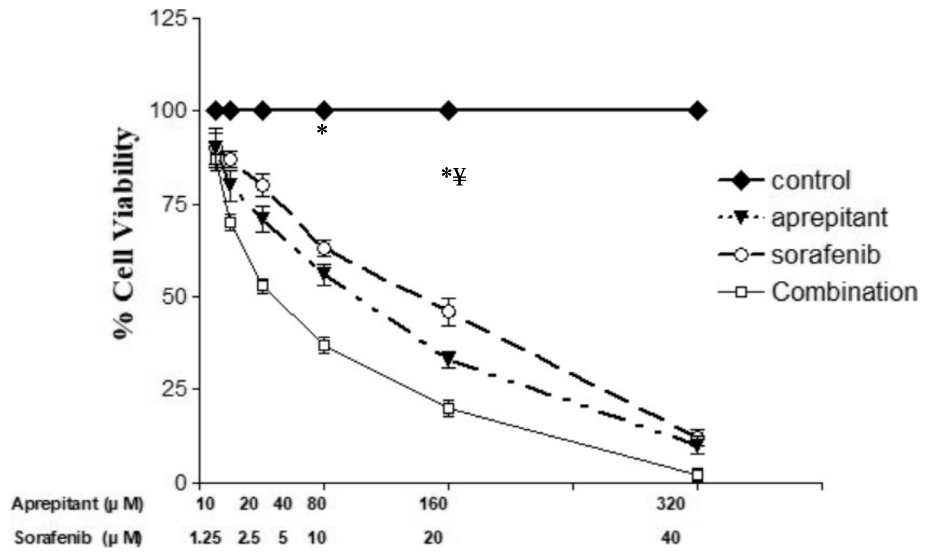

Fig. 1 This curve shows the antiproliferative effect of the aprepitant and sorafenib combination on HepG2 cells. The MTT test was utilized to create dose–response curves. For 72 h, cells were exposed to varying levels of sorafenib (1.25–40 µmol/l), aprepitant (10–320 µmol/l), and a fixed ratio of 1:8 for both. The outcomes of at least three separate experiments are represented by each point

the analysis of the median drug effect in computing CIs. The HepG2 cell line showed a synergistic effect with narrow confidence intervals (CIs = 0.71) when simultaneous combination doses were applied. Furthermore, in comparison to the concentrations of the two medications alone, we observed a dosage decrease in the IC50 values (DRI50) following the combination treatment in HepG2 cells: 3.9-fold for sorafenib and 2.1-fold for aprepitant.

Apoptosis assay

Flow cytometry was used to divide HepG2 cells into four quadrants following annexin/PI staining, as illustrated in Fig. 2: viable (annexin − /PI −), early apoptotic (annexin + / PI −), late apoptotic (annexin + /PI +), and necrotic (annexin − /PI +). In the control group (early and late apoptotic), the rate of apoptosis was 4.5 ± 0.7%. After receiving sorafenib and aprepitant alone or in combination,

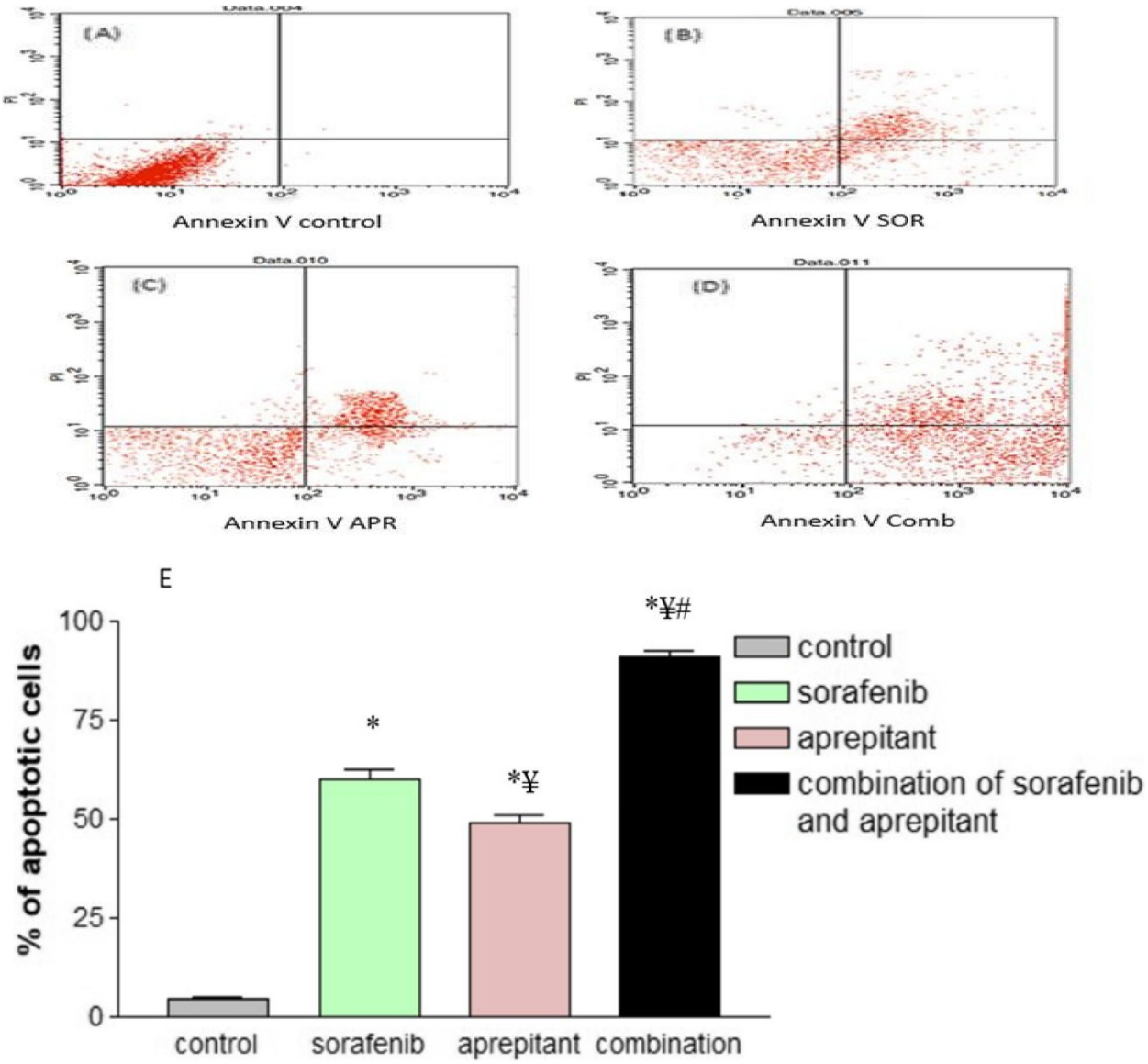

Fig. 2 The effect of treatment with sorafenib (14.02 μM), aprepitant (60.8 μM), and their combination for 72 h on HepG2 cell apoptosis. Examples of pictures from a flow cytometric examination of cell death following a 72-h exposure to various treatments using propidium iodide (PI) staining and annexin V coupled with fluorescein isothiocyanate (FITC). **A** Control, **B** sorafenib (SOR), **C** aprepitant (APR), and **D** combination (Comb). The top right quadrant reflects late apoptotic cells (annexin V + PI +), the bottom right quadrant reflects necrotic cells (annexin V − PI +), and the bottom left quadrant reflects viable cells (annexin V − PI −). **E** Bar graph illustrating the percentage of apoptotic HepG2 cells in response to various treatments. Significant differences were seen between the control group (*), sorafenib-treated group (¥ significant), and aprepitant-treated group (#). Data presented as mean ± SEM ($n = 3$)

the HepG2 cells' rate of apoptosis dramatically increased (59.9 ± 5%, 49.0 ± 3.7%, and 91.1 ± 2.5%, respectively). Additionally, compared to either drug-treated group, there was a notable rise in the proportion of the apoptotic population in the combination group ($p < 0.001$) (Figs. 2).

Combination of sorafenib with aprepitant leads to blockage of RAS/MAPK/ERK and PI3K/AKT/mTOR simultaneously

In the RAS/MAPK/ERK pathway, both sorafenib and aprepitant alone and in combination, in the current study, reduced the activity of ERK with more significant reduction observed in the combination group (116 ± 3.7, 129 ± 2.9, and 105 ± 2.4 pg/mg cellular protein, consequently), in comparison to the control group (154 ± 4.9 pg/mg cellular protein). However, the activity of AKT in the PI3K/AKT/mTOR pathway declined in both the sorafenib and aprepitant alone and in the combination groups (9.7 ± 0.3, 13.4 ± 0.5, and 6. 9 ± 0.6 U/mg protein cellular protein, respectively) with a more pronounced decrease in the combination group (25.2 ± 1.6 U/mg cellular protein) (Table 1). These results indicate that combining the two drugs is more beneficial in blocking both signaling pathways.

Combination of sorafenib with aprepitant has a synergistic antiangiogenic effect

Administration of either sorafenib or aprepitant alone led to a notable decline in the level of VEGF, in comparison to the untreated HepG2 cells (841.5 ± 12.3 pg/mg cellular protein, 920.3 ± 16.2 pg/mg cellular protein, and 987.6 ± 11.3 pg/mg cellular protein respectively). However, sorafenib demonstrated a more notable decline in the level of VEGF in comparison to the aprepitant-treated group ($p < 0.01$). The decrease in the level of VEGF was more substantial in the combination therapy group compared to the untreated, sorafenib, and aprepitant-treated groups (685.3 ± 5.3 pg/mg cellular protein, $p < 0.001$) (Table 1).

Potentiated anti-inflammatory effect of the combination therapy on HepG2

A significant decrease in NF-κB p-65 level in HepG2 cells was found in sorafenib and aprepitant-treated groups, in comparison to the control group (7.6 ± 0.1, 8.9 ± 0.5, and 12.2 ± 0.5 ng/mg cellular protein, respectively). Nevertheless, using sorafenib to treat HepG2 cells showed a more pronounced decline in NF-κB p-65 levels, in contrast to the aprepitant-treated group, $p < 0.001$. In the combination-treated group, the decrease in NF-κB p-65 levels was more significant compared to the untreated, sorafenib, and aprepitant-treated groups (5.0 ± 0.2 ng/mg cellular protein) (Table 1).

Aprepitant potentiated the antimetastatic effect of sorafenib on HepG2 cells

The control group showed the highest level of MMP-9 (7.6 ± 0.2 ng/mg cellular protein). Both sorafenib and aprepitant monotherapies caused a significant decrease in MMP-9 (5.7 ± 0.2 ng/mg cellular protein and 4.5 ± 0.1 ng/mg cellular protein, consequently), in contrast to the control group, thus hindering metastasis. A more favorable therapeutic response was observed in the combination group, in comparison to each drug, as the MMP-9 level decreased to 3.3 ± 0.1 ng/mg cellular protein *(*Table 1*)*.

Expression of NK1R in HCC and neighboring cirrhotic tissues

Thirty (60%) out of the 50 patients with HCC had immunostaining for NK1R that was strongly expressed. This was considerably greater than the expression seen in the cirrhotic tissues that were nearby (22%, 11/50, $p = 0.0002$). Figs (3 and 4) displays the degree of NK1R expression and the level of immune histochemistry staining.

Table 1 Effect of treatment with sorafenib (14.02 μM), aprepitant (60.8 μM), and their combination for 72 h, on the level of MMP-9, VEGF, NF-κB p-65, p-AKT, and p-ERK in lysates of HepG2 cells

	Control	Sorafenib	Aprepitant	Combination of sorafenib and aprepitant
MMP-9 (ng/mg protein)	7.6 ± 0.2	5.7 ± 0.2*	4.5 ± 0.1*¥	3.3 ± 0.1*¥#
VEGF (pg/mg cellular protein)	987.60 ± 11.30	841.50 ± 12.30*	920.30 ± 16.20*¥	685.30 ± 5.30*¥#
NF-κB p-65 (ng/mg cellular protein)	12.20 ± 0.5	7.60 ± 0.10*	8.90 ± 0.50*¥	5.0 ± 0.20*¥#
p-AKT (U/mg cellular protein)	25.2 ± 1.6	9.7 ± 0.3*	13.4 ± 0.5*¥	6.9 ± 0.6*¥#
p-ERK (pg/mg *cellular protein*)	154.3 ± 4.9	116 ± 3.7*	129 ± 2.9*¥	105 ± 2.4*¥#

* Significant from control group, ¥significant from sorafenib-treated group, #significant from aprepitant-treated group. Data presented as mean ± SEM ($n = 3$)

MMP-9, matrix metalloproteinase 9, *VEGF* vascular endothelial growth factor receptor, *NF-κB p-65* nuclear factor kappa-light-chain-enhancer of activated B cells, *p-AKT* phosphorylated activated kinase, *p-ERK* phosphorylated extracellular signal-regulated kinases

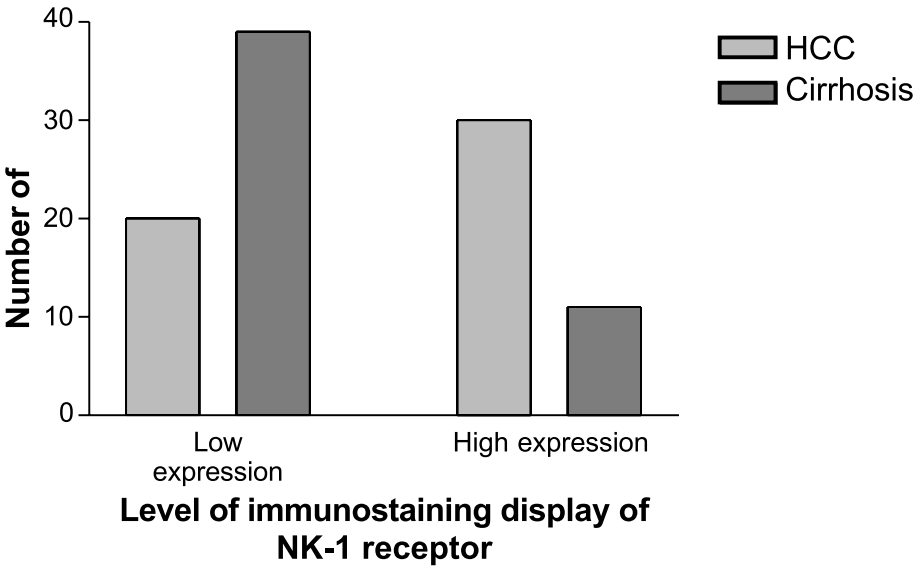

Fig. 3 Extent of immunostaining display of NK-1 receptor in Egyptian HCC vs cirrhotic cases

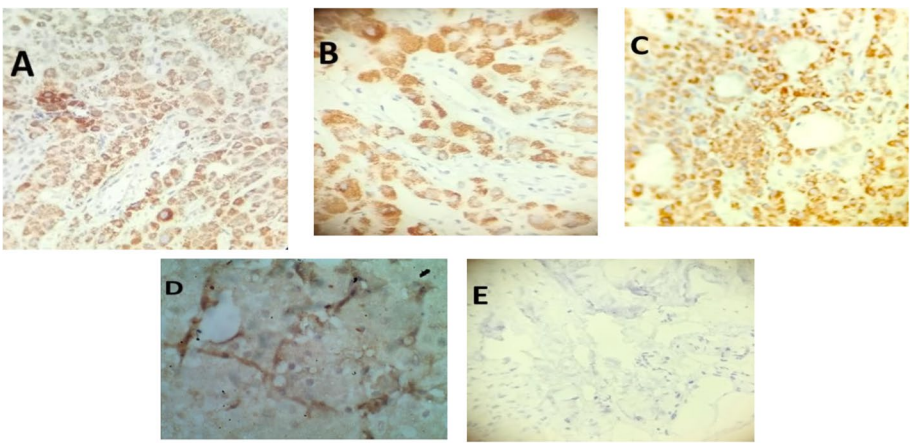

Fig. 4 **A** Strong cytoplasmic staining in more than 50% of tumor cells (score 5) (IHC, × 100). **B** High-power view of the previous image showing strong cytoplasmic staining in more than 50% of tumor cells (score 5) (IHC, × 400). **C** Moderate cytoplasmic staining in about 40% of tumor cells (score 4) (IHC, × 400)..**D** Weak cytoplasmic staining of Nk-1R in about 40% of tumor cells (score 3) (IHC, × 400). **E** Hepatic fibrosis showing negative staining of Nk1 receptor (IHC, × 400)

The patient characteristics and clinicopathological features in the HCC samples

The studied 50 HCC patients' mean age was 57.2 ± 10.8 years, with most of them being males (36 M, 14 F) (Table 2). Forty-five patients (90%) had a past infection of hepatitis B virus (HBV), and 47 patients (94%) had a history of liver cirrhosis, with the majority of the cases being viral induced: 46 (92%).

The value of AFP was high in all the patients (100%). In addition, 14 (28%) patients were found to have HCV. Patients who have portal hypertension and ascites were 6 (12%) and 17 (34%), respectively.

The majority of the patients have Child–Pugh stage A [35 (70%)] disease, while according to the TNM stage, low stage (I/II) and high stage (III/IV) distribution were 37 (74%) and 13 (26%), respectively.

The number of patients with NK + ve and NK − ve were 30 (60%) and 20 (40%), respectively (Table 2).

PFS and OS in patients with HCC

PFS (Fig. 5, Table 3) and OS (Fig. 6, Table 4) in HCC patients were evaluated by the K–M curve.

Table 2 Characteristics of 50 hepatocellular carcinoma (HCC) patients as regards various parameters ($n=50$)

	No. (%)
Sex	
Male	36 (72%)
Female	14 (28%)
Age (years)	
Mean ± SD	57.2 ± 10.8
Median (min.–max.)	56 (34–76)
HCV	14 (28%)
HBV	45 (90%)
AFP (high)	50 (100%)
Cirrhosis	47 (94%)
Etiology of cirrhosis	
Viral	46 (92%)
Viral + alcoholic	2 (4%)
Bilharzial	2 (4%)
Ascites	15 (30%)
Encephalopathy	0 (0%)
Portal HTN	6 (12%)
Child–Pugh	
A	35 (70%)
B	15 (30%)
TNM stage	
Low stage (I/II)	37 (74%)
High stage (III/IV)	13 (26%)
Progress	36 (72%)
Death	13 (26%)
NK	
NK – ve	20 (40%)
NK + ve	30 (60%)

HCV hepatitis C virus, *HBV* hepatitis B virus, *AFP* alpha fetoprotein, *NK* neurokinin

The mean PFS was 29.23 months, the median was 27 months, the 1-year PFS was 90%, and the 3-year PFS was 28%.

The mean OS was 44.60 months, the 1-year OS was 90%, and the 3-year OS was 74%.

Analysis of factors that influence PFS in patients with HCC

PFS was much shorter in female patients (Fig. 7), existence of HBV (Fig. 8), NK + ve (Fig. 9), the presence of cirrhosis ($p=0.008$), existence of ascites ($p=0.001$), and Child–Pugh [B] ($p=<0.001$). In contrast, PFS was not correlated with the presence of HCV ($p=0.223$), the existence of portal hypertension (PHT) ($p=0.051$), or high stage (III/IV) ($p=0.207$) in these patients.

Furthermore, a univariate Cox proportional hazards regression model analysis illustrated the following factors were associated with a worse prognosis: female patients, NK + ve, HBV presence, cirrhosis presence, ascites presence, and child Pugh [B] (Tables 5 and 6). The multivariate Cox proportional hazards regression model analysis including elements with a value of $p<0.05$ revealed that NK + ve, HBV, and female patients were independent predictors of worse PFS when the covariates with a value of $p<0.05$ were included.

Analysis of elements affecting OS in patients with HCC

OS was much shorter in female patients ($p=0.005$) (Fig. 10), the presence of cirrhosis ($p=0.008$)) (Fig. 11), the presence of portal HTN ($p=0.004$) (Fig. 12), and existence of HBV, while the presence of HCV ($p=0.378$) and the presence of ascites ($p=0.062$), Child–Pugh [B] ($p=0.101$) or high stage (III/IV) ($p=0.207$), and NK + ve ($p=0.506$) were not linked to OS.

Additionally, univariate Cox proportional hazards regression model analysis demonstrated that female patients ($p=0.005$), the presence of cirrhosis ($p=0.008$), the presence of portal HTN ($p=0.004$), and the presence of HBV ($p=<0.001$) were suggestive for poor PFS (Tables 7 and 8).

When the elements having a value of $p<0.05$ were included in the multivariate Cox proportional hazards regression model analysis, female patients ($p=0.005$), cirrhosis presence ($p=0.008$), and the presence of portal HTN ($p=0.004$) were noticed to be independent predictors for poor PFS.

Discussion

The majority of HCC patients presenting by intermediate and advanced stages do not respond well to standard therapeutic regimens, and as a result, the patients' mean survival time is less than 12 months after diagnosis. The intensity of sorafenib side effects is another drawback. Consequently, it is imperative to combine sorafenib with less toxic medicines that have demonstrated anti-cancer action to achieve greater therapeutic success and fewer unwanted side effects [27].

Aprepitant is one of the NK-1R antagonists that is safe and does not have any negative side effects. Presently, aprepitant is utilized as an antiemetic in clinical practice. Aprepitant exhibited numerous anticancer activities against different cancer types in both in vitro and in vivo research [28]. Robinson P. et al. recently proposed that aprepitant with chemotherapy might be effective when treating extremely aggressive osteosarcoma [29].

In 2019, Muñoz M. et al. studied NK-1R antagonists against malignant liver tumor (hepatoblastoma (HB)) where dual effects were observed in HB from NK-1R antagonists: reduced angiogenic activity and tumor volume [30].

As far as we are aware, this is the first research assessing the antitumor efficacy of aprepitant and sorafenib combination therapy in patients having HCC. The goal

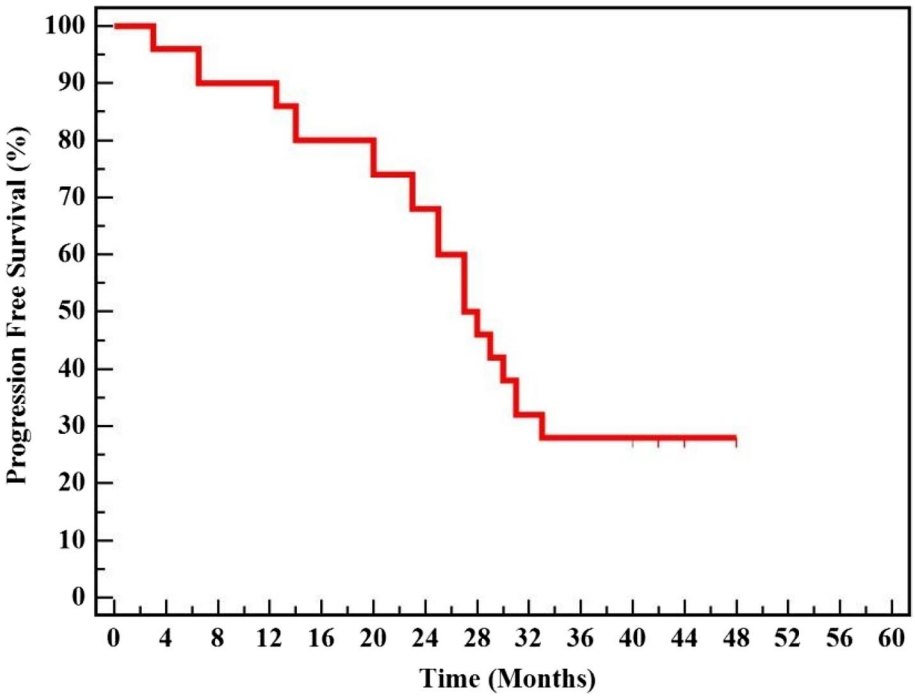

Fig. 5 K-M survival curve for PFS

Table 3 K-M survival curve for PFS

	Mean (months)	Median (months)	% 1 year	% 3 years	% end study
Progression-free survival	29.23	27.00	90.0%	28.0%	28.0%

of this research is to find potentially beneficial in vitro combinations of cytotoxic drugs that may eventually be brought to the clinic.

In the current research, we investigated the antitumor effect of both sorafenib and aprepitant alone and whether their combination has a synergistic effect on their antitumor activity in human HCC cells while lowering their doses than using higher doses of either of them alone.

The synergetic anti-tumor effects of aprepitant and sorafenib were demonstrated on the following: (i) cytotoxicity, (ii) apoptotic effect, (iii) blockage of RAS/MAPK/ERK and PI3K/AKT/mTOR, (iv) antiangiogenic effect, (v) anti-inflammatory effect, and (vi) anti-metastatic effect.

Aprepitant showed cytotoxic capability against HepG2 cells, according to our research, with IC_{50} 60.8 μM. This observation is in agreement with a recent study showing the cytotoxic effect of aprepitant in different hepatic malignancies such as hepatoblastoma [30] and intrahepatic cholangiocarcinoma cell lines [31]. Combination index for the combination of sorafenib and aprepitant at a fixed ratio of the two agents' doses (1:8), respectively, indicated that aprepitant synergized the cytotoxicity of sorafenib in HepG2 with a dose reduction in the IC50 values (DRI50) of 3.9-fold for sorafenib and of 2.1-fold for aprepitant in HepG2 cells, in contrast to the concentrations of either of the two drugs.

The capacity of internal or external stimuli to cause cell death is acknowledged for its enormous therapeutic potential; the combination of sorafenib and aprepitant enhanced apoptosis more than either of the two drugs alone as shown by flow cytometry using annexin V/FITC staining.

The primary mechanisms by which cells regulate their survival, differentiation, proliferation, metabolism, and motility as a result of external signals are through the RAS/MAPK/ERK and PI3K/AKT/mTOR signaling pathways [32].

Hence, the current research investigated the effect of adding aprepitant with sorafenib on RAS/MAPK/ERK and PI3K/AKT/mTOR signaling pathways to clarify the underlying mechanism behind the synergistic apoptotic interaction. In the current study, both sorafenib and aprepitant alone and in combination blocked these signaling pathways with a more significant reduction observed in the combination group in contrast to the

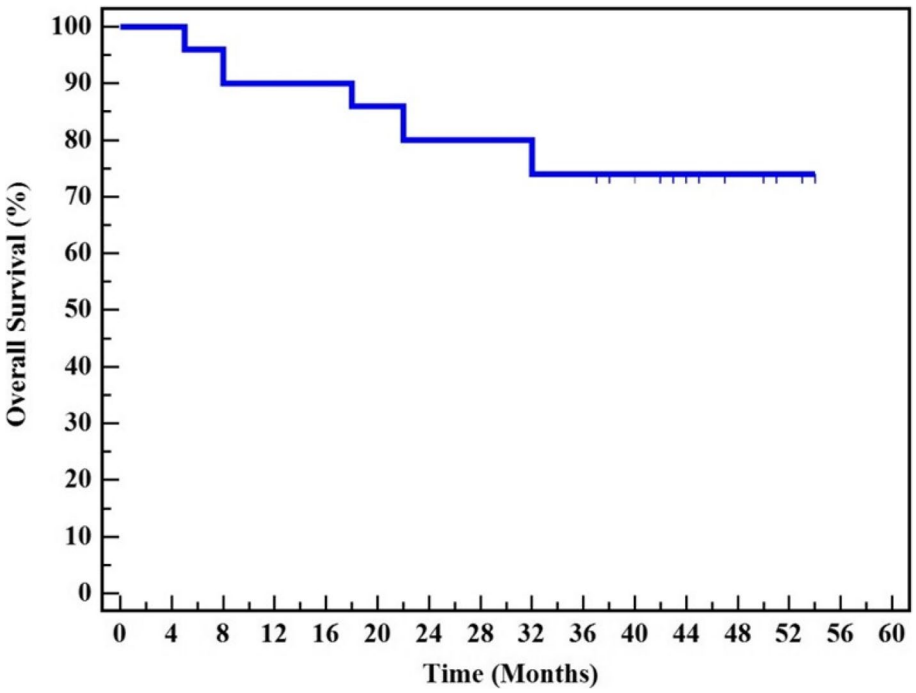

Fig. 6 K-M survival curve for OS

Table 4 K-M survival curve for OS

	Mean (months)	Median (months)	% 1 year	% 3 years	% end study
Overall survival	44.60	–	90.0%	74.0%	74.0%

control group. The repressive effect of sorafenib and aprepitant on these pathways was in line with previous studies performed on different types of cancer [33–36].

Furthermore, VEGFA expression is raised in many tumor types and is correlated with the prognosis of these tumors. It is strongly linked to the pathogenesis of numerous tumors, particularly vascular-rich, solid tumors. This mediator is inhibited by sorafenib (multitarget antitumor drug) [37]. Aprepitant also shows the same inhibitory effect on the level of VEGF as sorafenib in our work, in agreement with previous studies. This was proposed to be due blocking of the NK-1 receptor leading to inhibition of VEGF gene expression and the AP-1 transcription factor, which is a promotor for VEGF [38, 39]. Moreover, the combined therapy can achieve a synergistic result with higher inhibition of the expression of VEGFA which accordingly cause more inhibition of the proliferation of HCC cells, prevent the invasive ability, and enhance cell apoptosis.

The molecular pathways linking inflammation to carcinogenesis have been thoroughly examined in recent years. The NF-κB pathway is an important modulator of immune responses and inflammation that can also work as a promoter or tumor suppressor. Moreover, it has been documented that HCC invasion happens through nuclear factor-kappaB (NF-κB) over-expression. In HCC cells, sorafenib was previously shown to decrease the ERK/NF-κB pathway and hence inhibit TPA-induced MMP-9 and VEGF expressions, increasing overall survival [40].

In our study, treatment of HepG2 cells with sorafenib showed a pronounced decline in NF-κB p-65 levels compared to the aprepitant-treated group and a significant decline in MMP-9 levels. However, in the combination-treated group, the decrease in NF-κB p-65 and MMP-9 levels was more significant compared to the untreated, sorafenib, and aprepitant-treated groups with more hindrance of metastasis. Sorafenib is anticipated to repress the NF-κB p-65 and MMP-9 levels through blockage of the ERK/NF-κB pathway, while aprepitant decreases their level through blockage of the JNK and p38/MAPK pathway. Moreover, their combination presented a synergistic inhibitory effect on the inflammation and metastasis pathway [41, 42].

According to these data, we propose the repurposing of aprepitant for novel therapeutic usage as an antitumor agent. In the future, our findings noticed in vitro can be expanded to the in vivo model aiming to affirm the more effective synergistic anticancer effect of aprepitant and

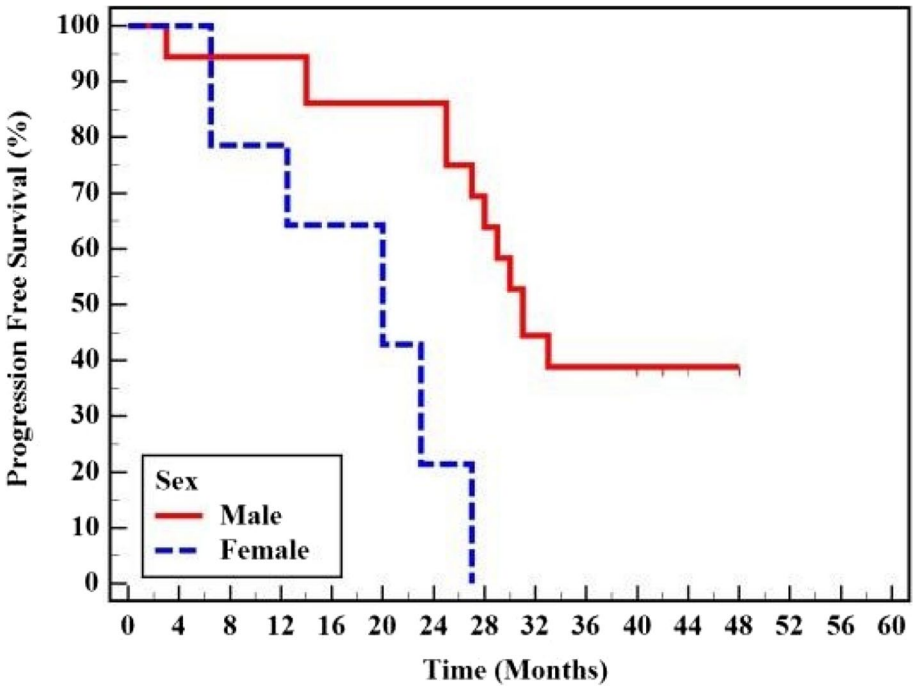

Fig. 7 K-M survival curve for PFS with sex

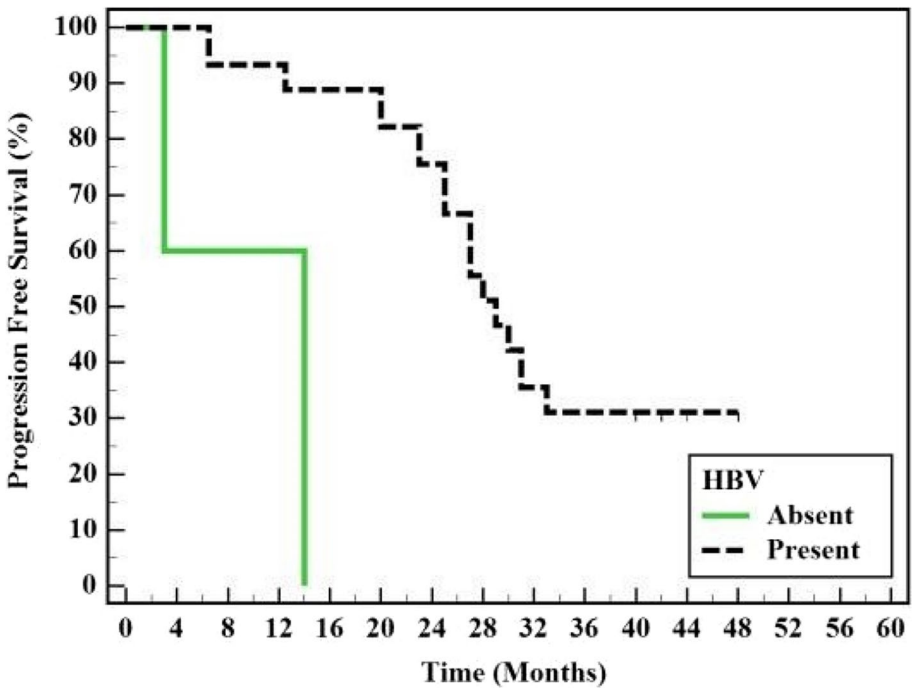

Fig. 8 K-M survival curve for PFS with HBV

sorafenib combination to reduce the dose of the active compound and consequently the undesirable effects of high doses of sorafenib.

Additionally, our study assessed the molecular targets in Egyptian HCC patients. In our patients, the mean PFS was 29.23 months, the 1-year PFS was 90%, and the 3-year PFS

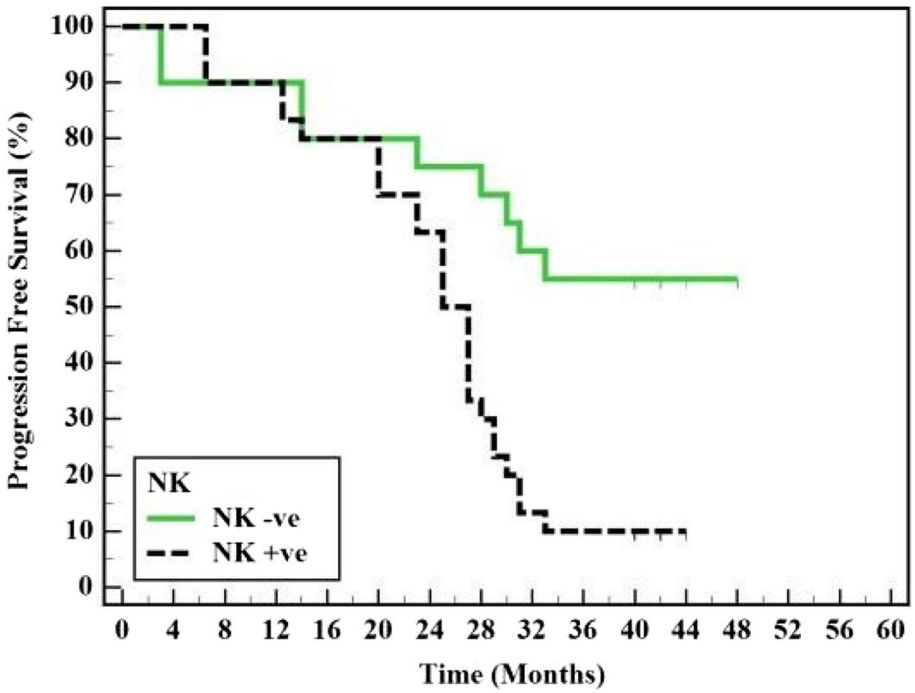

Fig. 9 K-M survival curve for PFS with NK

Table 5 Univariate and multivariate Cox regression analysis for the factors affecting PFS ($n = 36$ vs. 14)

	Univariate		#Multivariate	
	p	**HR (LL–UL 95% *CI*)**	***p***	**HR (LL–UL 95% *CI*)**
Female	< 0.001*	6.339 (2.796–14.370)	< 0.001*	12.783 (4.180–39.097)
Age (years)	0.016*	1.043 (1.008–1.079)	0.084	1.039 (0.995–1.086)
HCV presence	0.223	0.611 (0.276–1.351)		
HBV presence	< 0.001*	0.078 (0.022–0.269)	< 0.001*	0.018 (0.004–0.090)
Cirrhosis presence	0.008*	0.162 (0.042–0.627)		
Ascites presence	0.001*	3.085 (1.566–6.078)	0.294	1.681 (0.638–4.431)
Presence of **portal HTN**	0.051	2.416 (0.999–5.843)		
Child–Pugh	< 0.001*	5.564 (2.774–11.159)		
High stage (III/IV)	0.207	3310.4 (0.011–9.8×10^8)		
NK + ve	0.003*	3.216 (1.483–6.973)	0.030*	2.555 (1.096–5.954)

HCV hepatitis C virus, *HBV* hepatitis B virus, portal HTN portal hypertension, *NK* neurokinin. *HR* hazard ratio, *CI* confidence interval, *LL* lower limit, *UL* upper limit. #All significance for the asteriskvariables with $p < 0.05$ were included in the multivariate. *Statistically significant at $p < 0.05$

was 28%, while the mean OS was 44.60 months, the 1-year OS was 90%, and the 3-year OS was 74%.

The majority of our patients had HBV as the cause of liver cirrhosis which does not reflect the prevalence of viral hepatitis in Egypt where HCV is more prevalent. But it can be explained by the sample selection as we used archived paraffin blocks from 2010 to 2015 and applied many inclusion and exclusion criteria to fit in our study which incidentally was more applied to HBV patients. Additionally, the mean age for the patients was 57 years old, indicating that this group of patients was at high risk for HBV infection before the addition of the HBV vaccine to the Egyptian compulsory list of vaccination. Additionally, the campaign for anti-schistosomal treatment, which was done in Egypt in the 1980s, aided in the spread of HBV infection [43].

Moreover, two patients' liver cirrhosis was explained only by hepatic schistosomiasis. The role of schistosomal infection in the development of HCC is still controversial, but it has been suggested that *Schistosoma mansoni* infection may be a possible risk factor for HCC. Several mechanisms have been proposed including chronic inflammation resulting from prolonged infestation with

Table 6 Log rank for Progression Free Survival with different parameters

	Total no	Mean	*Media n*	% 1 year	% 3 year	% end of study	Log rank	
							χ^2	*P*
Sex								
Male	**36**	33.53	31.0	94.4%	38.9%	38.9%	26.30	< 0.00
Female	**14**	18.18	20.0	78.6%	0.0%	0.0%	0*	1*
HBV								
Absent	**5**	9.60	14.0	60.0%	0.0%	0.0%	27.97	< 0.00
Present	**45**	31.41	29.0	93.3%	31.1%	31.1%	7*	1*
NK								
NK − ve	**20**	35.35	–	90.0%	55.0%	55.0%	0.328*	0.001*
NK + ve	**30**	24.75	25.0	90.0%	10.0%	10.0%		

HBV hepatitis B virus, *NK* neurokinin

*Statistically significant

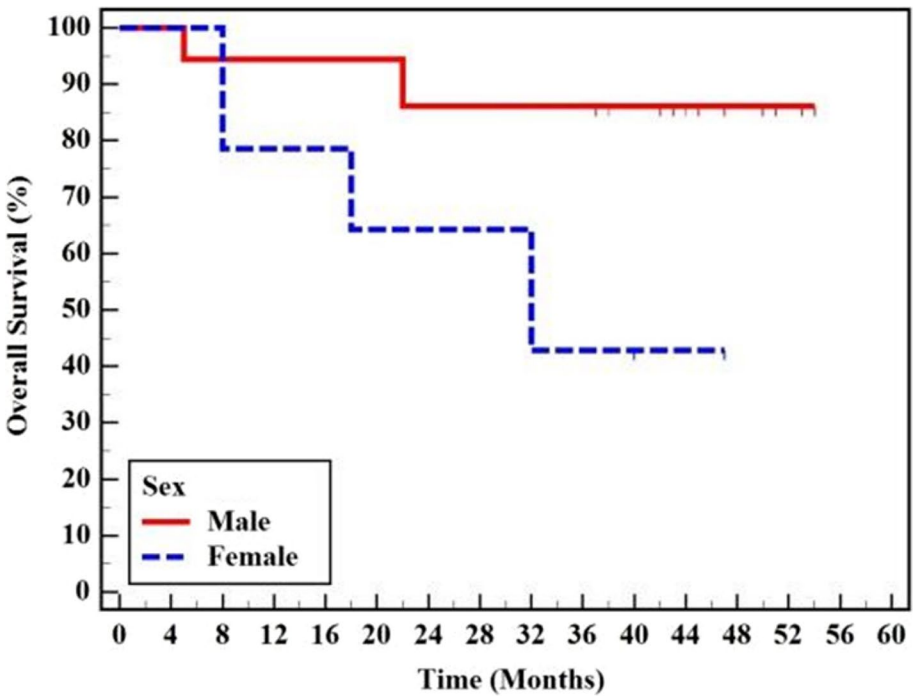

Fig. 10 K-M survival curve for OS with sex

the parasite leading to the development of oxidative stress and damaging effect on host cells, suppression of the immune system, and facilitating the evolution of oncogenes inside the host cells especially when co-infected with HCV or HBV [44].

We analyzed the risk factors for HCC progression and shorter OS with univariate and multivariate analyses.

The results of multivariate analysis in the present research showed that the female sex and NK + ve were significant independent factors in predicting worse PFS, while worse OS can be predicted by the female sex, the presence of cirrhosis, and the presence of portal HTN.

These findings support a prior study by Faitot F. et al. that found PH decreased overall survival (OS) in HCC patients waiting for liver transplantation [45]. Numerous studies, such as Lin C. W. et al. which examined important predictors of OS in patients with HCC following surgical resection, also found a correlation between cirrhosis and poor overall survival (OS), which is caused by worsening liver functions and fewer treatment options [46]

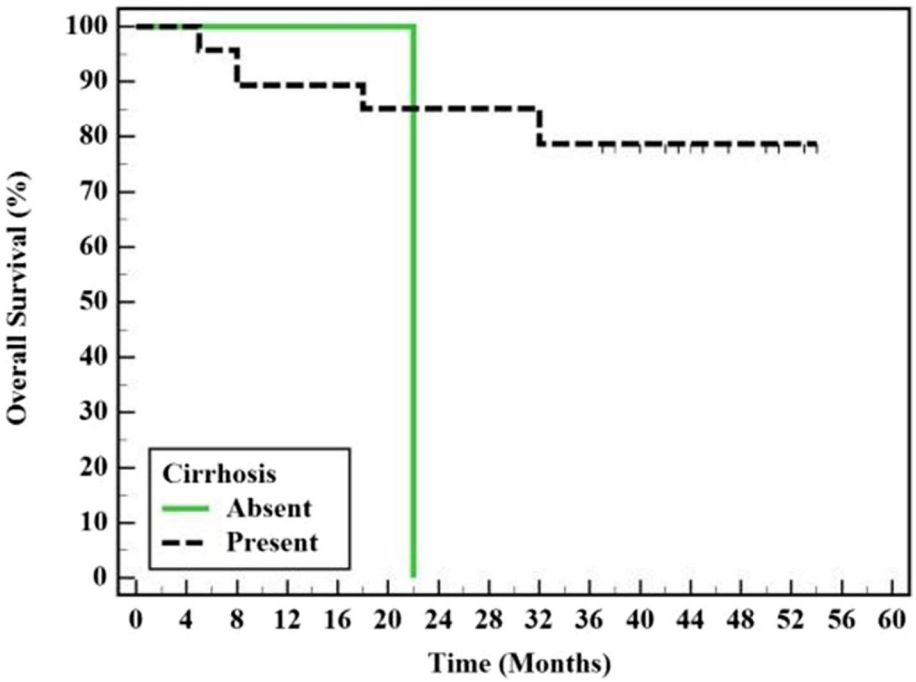

Fig. 11 K-M survival curve for OS with cirrhosis

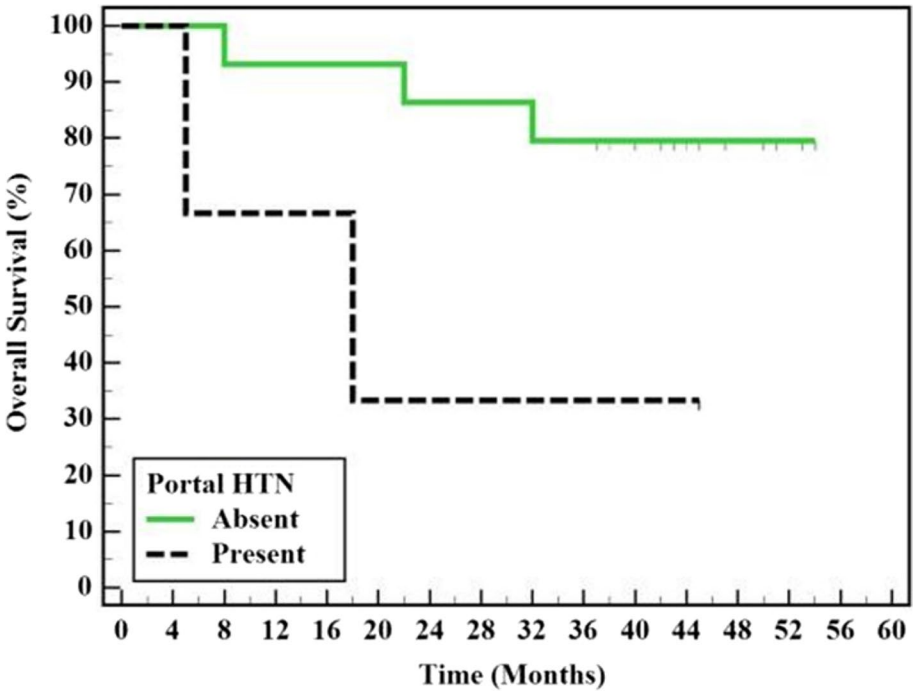

Fig. 12 K-M survival curve for OS with portal HTN

In our investigation, we noticed that patients with HCC had much higher levels of NK-1R immunohistochemistry expression than the cirrhotic tissues surrounding the tumor. This was associated with better response to NK-1R antagonists (including aprepitant). A higher progression-free survival was shown to be linked in the current investigation with greater intratumoral levels of natural killer cells (NK cells). Our findings are consistent with recent

Table 7 Univariate and multivariate Cox regression analysis for the elements affecting overall survival ($n = 13$ vs. 37)

	Univariate		#Multivariate	
	p	**HR (LL–UL 95% *CI*)**	***p***	**HR (LL–UL 95% *CI*)**
Female	0.005*	4.901 (1.597–15.040)	0.002*	13.162 (2.649–65.390)
Age (years)	0.244	1.033 (0.978–1.091)		
HCV presence	0.378	1.655 (0.540–5.074)		
HBV presence	< 0.001*	0.078 (0.022–0.269)		
Cirrhosis presence	0.008*	0.162 (0.042–0.627)	< 0.001*	0.021 (0.003–0.166)
Ascites presence	0.062	409.1(0.737–226,973.2)		
Presence of **portal HTN**	0.004*	5.789 (1.750–19.150)	0.001*	10.570 (2.479–45.076)
Child–Pugh [B]	0.101	920.6 (0.267–3,172,035.4)		
High stage (III/IV)	0.207	3310.4 (0.011–9.8×10^8)		
NK + ve	0.506	1.491 (0.459–4.845)		

HCV hepatitis C virus, *HBV* hepatitis B virus, portal HTN portal hypertension, *NK* neurokinin. *HR* hazard ratio, *CI* confidence interval, *LL* lower limit, *UL* upper limit. #All variables with $p < 0.05$ were included in the multivariate. *Statistically significant at $p < 0.05$

Table 8 Log rank for Overall Survival with different parameters

	Total no	Mean	Median	% 1 year	% 3 years	% end of study	Log rank	
							χ^2	*p*
Sex								
Male	**36**	48.61	–	94.4%	86.1%	86.1%	9.827*	0.002*
Female	**14**	31.29	32.00	78.6%	42.9%	42.9%		
Cirrhosis								
Absent	**3**	22.00	22.00	100.0%	0.0%	0.0%	9.425*	0.002*
Present	**47**	46.04	–	89.4%	78.7%	78.7%		
Portal HTN								
Absent	**44**	47.18	–	93.2%	79.5%	79.5%	10.937*	0.001*
Present	**6**	22.67	18.00	66.7%	33.3%	33.3%		

Portal HTN, portal hypertension. *Statistically significant at $p < 0.05$

research showing a negative association with tumor proliferation and a positive correlation between the density of intratumor NK-cells and tumor apoptosis [47].

Conclusion

Combined aprepitant and sorafenib may be a promising approach in HCC treatment.

Recommendation

Larger sample size to confirm our findings and possibility of doing clinical trials with its different phases using this combination therapy to test safety, effectiveness, and identify any side effects.

Abbreviations

PFS	Progression-free survival
OS	Overall survival
HCC	Hepatocellular carcinoma
NK-1	Neurokinin-1 receptor
FLT-3	FMS-like tyrosine kinase 3
HepG2	Hepatoblastoma cell line
PDGF-R	Platelet-derived growth factor receptor
VEGFR	Vascular endothelial growth factor receptor
Raf kinases	Rapidly accelerated fibrosarcoma kinases
MAPK	Mitogen-activated protein kinases
ERK	Extracellular signal-regulated kinases
IC50	Half-maximal inhibitory concentration
NF-κB	Nuclear factor kappa-light-chain enhancer of activated B cells
MMP-9	Matrix metalloproteinase 9
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
HB	Hepatoblastoma
HBV	Hepatitis B virus
HCV	Hepatitis C virus
AP-1	Activator protein-1
AKT	Activated kinase
PI3K	Phosphoinositide 3-kinase
mTOR	Mammalian target of rapamycin
DRI	Dosage reduction index
DMSO	Dimethyl sulfoxide

Author contributions

Wessam F. El Hadidy and Heba Essam Sedky: conceptualization, planning study design, major writing of themanuscript, practical work on Hep G2 cells. Hazem F, Mannaa and Yasmine Mahmoud Nabil : biochemical work, statistical analysis of results and contributed to writing of the manuscript. Mona Mohamed Nagy El wany: pathologypart in the study and writing of the manuscript. Mohamed A. Abdel Aziz, Eman Samy El Alfy And Yasmine Nagy Elwany: patient's data collection, contributed to writing of the manuscript and statistical analysis of results. All authors revised and approved the manuscript.

Funding
The authors declare that they did not receive any funding for this study.

Declarations

Ethics approval and consent to participate
This research was conducted in line with ethical principles. Before beginning the research, the Medical Research Institute, Alexandria University Ethical Committee, granted clearance on September 27, 2022, with approval serial number E/C.S/N.R9/2022.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Pharmacology and Experimental Therapeutics Department, Medical Research Institute, Alexandria University, Alexandria, Egypt. [2]Cancer Management and Research Department, Medical Research Institute, Alexandria University, Alexandria, Egypt. [3]Pathology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt. [4]Medical Biochemistry Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt. [5]Tropical Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

Received: 15 April 2024 Accepted: 20 June 2024
Published online: 12 July 2024

References

1. World Health Organization (2020) Data visualization tools for exploring the global cancer burden in 2020. World Health Organization, Geneva, Switzerland
2. International Agency for Research on Cancer (2018) A Tool That Predicts the Future Cancer Incidence and Mortality Burden Worldwide from the Current Estimates in 2018 Up Until 2040. International Agency for Research on Cancer (IARC)
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424
4. Sim H-W, Knox J (2018) Hepatocellular carcinoma in the era of immunotherapy. Curr Probl Cancer 42(1):40–48
5. Alqahtani A, Khan Z, Alloghbi A, S. Said Ahmed T, Ashraf M, M. Hammouda D (2019) Hepatocellular carcinoma: molecular mechanisms and targeted therapies. Medicina 55(9):526
6. Llovet J, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M et al (2016) Hepatocellular carcinoma Nat Rev Dis Primers 2:16018
7. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J-F et al (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359(4):378–390
8. Cervello M, Bachvarov D, Lampiasi N, Cusimano A, Azzolina A, McCubrey JA et al (2012) Molecular mechanisms of sorafenib action in liver cancer cells. Cell Cycle 11(15):2843–2855
9. Sonbol MB, Riaz IB, Naqvi SAA, Almquist DR, Mina S, Almasri J et al (2020) Systemic therapy and sequencing options in advanced hepatocellular carcinoma: a systematic review and network meta-analysis. JAMA oncology. 6(12):e204930
10. Munoz M, Covenas R (2013) Involvement of substance P and the NK-1 receptor in cancer progression. Peptides 48:1–9
11. Munoz M, Rosso M, Robles-Frias MJ, Salinas-Martín MV, Rosso R, González-Ortega A et al (2010) The NK-1 receptor is expressed in human melanoma and is involved in the antitumor action of the NK-1 receptor antagonist aprepitant on melanoma cell lines. Lab Invest 90(8):1259–1269
12. Berger M, Neth O, Ilmer M, Garnier A, Salinas-Martín MV, de Agustín Asencio JC et al (2014) Hepatoblastoma cells express truncated neurokinin-1 receptor and can be growth inhibited by aprepitant in vitro and in vivo. J Hepatol 60(5):985–994
13. Muñoz M, Coveñas R, Esteban F, Redondo M (2015) The substance P/NK-1 receptor system: NK-1 receptor antagonists as anti-cancer drugs. J Biosci 40:441–463
14. Majkowska-Pilip A, Halik PK, Gniazdowska E (2019) The significance of NK1 receptor ligands and their application in targeted radionuclide tumour therapy. Pharmaceutics 11(9):443
15. Aapro M, Carides A, Rapoport BL, Schmoll H-J, Zhang L, Warr D (2015) Aprepitant and fosaprepitant: a 10-year review of efficacy and safety. Oncologist 20(4):450–458
16. McAteer J, Davies J (1994) Basic cell culture technique and the maintenance of cell lines' in DAVIS, JM (Ed.):'basic cell culture. A practical approach'. IRL Press at Oxford University Press, Oxford, New York, Tokyo
17. Van Meerloo J, Kaspers GJ, Cloos J (2011) Cell sensitivity assays: the MTT assay. Cancer Cell Cult Methods Protoc 237–245
18. Bijnsdorp IV, Giovannetti E, Peters GJ (2011) Analysis of drug interactions. Cancer Cell Cult Methods Protoc 11:421–434
19. Carbajo-Pescador S, Ordoñez R, Benet M, Jover R, García-Palomo A, Mauriz J et al (2013) Inhibition of VEGF expression through blockade of Hif1α and STAT3 signalling mediates the anti-angiogenic effect of melatonin in HepG2 liver cancer cells. Br J Cancer 109(1):83–91
20. Granado-Serrano AB, Martiín MA, Bravo L, Goya L, Ramos S (2006) Quercetin induces apoptosis via caspase activation, regulation of Bcl-2, and inhibition of PI-3-kinase/Akt and ERK pathways in a human hepatoma cell line (HepG2). J Nutr 136(11):2715–2721
21. Smith PE, Krohn RI, Hermanson G, Mallia A, Gartner F et al (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150(2):76–85
22. Zhu P, Liu Z, Zhou J, Chen Y (2018) Tanshinol inhibits the growth, migration and invasion of hepatocellular carcinoma cells via regulating the PI3K-AKT signaling pathway. Onco Targets Ther 12:87–99
23. Muhammad RN, Sallam N, El-Abhar HS (2020) Activated ROCK/Akt/eNOS and ET-1/ERK pathways in 5-fluorouracil-induced cardiotoxicity: modulation by simvastatin. Sci Rep 10(1):14693
24. Hutz JE, Manning WA, Province MA, McLeod HL (2011) Genomewide analysis of inherited variation associated with phosphorylation of PI3K/AKT/mTOR signaling proteins. PLoS ONE 6(9):e24873
25. Fang L, Liu J, Wan L, Zhu F, Tan B, Zhang P (2016) Xinfeng capsule improves hypercoagulative state by inhibiting miR-155/NF-κB signaling pathway in patients with active ankylosing spondylitis. Xi bao yu fen zi Mian yi xue za zhi 32(8):1094–8
26. Chen X-Y, Ru G-Q, Ma Y-Y, Xie J, Chen W-Y, Wang H-J et al (2016) High expression of substance P and its receptor neurokinin-1 receptor in colorectal cancer is associated with tumor progression and prognosis. Onco Targets Ther 6:3595–3602
27. Youssef MM, Tolba MF, Badawy NN, Liu AW, El-Ahwany E, Khalifa AE et al (2016) Novel combination of sorafenib and biochanin-a synergistically enhances the anti-proliferative and pro-apoptotic effects on hepatocellular carcinoma cells. Sci Rep 6(1):30717
28. Muñoz M, Coveñas R (2020) The neurokinin-1 receptor antagonist aprepitant: an intelligent bullet against cancer? Cancers 12(9):2682
29. Robinson P, Rosso M, Muñoz M (2023) Neurokinin-1 receptor antagonists as a potential novel therapeutic option for osteosarcoma patients. J Clin Med 12(6):2135
30. Muñoz M, Rosso M, Coveñas R (2019) Neurokinin-1 receptor antagonists against hepatoblastoma. Cancers 11(9):1258
31. Yang Y, Cao X, Wang Y, Wu X, Zhou P, Miao L et al (2023) Neurokinin-1 receptor antagonist aprepitant regulates autophagy and apoptosis through ROS/JNK pathway in intrahepatic cholangiocarcinoma. Res Square 23:1–25
32. Mendoza MC, Er EE, Blenis J (2011) The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends Biochem Sci 36(6):320–328
33. Cao X, Yang Y, Zhou W, Wang Y, Wang X, Ge X et al (2023) Aprepitant inhibits the development and metastasis of gallbladder cancer via ROS and MAPK activation. BMC Cancer 23(1):471
34. Javid H, Asadi J, Zahedi Avval F, Afshari AR, Hashemy SI (2020) The role of substance P/neurokinin 1 receptor in the pathogenesis of esophageal squamous cell carcinoma through constitutively active PI3K/Akt/NF-κB signal transduction pathways. Mol Biol Rep 47:2253–2263
35. Zhang Y, Li G, Liu X, Song Y, Xie J, Li G et al (2018) Sorafenib inhibited cell growth through the MEK/ERK signaling pathway in acute promyelocytic leukemia cells. Oncol Lett 15(4):5620–5626
36. Lu M, Fei Z, Zhang G (2018) Synergistic anticancer activity of 20 (S)-ginsenoside Rg3 and sorafenib in hepatocellular carcinoma by modulating PTEN/Akt signaling pathway. Biomed Pharmacother 97:1282–1288

37. Qi Y, Song Y, Cai M, Li J, Yu Z, Li Y et al (2023) Vascular endothelial growth factor A is a potential prognostic biomarker and correlates with immune cell infiltration in hepatocellular carcinoma. J Cell Mol Med 27(4):538–552
38. Alsaeed MA, Ebrahimi S, Alalikhan A, Hashemi SF, Hashemy SI (2022) The Potential In Vitro Inhibitory Effects of Neurokinin-1 Receptor (NK-1R) Antagonist, Aprepitant, in Osteosarcoma Cell Migration and Metastasis. Biomed Res Int 2022:8082608
39. Momen Razmgah M, Ghahremanloo A, Javid H, AlAlikhan A, Afshari A-R, Hashemy SI (2022) The effect of substance P and its specific antagonist (aprepitant) on the expression of MMP-2, MMP-9, VEGF, and VEGFR in ovarian cancer cells. Mol Biol Rep 49(10):9307–9314
40. Chiang I-T, Liu Y-C, Wang W-H, Hsu F-T, Chen H-W, Lin W-J et al (2012) Sorafenib inhibits TPA-induced MMP-9 and VEGF expression via suppression of ERK/NF-κB pathway in hepatocellular carcinoma cells. In Vivo 26(4):671–681
41. Chuang H-Y, Tyan Y-S, Hwang J-J, Shih K-C, Lin W-C (2021) A combination of sorafenib and radiotherapy reduces NF-κB activity and growth of hepatocellular carcinoma in an orthotopic mouse model. Oncol Lett 21(4):1–9
42. Yang Y, Zhou W, Xu X, Ge X, Wang F, Zhang G-Q et al (2022) Aprepitant inhibits JNK and p38/MAPK to attenuate inflammation and suppresses inflammatory pain. Front Pharmacol 12:811584
43. Raslan E, AbdAllah M, Soliman S (2022) The prevalence and determinants of hepatitis B among Egyptian adults: a further analysis of a country-representative survey. Egyptian Liver Journal 12(1):46
44. Farahat Allam A, Fahmy Farag H, Youssef Shehab A, Soliman El Sahy A, Saleh Khalil S, et al (2023) Schistosoma Mansoni infection and hepatocellular carcinoma: A co-morbidity study. J Clin Images Med Case Rep 4(8):2527
45. Faitot F, Allard MA, Pittau G, Ciacio O, Adam R, Castaing D et al (2015) Impact of clinically evident portal hypertension on the course of hepatocellular carcinoma in patients listed for liver transplantation. Hepatology 62(1):179–187
46. Lin C-W, Chen Y-S, Lin C-C, Lee P-H, Lo G-H, Hsu C-C et al (2018) Significant predictors of overall survival in patients with hepatocellular carcinoma after surgical resection. PLoS ONE 13(9):e0202650
47. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH et al (2012) Trial watch: prognostic and predictive value of the immune infiltrate in cancer. Oncoimmunology 1(8):1323–1343

Publisher's Note