Original article

In vitro and in silico study on the effect of carvedilol and sorafenib alone and in combination on the growth and inflammatory response of melanoma cells

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ABSTRACT

Melanoma is an aggressive skin cancer. Increasing evidence has shown the role of β-adrenergic receptors in the pathogenesis of melanoma. Carvedilol is a widely used non-selective β-AR antagonist with potential anticancer activity. The purpose of the study was to estimate the influence of carvedilol and sorafenib alone and in combination on the growth and inflammatory response of C32 and A2058 melanoma cells. Furthermore, this study also aimed to predict the probable interaction of carvedilol and sorafenib when administered together. Predictive study of the interaction of carvedilol and sorafenib was performed using the ChemDIS-Mixture system. Carvedilol and sorafenib alone and in combination showed a growth inhibitory effect on cells. The greatest synergistic antiproliferative effect on both cell lines was observed at Car 5 μM combined with Sor 5 μM. Analysis *in silico* identified diseases, proteins, and metabolic pathways that can be affected by the interaction of carvedilol and sorafenib. The results obtained demonstrated that carvedilol and sorafenib modulated the secretion of IL-8 by IL-1β-stimulated by melanoma cell lines but the use of a combination of both drugs did not intensify the effect. In summary, the results presented indicate that the combination of carvedilol and sorafenib may have a promising anticancer effect on melanoma cells.

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1. Introduction

Cancers are among the most significant human health problems (Winitchaikul et al., 2021). Malignant melanoma is one of the most aggressive and chemotherapy resistant cancers (de Giorgi 2020). The incidence of new melanomas is increasing annually in numerous countries around the world (Rogers et al., 2015; Winitchaikul et al., 2021). In Poland, a total of 3689 new cases of melanoma and 1464 deaths from this disease were reported. The incidence during 2009 through 2019 for melanoma continued to increase (from 2562 to 3689 cases per year). Mortality patterns reflect trends in incidence, with increases for melanoma (Didkowska et al., 2009, 2019). The main causative agent for melanoma is ultra-violet (UV) radiation that causes DNA damage and synthesis of reactive oxygen species (Narendhirakannan and Hannah, 2013). Recent evidence also indicates a possible important role for chronic stress, involving environmental and psychological factors, in the development and spreading (Sanzo et al., 2010; Sinnya and De'Ambrosis, 2013).

Multiple studies have demonstrated the association between chronic stress or depression with various cancer pathogenesis and development (Mössinger and Kostev, 2023; Yang et al., 2019). Such effects can be partially mediated by stimulation of the autonomic nervous system that results in the secretion of norepinephrine (NE) and epinephrine (Epi). The effects of NE and Epi are mediated by stimulation of α- and β-adrenergic receptors (AR). Increasing experimental evidence indicates the role both Epi and NE in cancer progression and metastasis (Mravec et al., 2020). In particular, stimulation of β-AR signaling causes induction of cell proliferation, MMP synthesis, and the release of proangiogenic cytokines; therefore, it was suggested that β-AR antagonist drugs (β-blockers) may be protective against cancer progression. Recent retrospective studies linked the use of β-blockers with clinical benefits in cancer patients (Caparica et al., 2021; Powe and Entschladen, 2011). The positive correlation between β-blocker

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usage and outcome in malignancies has been shown in various cancers including ovarian (Watkins et al., 2015) and breast (Melhem-Bertrandt et al., 2011) cancer. Several studies demonstrated that β-blockers improve the outcomes of patients with melanoma and decrease the risk of recurrence and death of this disease (Lemeshow et al., 2011; Vojvodic et al., 2019). Melanoma is an immunogenic cancer expressing both α- and β- adrenergic receptors which can be activated during stress. The role of α1-AR expressed by melanoma cells is marginal, but β-ARs appear to play a greater role in the pathogenesis of melanoma (Colucci and Moretti, 2016; Sanzo et al., 2010).

Carvedilol is a commonly used non-selective β-AR antagonist (β-blocker) with α1-AR blocking activity It is recommended for the treatment of congestive heart failure and hypertension with some biological activities that are of interest in a cancer treatment context (Avila et al., 2018). Some studies showed that carvedilol treatment may reduce cancer risk (Gillis et al., 2021; Lin et al., 2015). Various pathways have been suggested to mediate the β-adrenergic signaling on tumour progression, including angiogenesis and inflammation (Chung et al., 2016). Since the β-ARs are involved in the pathogenesis of melanoma, it is interesting to find out whether carvedilol might influence melanoma cells alone or in cotreatment with anticancer drugs.

Sorafenib is a drug approved for the treatment of hepatocellular, renal cell, and thyroid carcinoma (Zhu, 2009). Its mechanisms of action involve the inhibition of Raf-1 and B-Raf serine/threonine kinases and receptor tyrosine kinases that participate in angiogenesis and tumour growth; however, sorafenib is not clinically used in melanoma in monotherapy due to its low efficacy.

Although several studies have illustrated the effectiveness of carvedilol and sorafenib as anticancerous agents individually, the combinatorial impact of these compounds has not yet been explored. The purpose of the study was to evaluate the influence of carvedilol and sorafenib alone and in combination on the growth of amelanotic and melanotic melanoma cells *in vitro*. Furthermore, the combined effect of carvedilol and sorafenib on human melanoma cells was also assessed by targeting the inflammatory response. This study also aimed to predict the possible interaction of carvedilol and sorafenib when administered together, as well as the pathways, diseases, and related genes that were influenced by the interaction of both drugs. This *in silico* predictive study was performed with the use of the ChemDIS-Mixture system.

2. Methods

2.1. Cell lines and culture conditions

Two human malignant melanoma cell lines were tested: the amelanotic C32 cell line and the melanotic A2058 cell line. C32 and A2058 cells (ATCC®, LGC Standards, Łomianki, Poland). Cells were cultured as monolayers in MEM medium (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (BioWest, Nualillé, France), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C, 5% CO_2. Before each experiment, cells were detached with 0.25% trypsin with 0.02% EDTA (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Preparation of drug solutions

Carvedilol (Car) and sorafenib (Sor)(Cayman Chemical, Ann Arbor, MI, USA) were dissolved in DMSO (Sigma-Aldrich, St. Louis, MO, USA) to obtain the stock solution (100 mM). Before use, the stock solutions were diluted in sterile culture medium to the concentrations studied. The concentration of DMSO in the culture media with pterostilbene was 0.01%. Control cells were treated with the same concentration of DMSO.

2.3. Cell growth assay

The influence of drugs studied alone and in combination on the growth of melanoma cells was analyzed by I*n Vitro Toxicology Assay Kit, Sulforhodamine* B (SRB) *based* (Sigma Aldrich). Cells were plated into 96-well plates at 2×10^3 cells per well. After 24 h, cultures were exposed to carvedilol (0.1–10 μM) and sorafenib (0.1–10 μM) alone and in combination. The growth of melanoma cells was measured after 72 h according to the manufacturer's protocol and expressed as a percentage of control. Car and Sor concentrations that inhibited cell growth by 50% (IC_{50}) compared to controls was determined by fitting a four-parameter logistic model (Hill equation) (GraphPad Prism version 7, San Diego, USA).

2.4. In silico analyse

The integrated chemogenomics-based system for analysing interacting proteins ChemDIS-Mixture version 2.4 (https://cw-tung.nhri.edu.tw/chemdis/) was used for the analysis of the potential effects of coexposure cancer cells to carvedilol and sorafenib. Enrichment analysis tools were applied and incorporated into ChemDIS-Mixture in order to evaluate functions (GO, gene ontology), pathways, and diseases (DO, disease ontology) from a given set of differentially expressed genes (Tung et al., 2018). For Car and Sor alone and in combination, probable interacting proteins were determined. Performed analyses are based upon hypergeometric tests with the Benjamini–Hochberg method for multiple-testing corrections with a corrected p-value < 0.05 (Du et al., 2009). For the prioritization of potential interaction effects, the joint p-value (Pjoint) was used, where p is the value for drug alone (Tung et al., 2018). The Pjoint value corresponds to the overall significance of the effect caused by carvedilol and sorafenib simultaneously.

2.5. IL-8 secretion

Melanoma cells were seeded in 24-well plates at a density of 1.5×10^5 cells. After 24 h, media were changed to media with 2% FBS and 10 ng/ml IL-1β and then cultured for 24 h. The cells were exposed to media with 10 ng/ml IL-1β and the drugs studied (carvedilol and sorafenib alone or in combination). Separate cultures were incubated only with media without IL-1β. The plates were then incubated for 48 h. IL-8 concentrations in the culture media were evaluated using the ELISA MAX™ Deluxe set (Biolegend Inc., San Diego, CA, USA) according to the producer's recommendations. The absorbances were measured with a Labtech LT-5000 plate reader. Cells were lysed in CelLytic (Sigma-Aldrich Co., St. Louis, MO) on ice for 15 min and centrifuged. In each collected supernatant, the concentration of total cellular protein was determined using the Bradford reagent (Sigma-Aldrich) according to the manufacturer's instruction. The IL-8 concentrations were normalized to the protein and shown as pg/mg protein.

2.6. Statistical analysis

All experiments were performed in triplicate. The values obtained are presented as means ± SD. All statistical analysis used the Statistica PL ver. 12.0 Software (StatSoft). To evaluate the distribution of the results Shapiro-Wilk test was done. One-way ANOVA with NIR's test was used for statistical comparisons. For all tests, the p-value of < 0.05 was considered statistically significant.

3. Results

3.1. The effect of Car and Sor on melanoma cells

The experiments first focused on the response of amelanotic (C32) and melanotic (A2058) melanoma cells to treatment with carvedilol and sorafenib. Cells were incubated with increasing concentrations of carvedilol or sorafenib (0.1–10 μM) for 72 h.

As shown in Fig. 1, carvedilol at concentrations ≤ 2.5 μM did not affect the growth of C32 and A2058 cells. The statistically significant decrease in the growth of both cell lines was evoked by carvedilol at higher concentrations (≥5 μM). Melanotic A2058 cells were more sensitive to carvedilol than amelanotic C32 cells, causing 98% inhibition at a concentration of 10 μM carvedilol, while in C32 cell cultures the inhibition was 62% in comparison to the control. The IC50 of 8.07 μM (C32 cells) and 5.85 μM (A2058 cells) showed their different sensitivity to carvedilol.

The effect of Sor on melanoma cells is shown in Fig. 2. C32 and A2058 cells exposed to sorafenib resulted in a substantial, dose-dependent decrease in growth. Sorafenib at concentrations of 0,1 and 1 μM did not influence the growth of C32 cells. However, it significantly decreased C32 cell growth at concentration ≥ 2.5 mM cells in a dose-dependent manner. Sorafenib at concentrations up to 2.5 μM did not affect A2058 cell growth. A significant reduction was observed in cultures exposed to higher concentrations (5 and 10 μM). For the C32 cell line, the IC50 was 6.67 μM. A higher IC value was obtained for A2058 cells (7.63 μM). Based on these results for further research, we decided to choose concentrations 2.5 and 5 μM for carvedilol as well as 1 and 5 μM for sorafenib.

3.2. The effect of carvedilol and sorafenib in combination on the growth of C32 and A2058 cells

To explore the possible synergistic effect of Car in combination with Sor on inhibition of melanoma cell growth, C32 and A2058 cells were treated with carvedilol (2.5; 5 μM) and sorafenib (1; 5 μM) in different combinations (Car2.5 + Sor1; Car5 + Sor1; Car2.5 + Sor5; Car5 + Sor5) as well as individually for 72 h. Both drugs in monotherapy and combination therapy inhibited melanoma cell growth (Fig. 3). The entire combination of carvedilol and sorafenib caused a statistically significant reduction in cell growth compared with control except for the combination Car2.5 + Sor1. The combination of two drugs led to a greater inhibition of cell viability than carvedilol or sorafenib alone in C32 cultures incubated with both drugs at concentration of 5 μM (Fig. 3A). In A2058 cell cultures such an effect was only observed in cultures treated with carvedilol and sorafenib at the concentration of 5 μM (Fig. 3B). The results obtained showed that the combinatorial drugs showed a synergistic repressive influence on the growth of melanoma cells and it varied in different concentrations of the drugs and the cell line. The types of drug interactions were calculated using CI (Combination Index). The CI above 1 represents an antagonistic effect, CI equal 1 indicates an additive effect, and CI below 1 indicates a synergistic effect of drugs. CI values for all combinatorial drug pairs used are shown in Table 1. The results showed that the synergistic inhibitory effect on C32 cells had the Car2.5 + Sor5 and Car5 + Sor5 combinations and the experimental CI values obtained for these combinations were 0.62 and 0.57 respectively. The melanotic A2058 cells were less susceptible to the combinatory effect of Car and Sor than the amelanotic C32 cells. As shown in Table 1, inhibition of A2058 cell growth was considerably enhanced only after treatment with carvedilol and sorafenib at the concentration of 5 μM (CI = 0.84).

3.3. In silico prognosis of the interaction of carvedilol and sorafenib via ChemDIS-mixture

In order to better predict the possible mechanisms of action causing the observed results, we analyzed the chemical-chemical interactions of both drugs with the use an online tool ChemDIS-Mixture. The analysis identified 845 proteins that can be influenced by carvedilol or/and sorafenib (Fig. 4). Of these, 336 are targeted only by carvedilol, 488 only by sorafenib, and 21 are shared by both studied drugs (carvedilol and sorafenib) (Table 2).

Among the proteins that interact with both drugs, there are molecules related to the regulation of proliferation and apoptosis (eg MCL1 apoptosis regulator, BCL2 antagonist), MAP kinase pathway (mitogen-activated kinase 1, 3 and 14), transcription factors (STAT3, hypoxia-inducible factor alpha subunit) and vascular endothelial growth factor VEGF.

The ontology analyze gene (GO) was also performed to obtain an overview of the essential pathways influenced by the potential
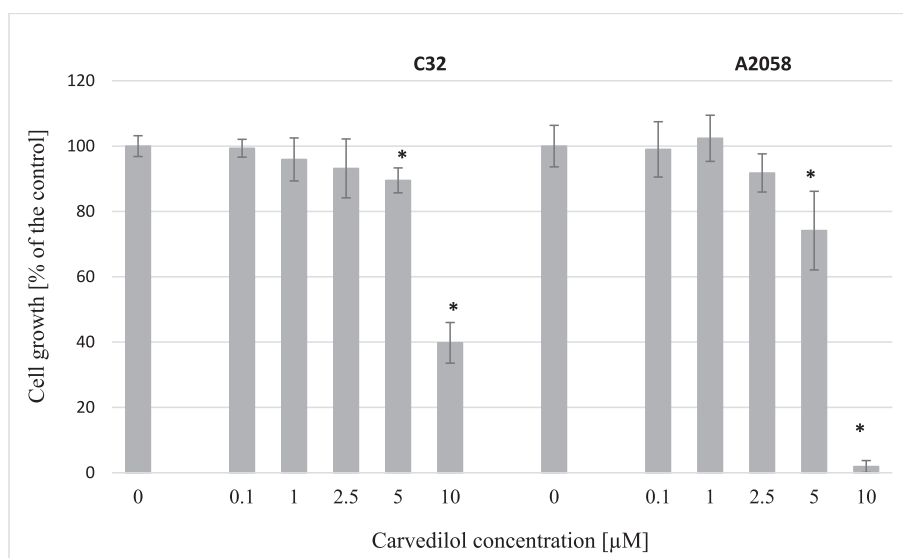

Fig. 1. The growth inhibitory influence of carvedilol on melanoma cells after 72 h. The results are expressed as a percentage of untreated control (the means ± SD; *p < 0.05 vs. control).
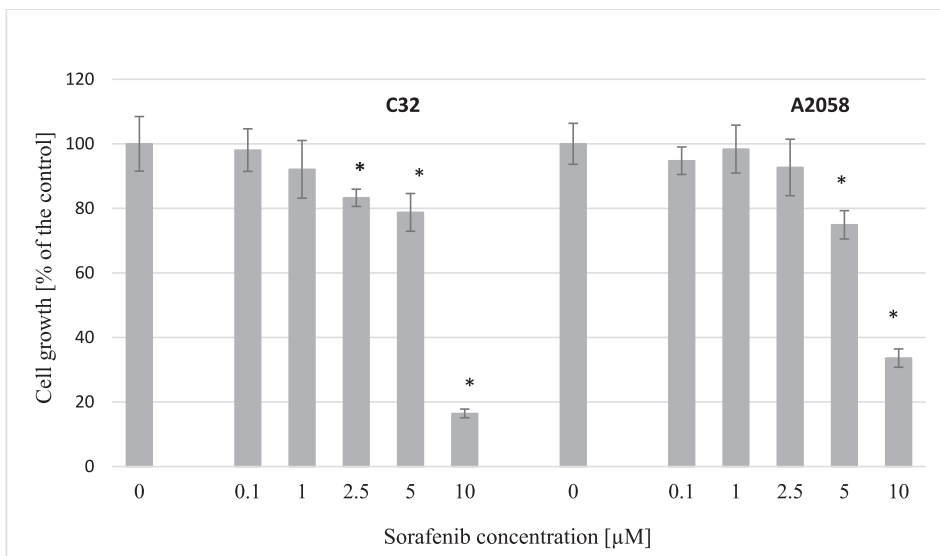
Fig. 2. Influence of sorafenib on the growth of melanoma C32 and A2058 cells after 72 h. The results are expressed as a percentage of untreated control (the means ± SD; *p < 0.05 vs. control).
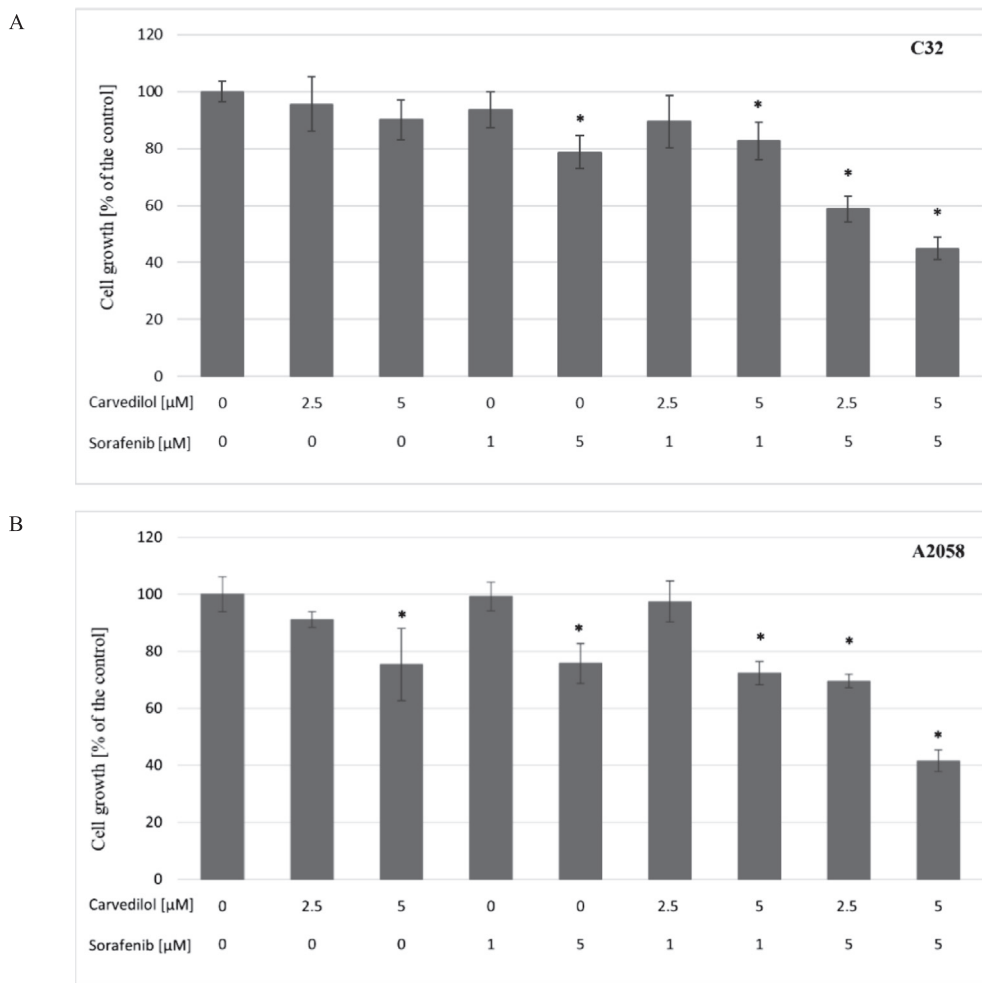

Fig. 3. Growth inhibitory effect of carvedilol and sorafenib or both drugs simultaneously at various concentrations on C32 (A) and A2058 (B) cells after 72 h. The results are expressed as a percentage of untreated control (the means ± SD; *p < 0.05 vs. control).

Table 1
Combination Index (CI) values of drug combinations in melanoma C32 and A2058 cells.

Cell line	Drugs	CI value
C32 cell line	Car (2.5 μM) + Sor (1 μM)	1.01
	Car (5 μM) + Sor (1 μM)	0.94
	Car (2.5 μM) + Sor (5 μM)	0.62
	Car (5 μM) + Sor (5 μM)	0.57
A2058 cell line	Car (2.5 μM) + Sor (1 μM)	3.80
	Car (5 μM) + Sor (1 μM)	0.92
	Car (2.5 μM) + Sor (5 μM)	1.09
	Car (5 μM) + Sor (5 μM)	0.84

interaction with carvedilol and sorafenib. The analysis recognized 1744 drugs-responsive genes: 1086 to sorafenib, 430 to carvedilol, and 228 shared between both drugs (Fig. 4). The summarized

information is presented in Table 3. The results obtained indicated that an interaction of carvedilol and sorafenib is mainly related to proliferation, apoptosis, intracellular transduction pathways, and inflammatory response. The prediction of the main pathways affected by the simultaneous use of carvedilol and sorafenib was also performed using the ChemDIS-Mixture pathway tool. The results obtained showed 25 shared metabolic pathways indicating that the combination of these studied drugs influences the signaling pathways connected with IL (e.g. IL-4, IL-10), chemokines as well as modulates activity of kinases such as MAP, ERK, PI3K, PKC (Tab. 3). Based on these results, it seems important to study the influence of cotreatment of carvedilol and sorafenib on inflammation.

The main objective of the study was to evaluate the combinatorial impact of carvedilol and sorafenib on melanoma cells.
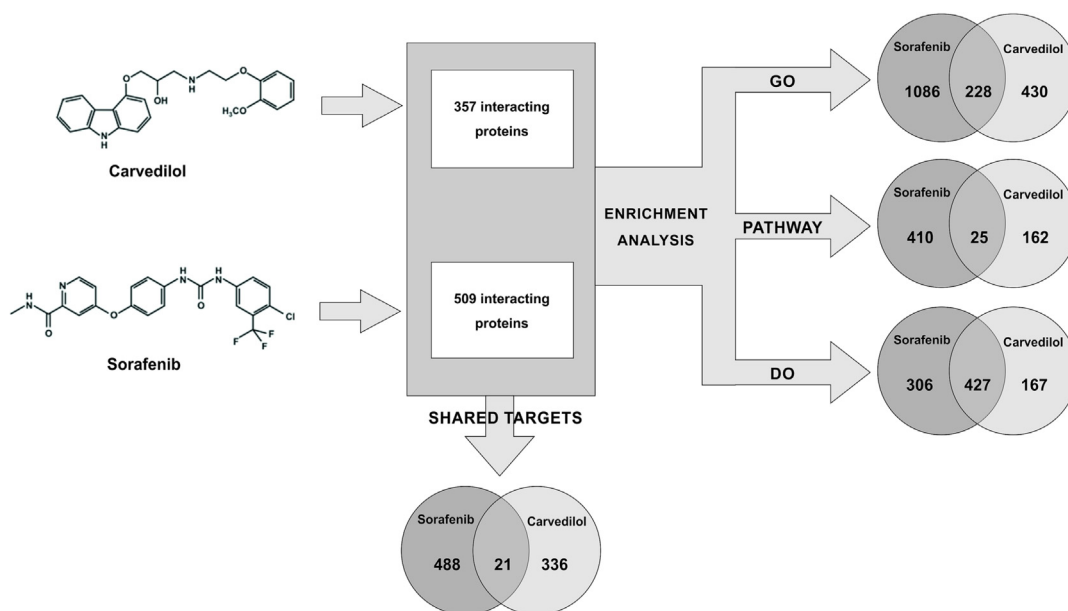

Fig. 4. Diagrammatic representation of the ChemDIS-Mixture analysis for carvedilol and sorafenib.

Table 2
List of common protein interacting with carvedilol and sorafenib simultaneously.

Ensembl protein ID	Gene Symbol	Gene name	Gene ID	Score sorafenib	Score carvedilol
ENSP00000358022	TM	MCL1 apoptosis regulator (BCL2 family apoptosis regulator)	4170	0.979	0.208
ENSP00000215832	p41	mitogen-activated protein kinase 1	5594	0.964	0.700
ENSP00000264657	STAT3	signal transducer and activator of transcription 3	6774	0.962	0.183
ENSP00000361125	VEGFA	vascular endothelial growth factor A	7422	0.909	0.816
ENSP00000229794	RK	mitogen-activated protein kinase 14	1432	0.878	0.170
ENSP00000265724	ABCB1	ATP binding cassette subfamily B member 1	5243	0.854	0.747
ENSP00000304845	UDPGT	UDP glucuronosyltransferase family 1 member A1	54,658	0.830	0.700
ENSP00000263025	ERK-1	mitogen-activated protein kinase 3	5595	0.822	0.700
ENSP00000329623	BCL2	BCL2, apoptosis regulator	596	0.784	0.208
ENSP00000353878	BAK1	BCL2 antagonist/killer 1	578	0.764	0.208
ENSP00000221930	TGFB1	transforming growth factor beta 1	7040	0.700	0.150
ENSP00000302564	Bcl-X	BCL2 like 1	598	0.447	0.208
ENSP00000258400	HTR2B	5-hydroxytryptamine receptor 2B	3357	0.410	0.710
ENSP00000338018	HIF1A	hypoxia inducible factor 1 alpha subunit	3091	0.295	0.800
ENSP00000386884	WHIMS	C-X-C motif chemokine receptor 4	7852	0.293	0.274
ENSP00000314132	BOK	BOK, BCL2 family apoptosis regulator	666	0.239	0.208
ENSP00000267953	HBPA1	BCL2-related protein A1	597	0.239	0.346
ENSP00000309132	BCLG	BCL2 like 14	79,370	0.239	0.208
ENSP00000276198	HTR1C	5-hydroxytryptamine receptor 2C	3358	0.180	0.609
ENSP00000310036	CD34	CD34 molecule	947	0.174	0.189
ENSP00000219070	MMP2	matrix metallopeptidase 2	4313	0.159	0.800

Table 3

The selected results of enrichment analysis for investigating the interaction between carvedilol and sorafenib with the use of gene ontology (GO), pathways and disease ontology (DO) tools.

GO analysis							
ID	Description	Gene Ratio	Adj. P sorafenib	Gene Ratio	Adj. P carvedilol	Adj. Pjoint	
GO:0005887	integral component of plasma membrane	72/491	7.29E-07	148/344	3.57E-72	2.60E-78	
GO:0005886	plasma membrane	146/491	1.82E-04	213/344	4.67E-56	8.51E-60	
GO:0042493	response to drug	43/489	1.33E-16	32/339	3.67E-13	4.89E-29	
GO:0070374	positive regulation of ERK1 and ERK2 cascade	33/489	1.47E-16	17/339	5.84E-07	8.55E-23	
GO:0046982	protein heterodimerization activity	55/488	7.27E-18	22/341	5.23E-04	3.80E-21	
GO:0010628	positive regulation of gene expression	36/489	2.11E-14	17/339	4.66E-05	9.85E-19	
GO:0008630	intrinsic apoptotic signaling pathway in response to DNA damage	15/489	1.51E-11	9/339	1.42E-06	2.14E-17	
GO:0043410	positive regulation of MAPK cascade	20/489	2.23E-12	10/339	2.63E-05	5.86E-17	
GO:0003924	GTPase activity	25/488	1.38E-07	21/341	7.30E-08	1.01E-14	
GO:0006915	apoptotic process	51/489	1.42E-12	18/339	2.73E-02	3.88E-14	
GO:0071456	cellular response to hypoxia	20/489	6.38E-11	8/339	1.80E-03	1.15E-13	
GO:0006954	inflammatory response	23/489	1.69E-03	32/339	1.21E-10	2.05E-13	
GO:0008283	cell proliferation	40/489	7.09E-12	13/339	3.37E-02	2.39E-13	
GO:0007166	cell surface receptor signaling pathway	17/489	4.64E-03	27/339	1.35E-10	6.24E-13	
GO:0005525	GTP binding	28/488	2.67E-05	27/341	8.76E-08	2.34E-12	
GO:0001666	response to hypoxia	19/489	2.08E-06	16/339	1.39E-06	2.90E-12	
GO:0042803	protein homodimerization activity	51/488	2.88E-08	27/341	3.42E-03	9.85E-11	
Pathway analysis							
ID	Description		Adj. P sorafenib	Adj. P carvedilol	Adj. Pjoint		
hsa04540	Gap junction		4.96E-24	0.001782	8.84E-27		
R-HSA-6785807	Interleukin-4 and 13 signalling		4.53E-15	3.71E-06	1.68E-20		
R-HSA-456926	Thrombin signalling through proteinase activated receptors (PARs)		0.030368	1.76E-13	5.33E-15		
hsa04062	Chemokine signalling pathway		5.06E-06	7.36E-08	3.72E-13		
R-HSA-450341	Activation of the AP-1 family of transcription factors		1.28E-09	0.003713	4.77E-12		
R-HSA-392451	G beta: gamma signalling through PI3Kgamma		0.010054	1.02E-08	1.03E-10		
R-HSA-5674499	Negative feedback regulation of MAPK pathway		1.84E-08	0.013159	2.42E-10		
R-HSA-982772	Growth hormone receptor signaling		6.50E-06	0.031663	2.06E-07		
R-HSA-111453	BH3-only proteins associate with and inactivate anti-apoptotic BCL-2 members		2.26E-05	0.02573	5.81E-07		
R-HSA-198753	ERK/MAPK targets		0.00055	0.013159	7.23E-06		
R-HSA-162658	Golgi Cisternae Pericentriolar Stack Reorganization		0.015389	0.0009	1.39E-05		
R-HSA-881907	Gastrin-CREB signalling pathway via PKC and MAPK		0.004948	0.002856	1.41E-05		
R-HSA-74749	Signal attenuation		0.006532	0.003713	2.43E-05		
R-HSA-6783783	Interleukin-10 signalling		0.010936	0.003713	4.06E-05		
DO analysis							
ID	Description		Adj. P sorafenib	Adj. P carvedilol	Adj. Pjoint		
DOID:1240	leukemia		8.68E-45	8.22E-04	7.13E-48		
DOID:0060083	immune system cancer		1.79E-44	1.34E-03	2.40E-47		
DOID:2531	hematologic cancer		5.63E-44	1.05E-03	5.90E-47		
DOID:1909	melanoma		9.02E-43	1.06E-04	9.52E-47		
DOID:0050615	respiratory system cancer		1.45E-41	6.76E-04	9.78E-45		
DOID:3119	gastrointestinal system cancer		1.81E-40	2.69E-04	4.88E-44		
DOID:1324	lung cancer		5.94E-41	1.02E-03	6.03E-44		
DOID:3905	lung carcinoma		4.12E-41	3.28E-03	1.35E-43		
DOID:1749	squamous cell carcinoma		1.45E-41	2.74E-02	3.96E-43		
DOID:0050687	cell type cancer		3.15E-41	1.86E-02	5.85E-43		

Therefore, we use the ChemDIS Mixture Disease Ontology (DO) tool to identify diseases for which the combination of carvedilol and sorafenib could be beneficial. In Table 3 are shown the ten most relevant DO terms. It is worth emphasizing that the results revealed the high probability of carvedilol and sorafenib interactions in various malignancies. Among the DO terms shared by both carvedilol and sorafenib melanoma is the fourth place out of 427 common disease entities.

3.4. The effect of carvedilol and sorafenib alone and in combination on interleukin 8

Based on the results obtained *in silico,* further studies of the influence of carvedilol-sorafenib on cytokine secretion are reasonable. IL-8 is an important multifunctional chemokine that is not normally produced by melanocytes, but modulates the pathogenesis of melanoma (Singh et al., 2010). The studied melanoma cells constitutively secreted IL-8 at a concentration of 65 ± 7 pg/mg protein (C32 cells) and 147 ± 13 pg/mg protein (A2058 cells), which confirms the potential of melanoma cells to secrete this chemoat-

tractant spontaneously. Basal IL-8 secretion by C32 and A2058 cells was significantly enhanced upon activation with IL-1β, cytokine that are engaged in cancer pathogenesis and invasiveness, to the amount of 9 539 ± 292 and 59 096 ± 6 782 pg/mg protein, respectively.

To clarify whether carvedilol and sorafenib modulate IL-8 secretion by IL-1β-stimulated melanoma cells the concentration of this chemokine secreted into culture media was examined. Treatment of C32 cells with carvedilol did not affect the IL-8 secretion from IL1β-stimulated cells (Fig. 5A). However, carvedilol had a statistically significant suppressive effect on IL-8 release by IL-1β-stimulated A2058 cells causing its 26.64% and 44.76% concentration-depended reduction (2.5 and 5 μM carvedilol, respectively) in relation to the control (Fig. 5B).

Sorafenib significantly decreased the release of IL-8 by both cell lines. Incubation of stimulated C32 cells with 1 and 5 μM sorafenib resulted in a progressive suppressive effect with respect to IL-8 secretion (Fig. 5a), as manifested by inhibition of 45.72% and 82.84%. Sorafenib also had an inhibitory influence on IL-8 release by IL-1β stimulated A2058 cells stimulated with IL-1β, i.e.,
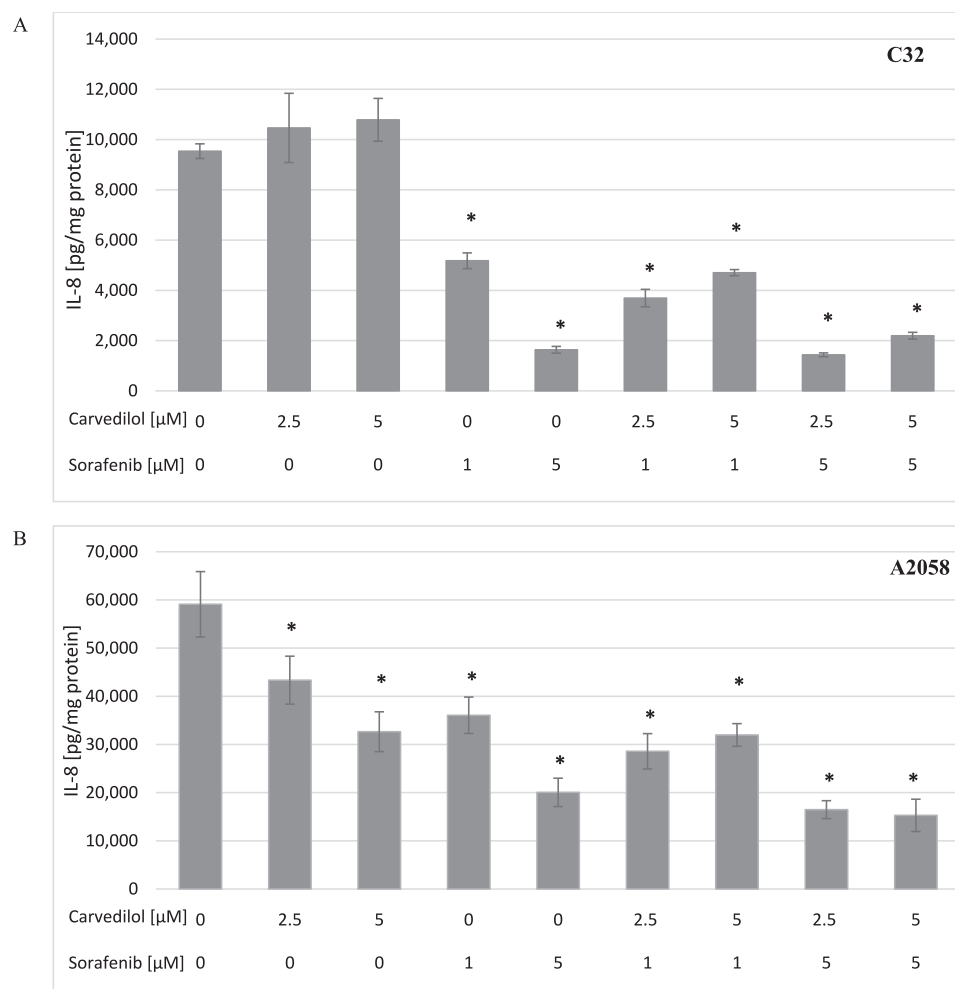
Fig. 5. IL-8 secretion by IL-1β- stimulated C32 (A) and A2058 (B) cells exposed to carvedilol and sorafenib, *p < 0.05 versus untreated cells.

34.13% (1 μM) and 72.48% (5 μM), respectively (Fig. 5B). The inhibitory effect of this drug was more pronounced against C32 cells.

As shown in Fig. 5, the influence of simultaneous treatment with carvedilol and sorafenib on IL-8 secretion was also studied. Under these conditions, IL-8 levels dropped below the monitored levels following the treatment C32 and A2058 cells with IL-1β alone.

Incubation of IL-1β-stimulated C32 cells with 2.5 μM carvedilol together with 1 μM sorafenib significantly potentiate the inhibition of IL-8 expression compared to cultures incubaed with 1 μM sorafenib (p = 0.027). However, exposure of these cells to 5 μM carvedilol together with 1 μM sorafenib did not pronounced the inhibitory effect of these drugs on IL-8 release (Fig. 5A). Incubation of stimulated C32 cells with 5 μM sorafenib alone or 5 μM sorafenib and carvedilol caused a strong inhibition of IL-8 (>76%), however, there were no significant differences between IL and 8 concentrations in media collected from cultures incubated with 5 μM sorafenib alone and with Car2.5 + Sor5 and Car5 + Sor5 (Fig. 5A).

Cotreatment of IL-1β-stimulated A2058 cells with carvedilol with sorafenib had a supressive effect on IL-8 realesed in relation to cells exposed to sorafenib alone (Fig. 5B). However, the differences among the inhibitory effects of sorafenib alone and sorafenib plus carvedilol were not statistically significant.

The results obtained demonstrated that carvedilol and sorafenib modulated IL-8 expression at protein level in IL-1β-stimulated A2058 and C32 cell lines. However, the use of a combination of both drugs did not intensify the effect on secretion compared to when the drugs were used separately.

4. Discussion

Drug repurposing is a relatively new concept of applying known drugs to new indications for purposes different from their main indication. Not only does it shorten the time required for the clinical application of new drugs based on the results of drug and toxicology tests, but it also generates lower costs. Therefore, current research focusses on the search for known drugs that show anticancer activity, as well as the possibility of their use in combination therapy with drugs already used in oncological therapy. Combination therapy is widely used in the treatment of various diseases and relies on the positive consequences of pharmacodynamic interactions (additive or synergistic) between drugs, causing more efficient cures. In this therapy, drugs are used in lower quantities and interact with different molecular paths. The advantages of combination therapy include decreasing the doses of drugs, their toxicity, and the improvement of effectiveness (Kamran et al., 2022).

Therefore, this study aimed to estimate the antineoplastic action of carvedilol, a well-known drug, used for years in clinical practice for medical indications other than cancer, as well as the possible synergistic interaction with the drug used in oncological

therapy. Carvedilol is one of the most commonly used and tolerable β-blocker for hypertension and arrhythmic. Studies have confirmed that carvedilol and others β-blockers show new possible clinical uses in cancer prevention and treatment, which seems very useful (Lin et al., 2015; Peixoto et al., 2020). The effect of antagonist of β-AR receptors on cancer development involved inhibition of cancer cell growth, as well as vascular events (Qiao et al., 2018; Tang et al., 2013). It also have presented that carvedilol inhibits the growth and proliferation of numerous cancer cells *in vitro*. Stanojkovic et al. (Stanojkovic et al., 2005) have shown that carvedilol at concentrations of 0.1 μM to 50 μM inhibited the growth of MDA-MB-361 breast cancer, HeLa cervical cancer, and myelogenous leukemia K562 cells with the IC values 35.04 μM, 30.56 μM and 22.66 μM, respectively. Other reports showed that carvedilol suppressed the glioma, colon, and breast cancer cells growth and invasion (Coelho et al., 2015; Erguven, 2010; Ma et al., 2018). The cancer-related effects of carvedilol against melanoma have not been extensively studied. Since stress-related catecholamines have been shown to maintain the progression of melanoma, the possibility of using β-blockers as a therapeutic intervention for melanoma drugs in cancer is gaining attention. Despite many studies that confirm the antitumor effect of other non-selective β-blocker propranolol against melanoma cells (Zhou et al., 2016), reports of the effects on these cells are limited. The current study establishes the growth inhibitory effect of carvedilol at concentrations ≥ 5 μM on C32 amelanotic cells and A2058 melanotic melanoma cells after 72 h of treatment. Furthermore, melanotic A2058 cells were more sensitive to carvedilol than amelanotic cells. The results obtained correspond to those published by other researchers. Stanjkovic et al. (Stanojkovic et al., 2005) showed an inhibition of FemX melanoma cell growth with the IC50 value 32.17 μM after 48 h exposure to carvedilol. The weaker effect of this drug on melanoma cells (higher IC50 values) could probably be due to a shorter treatment incubation period (48 h). Carvedilol (10–50 μM) substantially inhibited growth and compactness of spheroids grown from Mel270, 92–1, UPMD2 or UPMM3 uveal melanoma cells (Farhoumand et al., 2022). Furthermore, carvedilol applied orally significantly inhibited the growth of the A375 melanoma xenograft in SCID mice (Cleveland et al., 2018). Based on our results and reports cited, it seems important to perform more detailed studies focusing on the activity of carvedilol against various melanoma cells and the relationship between the melanin content and the carvedilol-mediated effect.

Interestingly, β-blockers also displayed synergistic effects with antitumor drugs. Thus far, several studies have demonstrated an enhanced efficacy of anticancer drugs combined with propranolol. Wei et al. (Wei et al., 2016) established that propranolol increased the cytotoxicity of vemurafenib and sensitized cells to the of this tyrosine kinase inhibitor (TKI) in thyroid cancer. Other studies showed a synergistic effect of the combination of propranolol and other TKI sunitinib on melanoma cells (Kuang et al., 2018). Studies have also shown the ability of carvedilol to enhance the activity of anticancer drugs, for example, it increased the efficacy of vincristine treatment against neuroblastoma (Pasquier et al., 2013).

With the growing interest in finding new possible anticancer combination therapies, our objective was to test the ability of carvedilol to synergize with the cancer treating drug sorafenib. Since receptor and nonreceptor TK become clinically valuable target molecules for cancer therapy, our study examined not only the influence of carvedilol alone against melanoma cells but also in combination with TKI sorafenib. Studies have shown antitumor activity of sorafenib against various cancers such as breast cancer (Zafrakas et al., 2016), colorectal cancer (Kacan et al., 2016), and melanoma (Takeda et al., 2021). The current study establishes the significant growth inhibitory effect of sorafenib on C32 amelanotic and A2058 melanotic melanoma cells after 72 h of treatment with IC50 values of 6.67 μM and 7.63 μM respectively. Xia et al. (Xia et al., 2016) showed the antiproliferative activity of sorafenib against two melanoma cell lines (SK-MEL-2 and SK-MEL-30) with IC50 values of 3.91 μM and 3.69 μM. Sorafenib was supposed to be a promising drug for melanoma therapy due to its ability to inhibit BRAF, a protein commonly altered in melanoma. Nevertheless, clinical trials of sorafenib showed modest effectiveness in melanoma (Egberts et al., 2008), which restricts the therapeutic use of sorafenib for the melanoma treatment. Drugs that inhibit kinases, such as sorafenib, are frequently co-administered with other drugs to improve the efficiency of anticancer therapy and to treat comorbidities or to alleviate some of the side-effects. Therefore, new strategies for sorafenib in the treatment of melanoma are necessary to increase its efficacy. Although sorafenib has shown little promise as a single agent in melanoma patients, it is still believed about the possibility of its use in combination therapy. The antiproliferative effects of sorafenib against melanoma cells have been shown to improve in the presence of the commonly used drug fluvastatin (Zhang et al., 2011). Furthermore, sorafenib has also been shown to sensitize melanoma cell to vemurafenib through the induction of cell death (Tang et al., 2020). Clinical trials indicate hypertension as one of the common side effects occurring in sorafenib-treated patients (Wu et al., 2008). Thus, it seems reasonable to use sorafenib in combination with a blood pressure lowering drugs with anticancer potential like carvedilol.

The impact of the combination of carvedilol and sorafenib on melanoma cell growth *in vitro* has not yet been explored. We demonstrated that combination of carvedilol and 5 μM sorafenib showed a synergistic growth inhibitory effect on C32 amelanotic cells with CI values ≤ 0.62. Although A2058 melanotic cells were less sensitive to these combinations and a synergistic effect was observed only after treatment with drugs at the concentrations of 5 μM (CI = 0.84). The results presented have shown for the first time the synergistic antiproliferative effects of carvedilol and sorafenib on melanoma cells. To the best of our knowledge, no studies on the effects of carvedilol and sorafenib on melanoma cells *in vitro* have been published. However, Erguven et al. (Erguven, 2010) demonstrated the ability of carvedilol at concentration 10 μM to enhance anticancer effects of imatinib mesylate, another TKI, against C6 glioma cells.

Our results showed a positive effect of combining carvedilol and sorafenib with respect to amelanotic and melanotic melanoma cell growth. To predict the potential effects and molecular mechanism of coadministration of these two drugs, we performed *in silico* analysis using the ChemDIS-Mixture system. Analysis of the probability of pharmacological interactions of carvedilol and sorafenib when used in various disorders confirmed the high probability of effectiveness of such a combination in melanoma. The results obtained point out the relevance of further studies on the effects of these drugs on the treatment of malignant melanoma. Additionally, *in silico* analysis suggested that the observed synergistic effect of cotreatment of C32 and A2058 cells with carvedilol and sorafenib could be related to some of the 21 potential targets responsible for the interaction effect. Among them, one-third belong to the members of the BCL2 family proteins, which play the role in various cell death. Antiapoptotic Bcl2 proteins include Mcl-1 (Bcl2 family apoptosis regulator) and Bcl-XL, while proteins such as Bak and Bax are pro-apoptotic. Interactions among these proteins influence the fate of cells. The antiapoptotic role of Mcl-1 is crucial for cell survival, and its overexpression has been described in a wide range of human tumors, including melanoma. Mcl-1 can antagonize the pro-apoptotic action of various Bcl2 proteins. Due to such neutralization, Mcl-1 can balance for the loss of other anti-apoptotic Bcl2 proteins in cancer cells, proposing it as a possible significant regulator of anti-apoptotic regulator in tumor cells

(Senichkin et al., 2020). Recent studies have shown that Mcl-1 silencing represents an appropriate approach to increase the cytotoxicity of a TRAIL-armed oncolytic adenovirus in melanoma cells (Tolksdorf et al., 2021). The expression of the *MCL1* gene is controlled by many transcription factors (e.g., HIF-1 and STAT3) (Senichkin et al., 2020).

Based on the outcomes of ChemDis-Mixture analysis among shared proteins interacting with both carvedilol and sorafenib, there are also two transcription factors: STAT3 and HIF1A. Therefore, it can be hypothesised that the effects of these compounds may be associated with modulation of the activity of these transcription factors. In melanoma cells, STAT3 has been shown to be essential for promotion and progression, not only by regulating melanoma cell growth and apoptosis, but also by increasing its invasiveness and ability to avoid the body's immune response. The action of STAT3 action has also been associated with an increased probability of relapse of melanoma after therapy (Lesinski, 2013). HIF1A is the main factor that plays a role in the adaptive response to alterations in oxygen level. It is a key factor in cancer development and its overexpression is associated with the progression of many cancers, including melanoma. It coordinates gene expression as a result of reduced oxygen tension. Translocation of HIF1A to the nucleus modulates the transcription of genes involved in the synthesis of, among others, proangiogenic factors (Kuphal et al., 2010; Lequeux et al., 2021). HIF1A expression is regulated by the AKT/PI3K and the MAPK/ERK pathways (Zhang et al., 2020). It is worth remarking that the analysis of carvedilol and sorafenib by ChemDis-Mixture revealed mitogen-activated kinases as shared targets of these drugs. Furthermore, enriched analysis suggests that he simultaneous use of carvedilol and sorafenib potentially influences signalling transduction pathways (especially MAP and PI3K kinases and GTPase activity) and plays a role in inflammation.

Due to the fact that the performed *in silico* study have shown that cotreatment with carvedilol and sorafenib could influence the inflammatory response, further studies focused on the influence of both drugs on pro-inflammatory cytokines. As mentioned above, stress influences the immune-inflammatory interplay and can alter the melanoma growth by secretion of neuroendocrine factors such as β-adrenergic agonists (Sinnya and De'Ambrosis, 2013). β-AR are upregulated in melanoma and their stimulation releases pro-cancerogenic interleukins such as IL-8 (Moretti et al., 2013). IL-8 is an important multifunctional chemokine that is not normally produced by melanocytes, but modulates the pathogenesis of melanoma (Singh et al., 2010). The expression of IL-8 has been observed after activation with cytokines, creating the premises of pro-inflammatory mechanisms activation such as IL-1β. In contrast, melanoma cells secrete IL-8, which acts as an autocrine factor, activating oncogenic signaling pathways and pre-metastatic traits (Filimon et al., 2021). The function of IL-8 in this skin cancer is constantly expanding. Its presence promotes a growth environment for the melanoma and increases the survival and metastatic potential of melanoma cells. The importance of IL-8 in the progression of melanoma is related to its proangiogenic, chemotactic, and autocrine stimulant activity (Filimon et al., 2021; Yang et al., 2009). The activity of IL-8 in the microenvironment of melanoma leads to the accumulation of immune cells, intravasation of melanoma cells, and their metastasis. Furthermore, IL-8 reprograms cell phenotypes, making them extremely active (Filimon et al., 2021).

With regards to the widely recognized role of IL-8 in the progression as well as the results of an *in silico* study which demonstrated that many proteins and pathways affected by both drugs are related to this chemokine, we decided to evaluate the influence of carvedilol and sorafenib alone and in combination on the secretion of interleukin 8 (IL-8) by C32 and A2058 melanoma cells stim-

ulated with IL-1β. IL-1β is an important cytokine engaged in cancer growth and metastasis. Numerous studies have confirmed its role in the pathogenesis of melanoma. It promotes the growth and invasiveness of melanoma by increasing oxidative stress, influencing inflammatory factors, and inhibiting the expression of cell cycle inhibitors (Lewis et al., 2006; Qin et al., 2011). IL-1β secreted by melanoma cells, together with IL-8 stimulated by it, induce macrophage cytokine secretion and participated in metastasis (Okamoto et al., 2010).

The present results indicated that carvedilol can modulate the immunologic function of melanoma cells by inhibition of IL-1β-stimulated IL-8 expression; however, the response of the studied amelanotic and melanotic melanoma cells differed. Carvedilol negatively regulated IL-8 release by A2058 melanotic cells, while IL-8 secretion was essentially unchanged after treatment of amelanotic C32 cells with carvedilol. Since it is known that activation of β-AR signaling can stimulate IL-8 secretion, it is remarkable to determine the effect of β-blockers on the release of this chemokine. It is not well known whether the effect of these drugs involves suppression of IL-8 in addition to suppression of increased sympathetic activity. The versatile anti-inflammatory properties of carvedilol have previously been observed. Carvedilol has been shown that carvedilol reduced IL-1β-mediated overexpression of MMP in chondrocytes (Li et al., 2016). Furthermore, it suppressed plasma levels of proinflammatory cytokines IL-6 and TNF-α in ischaemic and non-ischaemic patients (Kurum et al., 2007). Furthermore, carvedilol decreased the concentration of myocardial IL-1β and IL-8 in alleviating viral myocarditis in mice (Wang et al., 2014). Nevertheless, there is currently a lack of reports on the influence of carvedilol on IL8 secretion by cancer cells, especially melanoma cells. Liang et al.(Liang et al., 2021) find that topical administration of R-carvedilol in the concentration range of 0.1–10 μM decreased UV-induced expression of IL-1β and IL-6 in SKH-1mice. The authors suggested that this mechanism of action might be important in carvedilol-mediated skin cancer prevention. However, a reduction of IL-8 expression in melanoma cells was observed after the use of another β-blocker, propranolol. Treatment of three melanoma cell lines with propranolol entirely revoked the norepinephrine induced IL-8 mRNA levels (Yang et al., 2009). Our results on the influence of carvedilol on IL-8 secretion are novel in describing its possible anticancer action against melanoma. However, considering the different sensitivity of the studied melanoma cells to the inhibitory effect of carvedilol on IL-8 secretion, future studies should focus on determining the cellular mechanisms of its anti-inflammatory activity.

We also investigated the possible synergistic effect of sorafenib and carvedilol on IL-8 secretion. While sorafenib alone significantly inhibits IL-1β-stimulated secretion of IL-8 by C32 and A2058 cells, we did not observe the synergistic activity of carvedilol and sorafenib on the secretion of IL-8 by these melanoma cells. Thus, it could be suggested that the observed synergistic effect of two drugs was not correlated with this chemokine.

5. Conclusion

The presented study reveals for the first time that the combination of carvedilol and sorafenib may have a promising anticancer effect on melanoma cells. While ChemDIS-Mixture is a useful tool for estimation of the potential interactions caused by cotreatment with two or more drugs, confirmation of the results is needed in the form of *in vitro* or/and *in vivo* studies. Further experiments are essential to evaluate the underlying mechanism of action of the combination and to confirm the anticancer effect of cotreatment with carvedilol and sorafenib against melanoma cells.

Funding

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Avila, M.S., Ayub-Ferreira, S.M., de Barros Wanderley, M.R., das Dores Cruz, F., Gonçalves Brandão, S.M., Rigaud, V.O.C., Higuchi-dos-Santos, M.H., Hajjar, L.A., Kalil Filho, R., Hoff, P.M., Sahade, M., Ferrari, M.S.M., de Paula Costa, R.L., Mano, M.S., Bittencourt Viana Cruz, C.B., Abduch, M.C., Lofrano Alves, M.S., Guimaraes, G.V., Issa, V.S., Bittencourt, M.S., Bocchi, E.A., 2018. Carvedilol for Prevention of Chemotherapy-Related Cardiotoxicity. J. Am. Coll. Cardiol. 71, 2281–2290. https://doi.org/10.1016/j.jacc.2018.02.049.

Caparica, R., Bruzzone, M., Agostinetto, E., De Angelis, C., Fêde, Â., Ceppi, M., de Azambuja, E., 2021. Beta-blockers in early-stage breast cancer: a systematic review and meta-analysis. ESMO Open 6,. https://doi.org/10.1016/j.esmoop.2021.100066 100066.

Chung, J.F., Lee, S.J., Sood, A.K., 2016. Immunological and pleiotropic effects of individual β-blockers and their relevance in cancer therapies. Expert Opin. Investig. Drugs 25, 501–505. https://doi.org/10.1517/13543784.2016.1164141.

Cleveland, K.H., Yeung, S., Huang, K.M., Liang, S., Andresen, B.T., Huang, Y., 2018. Phosphoproteome profiling provides insight into the mechanism of action for carvedilol-mediated cancer prevention. Mol. Carcinog. 57, 997–1007. https://doi.org/10.1002/mc.22820.

Coelho, M., Moz, M., Correia, G., Teixeira, A., Medeiros, R., Ribeiro, L., 2015. Antiproliferative effects of β-blockers on human colorectal cancer cells. Oncol. Rep. 33, 2513–2520. https://doi.org/10.3892/or.2015.3874.

Colucci, R., Moretti, S., 2016. The role of stress and beta-adrenergic system in melanoma: current knowledge and possible therapeutic options. J. Cancer Res. Clin. Oncol. 142, 1021–1029. https://doi.org/10.1007/s00432-015-2078-z.

Didkowska, J., Wojciechowska, U., Olasek, P., Caetano dos Santos, F., Michałek, I, 2019. Cancer in Poland in 2019. Polish National Cancer Registry.

Didkowska, J., Wojciechowska, U., Zatoński, W., 2009. Cancer in Poland in 2009. Polish National Cancer Registry.

Du, P., Feng, G., Flatow, J., Song, J., Holko, M., Kibbe, W.A., Lin, S.M., 2009. From disease ontology to disease-ontology lite: statistical methods to adapt a general-purpose ontology for the test of gene-ontology associations. Bioinformatics 25, i63–i68. https://doi.org/10.1093/bioinformatics/btp193.

Egberts, F., Kähler, K.C., Livingstone, E., Hauschild, A., 2008. Metastatic melanoma: scientific rationale for sorafenib treatment and clinical results. Onkologie 31, 398–403. https://doi.org/10.1159/000137714.

Erguven, 2010. Carvedilol in glioma treatment alone and with imatinib in vitro. Int. J. Oncol. 36. https://doi.org/10.3892/ijo_00000563.

Farhoumand, L.S., Fiorentzis, M., Kraemer, M.M., Sak, A., Stuschke, M., Rassaf, T., Hendgen-Cotta, U., Bechrakis, N.E., Berchner-Pfannschmidt, U., 2022. The adrenergic receptor antagonist carvedilol elicits anti-tumor responses in uveal melanoma 3D tumor spheroids and may serve as co-adjuvant therapy with radiation. Cancers 14, 3097. https://doi.org/10.3390/cancers14133097.

Filimon, A., Preda, I.A., Boloca, A.F., Negroiu, G., 2021. Interleukin-8 in Melanoma Pathogenesis, Prognosis and Therapy—An Integrated View into Other Neoplasms and Chemokine Networks. Cells 11, 120. https://doi.org/10.3390/cells11010120.

Gillis, R.D., Botteri, E., Chang, A., Ziegler, A.I., Chung, N.-C., Pon, C.K., Shackleford, D.M., Andreassen, B.K., Halls, M.L., Baker, J.G., Sloan, E.K., 2021. Carvedilol blocks neural regulation of breast cancer progression in vivo and is associated with reduced breast cancer mortality in patients. Eur. J. Cancer 147, 106–116. https://doi.org/10.1016/j.ejca.2021.01.029.

Kacan, T., Nayir, E., Altun, A., Kilickap, S., Babacan, N.A., Ataseven, H., Kaya, T., 2016. Antitumor activity of sorafenib on colorectal cancer. J. Oncol. Sci. 2, 53–57. https://doi.org/10.1016/j.jons.2016.07.008.

Kamran, S., Sinniah, A., Chik, Z., Alshawsh, M.A., 2022. Diosmetin Exerts Synergistic Effects in Combination with 5-Fluorouracil in Colorectal Cancer Cells. Biomedicines 10, 531. https://doi.org/10.3390/biomedicines10030531.

Kuang, X., Qi, M., Peng, C., Zhou, C., Su, J., Zeng, W., Liu, H., Zhang, J., Chen, M., Shen, M., Xie, X., Li, F., Zhao, S., Li, Q., Luo, Z., Chen, J., Tao, J., He, Y., Chen, X., 2018. Propranolol enhanced the anti-tumor effect of sunitinib by inhibiting proliferation and inducing G0/G1/S phase arrest in malignant melanoma. Oncotarget 9, 802–811. https://doi.org/10.18632/oncotarget.22696.

Kuphal, S., Winklmeier, A., Warnecke, C., Bosserhoff, A.-K., 2010. Constitutive HIF-1 activity in malignant melanoma. Eur. J. Cancer 46, 1159–1169. https://doi.org/10.1016/j.ejca.2010.01.031.

Kurum, T., Tatli, E., Yuksel, M., 2007. Effects of carvedilol on plasma levels of pro-inflammatory cytokines in patients with ischemic and nonischemic dilated cardiomyopathy. Tex. Heart Inst. J. 34, 52–59.

Lemeshow, S., Sørensen, H.T., Phillips, G., Yang, E.V., Antonsen, S., Riis, A.H., Lesinski, G.B., Jackson, R., Glaser, R., 2011. β-blockers and survival among danish patients with malignant melanoma: a population-based cohort study. Cancer Epidemiol. Biomarkers Prev. 20, 2273–2279. https://doi.org/10.1158/1055-9965.EPI-11-0249.

Lequeux, A., Noman, M.Z., Xiao, M., Van Moer, K., Hasmim, M., Benoit, A., Bosseler, M., Viry, E., Arakelian, T., Berchem, G., Chouaib, S., Janji, B., 2021. Targeting HIF-1 alpha transcriptional activity drives cytotoxic immune effector cells into melanoma and improves combination immunotherapy. Oncogene 40, 4725–4735. https://doi.org/10.1038/s41388-021-01846-x.

Lesinski, G.B., 2013. The potential for targeting the STAT3 pathway as a novel therapy for melanoma. Future Oncol. 9, 925–927. https://doi.org/10.2217/fon.13.83.

Li, Z., Liu, B., Wang, B., Liu, Y., Zhang, Y., Tian, F., Li, B., Zhao, D., 2016. Carvedilol suppresses cartilage matrix destruction. Biochem. Biophys. Res. Commun. 480, 309–313. https://doi.org/10.1016/j.bbrc.2016.10.032.

Liang, S., Shamim, M.A., Shahid, A., Chen, M., Cleveland, K.H., Parsa, C., Orlando, R., Andresen, B.T., Huang, Y., 2021. Prevention of Skin Carcinogenesis by the Non-β-blocking R-carvedilol Enantiomer. Cancer Prev. Res. (Phila. Pa.) 14, 527–540. https://doi.org/10.1158/1940-6207.CAPR-20-0609.

Lin, C.-S., Lin, W.-S., Lin, C.-L., Kao, C.-H., 2015. Carvedilol use is associated with reduced cancer risk: a nationwide population-based cohort study. Int. J. Cardiol. 184, 9–13. https://doi.org/10.1016/j.ijcard.2015.02.015.

Ma, Z., Liu, X., Zhang, Q., Yu, Z., Gao, D., 2018. Carvedilol suppresses malignant proliferation of mammary epithelial cells through inhibition of the ROS-mediated PI3K/AKT signaling pathway. Oncol. Rep. https://doi.org/10.3892/or.2018.6873.

Melhem-Bertrandt, A., Chavez-MacGregor, M., Lei, X., Brown, E.N., Lee, R.T., Meric-Bernstam, F., Sood, A.K., Conzen, S.D., Hortobagyi, G.N., Gonzalez-Angulo, A.-M., 2011. Beta-Blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. J. Clin. Oncol. 29, 2645–2652. https://doi.org/10.1200/JCO.2010.33.4441.

Moretti, S., Massi, D., Farini, V., Baroni, G., Parri, M., Innocenti, S., Cecchi, R., Chiarugi, P., 2013. β-adrenoceptors are upregulated in human melanoma and their activation releases pro-tumorigenic cytokines and metalloproteases in melanoma cell lines. Lab. Invest. 93, 279–290. https://doi.org/10.1038/labinvest.2012.175.

Mössinger, H., Kostev, K., 2023. Depression is associated with an increased risk of subsequent cancer diagnosis: a retrospective cohort study with 235,404 patients. Brain Sci. 13, 302. https://doi.org/10.3390/brainsci13020302.

Mravec, B., Horvathova, L., Hunakova, L., 2020. Neurobiology of Cancer: the Role of β-adrenergic receptor signaling in various tumor environments. Int. J. Mol. Sci. 21, 7958. https://doi.org/10.3390/ijms21217958.

Narendhirakannan, R.T., Hannah, M.A.C., 2013. Oxidative Stress and Skin Cancer: An Overview. Indian J. Clin. Biochem. 28, 110–115. https://doi.org/10.1007/s12291-012-0278-8.

Okamoto, M., Liu, W., Luo, Y., Tanaka, A., Cai, X., Norris, D.A., Dinarello, C.A., Fujita, M., 2010. Constitutively Active Inflammasome in Human Melanoma Cells Mediating Autoinflammation via Caspase-1 Processing and Secretion of Interleukin-1β. J. Biol. Chem. 285, 6477–6488. https://doi.org/10.1074/jbc.M109.064907.

Pasquier, E., Street, J., Pouchy, C., Carre, M., Gifford, A.J., Murray, J., Norris, M.D., Trahair, T., Andre, N., Kavallaris, M., 2013. β-blockers increase response to chemotherapy via direct antitumour and anti-angiogenic mechanisms in neuroblastoma. Br. J. Cancer 108, 2485–2494. https://doi.org/10.1038/bjc.2013.205.

Peixoto, R., de Pereira, M.L., Oliveira, M., 2020. Beta-blockers and cancer: where are we? Pharmaceuticals 13, 105. https://doi.org/10.3390/ph13060105.

Powe, D.G., Entschladen, F., 2011. Using β-blockers to inhibit breast cancer progression. Nat. Rev. Clin. Oncol. 8, 511–512. https://doi.org/10.1038/nrclinonc.2011.123.

Qiao, G., Chen, M., Bucsek, M.J., Repasky, E.A., Hylander, B.L., 2018. Adrenergic signaling: a targetable checkpoint limiting development of the antitumor immune response. Front. Immunol. 9, 164. https://doi.org/10.3389/fimmu.2018.00164.

Qin, Y., Ekmekcioglu, S., Liu, P., Duncan, L.M., Lizée, G., Poindexter, N., Grimm, E.A., 2011. Constitutive Aberrant Endogenous Interleukin-1 Facilitates Inflammation and Growth in Human Melanoma. Mol. Cancer Res. 9, 1537–1550. https://doi.org/10.1158/1541-7786.MCR-11-0279.

Rogers, H.W., Weinstock, M.A., Feldman, S.R., Coldiron, B.M., 2015. Incidence estimate of nonmelanoma skin cancer (Keratinocyte Carcinomas) in the US Population, 2012. JAMA Dermatol. 151, 1081. https://doi.org/10.1001/jamadermatol.2015.1187.

Sanzo, M., Colucci, R., Arunachalam, M., Berti, S., Moretti, S., 2010. Stress as a possible mechanism in melanoma progression. Dermatol. Res. Pract. 2010, 1–4. https://doi.org/10.1155/2010/483493.

Senichkin, V.V., Streletskaia, A.Y., Gorbunova, A.S., Zhivotovsky, B., Kopeina, G.S., 2020. Saga of Mcl-1: regulation from transcription to degradation. Cell Death Differ. 27, 405–419. https://doi.org/10.1038/s41418-019-0486-3.

Singh, S., Singh, A.P., Sharma, B., Owen, L.B., Singh, R.K., 2010. CXCL8 and its cognate receptors in melanoma progression and metastasis. Future Oncol. 6, 111–116. https://doi.org/10.2217/fon.09.128.

Sinnya, S., De'Ambrosis, B., 2013. Stress and melanoma: increasing the evidence towards a causal basis. Arch. Dermatol. Res. 305, 851–856. https://doi.org/10.1007/s00403-013-1373-2.

Stanojkovic, T.P., Zizak, Z., Mihailovic-Stanojevic, N., Petrovic, T., Juranic, Z., 2005. Inhibition of proliferation on some neoplastic cell lines-act of carvedilol and captopril. J. Exp. Clin. Cancer Res. CR 24, 387–395.

Takeda, T., Tsubaki, M., Kato, N., Genno, S., Ichimura, E., Enomoto, A., Imano, M., Satou, T., Nishida, S., 2021. Sorafenib treatment of metastatic melanoma with c-Kit aberration reduces tumor growth and promotes survival. Oncol. Lett. 22, 827. https://doi.org/10.3892/ol.2021.13089.

Tang, J., Li, Z., Lu, L., Cho, C.H., 2013. β-Adrenergic system, a backstage manipulator regulating tumour progression and drug target in cancer therapy. Semin. Cancer Biol. 23, 533–542. https://doi.org/10.1016/j.semcancer.2013.08.009.

Tang, F., Li, S., Liu, D., Chen, J., Han, C., 2020. Sorafenib sensitizes melanoma cells to vemurafenib through ferroptosis. Transl. Cancer Res. 9, 1584–1593. https://doi.org/10.21037/tcr.2020.01.62.

Tolksdorf, B., Zarif, S., Eberle, J., Hazini, A., Dieringer, B., Jönsson, F., Kreppel, F., Kurreck, J., Fechner, H., 2021. Silencing of Mcl-1 overcomes resistance of melanoma cells against TRAIL-armed oncolytic adenovirus by enhancement of apoptosis. J. Mol. Med. 99, 1279–1291. https://doi.org/10.1007/s00109-021-02081-3.

Tung, C.-W., Wang, C.-C., Wang, S.-S., Lin, P., 2018. ChemDIS-Mixture: an online tool for analyzing potential interaction effects of chemical mixtures. Sci. Rep. 8, 10047. https://doi.org/10.1038/s41598-018-28361-6.

Vojvodic, A., Vojvodic, P., Vlaskovic-Jovicevic, T., Sijan, G., Dimitrijevic, S., Peric-Hajzler, Z., Matovic, D., Uwe, W., Tirant, M., Nguyen, V.T., Fioranelli, M., Lotti, T., 2019. Beta Blockers and Melanoma. Open Access Maced. J. Med. Sci. 7, 3110–3112. https://doi.org/10.3889/oamjms.2019.782.

Wang, D., Chen, Y., Jiang, J., Zhou, A., Pan, L., Chen, Q., Qian, Y., Chu, M., Chen, C., 2014. Carvedilol has stronger anti-inflammation and anti-virus effects than metoprolol in murine model with coxsackievirus B3-induced viral myocarditis. Gene 547, 195–201. https://doi.org/10.1016/j.gene.2014.06.003.

Watkins, J.L., Thaker, P.H., Nick, A.M., Ramondetta, L.M., Kumar, S., Urbauer, D.L., Matsuo, K., Squires, K.C., Coleman, R.L., Lutgendorf, S.K., Ramirez, P.T., Sood, A.K., 2015. Clinical impact of selective and nonselective beta-blockers on survival in patients with ovarian cancer. Cancer 121, 3444–3451. https://doi.org/10.1002/cncr.29392.

Wei, W.-J., Shen, C.-T., Song, H.-J., Qiu, Z.-L., Luo, Q.-Y., 2016. Propranolol sensitizes thyroid cancer cells to cytotoxic effect of vemurafenib. Oncol. Rep. 36, 1576–1584. https://doi.org/10.3892/or.2016.4918.

Winitchaikul, T., Sawong, S., Surangkul, D., Srikummool, M., Somran, J., Pekthong, D., Kamonlakorn, K., Nangngam, P., Parhira, S., Srisawang, P., 2021. Calotropis gigantea stem bark extract induced apoptosis related to ROS and ATP production in colon cancer cells. PLOS ONE 16, e0254392.

Wu, S., Chen, J.J., Kudelka, A., Lu, J., Zhu, X., 2008. Incidence and risk of hypertension with sorafenib in patients with cancer: a systematic review and meta-analysis. Lancet Oncol. 9, 117–123. https://doi.org/10.1016/S1470-2045(08)70003-2.

Xia, Y., Li, Y., Westover, K.D., Sun, J., Chen, H., Zhang, J., Fisher, D.E., 2016. Inhibition of Cell Proliferation in an NRAS Mutant Melanoma Cell Line by Combining Sorafenib and α-Mangostin. PLOS ONE 11, e0155217.

Yang, E.V., Kim, S., Donovan, E.L., Chen, M., Gross, A.C., Webster Marketon, J.I., Barsky, S.H., Glaser, R., 2009. Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: Implications for stress-related enhancement of tumor progression. Brain. Behav. Immun. 23, 267–275. https://doi.org/10.1016/j.bbi.2008.10.005.

Yang, T., Qiao, Y., Xiang, S., Li, W., Gan, Y., Chen, Y., 2019. Work stress and the risk of cancer: A meta-analysis of observational studies. Int. J. Cancer 144, 2390–2400. https://doi.org/10.1002/ijc.31955.

Zafrakas, M., Papasozomenou, P., Emmanouilides, C., 2016. Sorafenib in breast cancer treatment: A systematic review and overview of clinical trials. World J. Clin. Oncol. 7, 331. https://doi.org/10.5306/wjco.v7.i4.331.

Zhang, S., Doudican, N.A., Quay, E., Orlow, S.J., 2011. Fluvastatin enhances sorafenib cytotoxicity in melanoma cells via modulation of AKT and JNK signaling pathways. Anticancer Res. 31, 3259–3265.

Zhang, Q., Han, Z., Zhu, Y., Chen, J., Li, W., 2020. Role of hypoxia inducible factor-1 in cancer stem cells (Review). Mol. Med. Rep. 23, 1–1. https://doi.org/10.3892/mmr.2020.11655.

Zhou, C., Chen, X., Zeng, W., Peng, C., Huang, G., Li, X., Ouyang, Z., Luo, Y., Xu, X., Xu, B., Wang, W., He, R., Zhang, X., Zhang, L., Liu, J., Knepper, T.C., He, Y., McLeod, H. L., 2016. Propranolol induced G0/G1/S phase arrest and apoptosis in melanoma cells via AKT/MAPK pathway. Oncotarget 7, 68314–68327. https://doi.org/10.18632/oncotarget.11599.

Zhu, A.X., 2009. Predicting the response to sorafenib in hepatocellular carcinoma: where is the evidence for phosphorylated extracellular signaling-regulated kinase (pERK)? BMC Med. 7, 42. https://doi.org/10.1186/1741-7015-7-42.

Lewis, A.M., Varghese, S., Xu, H., Alexander, H.R., 2006. Interleukin-1 and cancer progression: the emerging role of interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment. J. Transl. Med. 4, 48. https://doi.org/10.1186/1479-5876-4-48.