**Name of Journal:** *World Journal of Clinical Oncology*

**Manuscript NO:** 65070

**Manuscript Type:** REVIEW

**Therapeutic potential of thymoquinone in combination therapy against cancer and cancer stem cells**

Fatfat Z *et al*. Thymoquinone in combination cancer therapy

Zaynab Fatfat, Maamoun Fatfat, Hala Gali-Muhtasib

**Zaynab Fatfat, Maamoun Fatfat, Hala Gali-Muhtasib,** Department of Biology, American University of Beirut, Beirut 1107 2020, Lebanon

**Hala Gali-Muhtasib,** Center for Drug Discovery, American University of Beirut, Beirut 1107 2020, Lebanon

**Author contributions:** Fatfat Z and Fatfat M reviewed the literature and drafted the manuscript; Gali-Muhtasib H initiated the idea and revised the manuscript; all authors have read and approved the final manuscript.

**Corresponding author: Hala Gali-Muhtasib, PhD, Professor,** Department of Biology, and Center for Drug Discovery, American University of Beirut, Bliss Street, Beirut 1107 2020, Lebanon. amro@aub.edu.lb

**Received:** February 27, 2021

**Revised:** April 11, 2021

**Accepted:** June 18, 2021

**Published online:** July 24, 2021

**Abstract**

The long-term success of standard anticancer monotherapeutic strategies has been hampered by intolerable side effects, resistance to treatment and cancer relapse. These monotherapeutic strategies shrink the tumor bulk but do not effectively eliminate the population of self-renewing cancer stem cells (CSCs) that are normally present within the tumor. These surviving CSCs develop mechanisms of resistance to treatment and refuel the tumor, thus causing cancer relapse. To ensure durable tumor control, research has moved away from adopting the monotreatment paradigm towards developing and using combination therapy. Combining different therapeutic modalities has demonstrated significant therapeutic outcomes by strengthening the anti-tumor potential of monotreatment against cancer and cancer stem cells, mitigating their toxic adverse effects, and ultimately overcoming resistance. Recently, there has been growing interest in combining natural products from different sources or with clinically used chemotherapeutics to further improve treatment efficacy and tolerability. Thymoquinone (TQ), the main bioactive constituent of *Nigella sativa,* has gained great attention in combination therapy research after demonstrating its low toxicity to normal cells and remarkable anticancer efficacy in extensive preclinical studies in addition to its ability to target chemoresistant CSCs. Here, we provide an overview of the therapeutic responses resulting from combining TQ with conventional therapeutic agents such as alkylating agents, antimetabolites and antimicrotubules as well as with topoisomerase inhibitors and non-coding RNA. We also review data on anticancer effects of TQ when combined with ionizing radiation and several natural products such as vitamin D3, melatonin and other compounds derived from Chinese medicinal plants. The focus of this review is on two outcomes of TQ combination therapy, namely eradicating CSCs and treating various types of cancers. In conclusion, the ability of TQ to potentiate the anticancer activity of many chemotherapeutic agents and sensitize cancer cells to radiotherapy makes it a promising molecule that could be used in combination therapy to overcome resistance to standard chemotherapeutic agents and reduce their associated toxicities.

**Key Words:** Thymoquinone; Combination therapy; Cancer cells; Cancer stem cells; Conventional cancer therapy; Natural products

**©The** **Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Citation:** Fatfat Z, Fatfat M, Gali-Muhtasib H. Therapeutic potential of thymoquinone in combination therapy against cancer and cancer stem cells. *World J Clin Oncol* 2021; 12(7): 522-543

**URL:** https://www.wjgnet.com/2218-4333/full/v12/i7/522.htm

**DOI:** https://dx.doi.org/10.5306/wjco.v12.i7.522

**Core Tip:** There has been great interest in integrating thymoquinone (TQ) in combination therapy particularly to target cancer stem cells, which are known to be responsible for resistance to treatment and cancer recurrence. The combination of TQ with standard chemotherapeutics and other natural products has exhibited promising anticancer responses. TQ was also shown to sensitize cancer cells to radiotherapy and help in overcoming major limitations that restrict the potency of chemotherapy, which are chemoresistance and treatment associated toxic effects.

**INTRODUCTION**

Cancer incidence and mortality are still growing worldwide despite the monumental efforts and the significant progress made in developing therapeutic strategies and improving detection techniques for combatting this disease. Around 19 million new cases and nearly 10 million deaths are estimated globally in 2020[1]. The conventional therapeutic strategies used to treat cancer are surgery, radiotherapy and chemotherapy, in addition to targeted and hormonal therapy. The effectiveness of these approaches has been found to be limited when used in monotherapy strategies due to cancer resistance, tumor relapse and treatment-induced toxicities[2-6]. Ample evidence has demonstrated that the intratumoral heterogeneity is a prominent contributor to cancer resistance to monotherapy and tumor recurrence[7]. The tumor consists of a heterogeneous population of cells that show distinct genetic, epigenetic, and phenotypic features in addition to different sensitivity to the standard therapeutic modalities[8-11]. A growing body of literature has supported the role of cancer stem cells (CSCs) in generating this intratumoral heterogeneity. These CSCs are characterized by their ability to self- renew and to differentiate into various lineages of cancer cells composing the tumor. They are also resistant to the widely used therapeutics measures[12].

Over the last few decades, there has been increased interest in combining cancer treatments rather than using single therapeutic agents. A monotherapeutic strategy having one mode of action eradicates only one subpopulation of tumor cells. Other subpopulations which are less sensitive can escape the treatment and reform a resistant tumor, thus resulting in cancer relapse and treatment failure. In contrast, combined therapeutic agents act simultaneously on multiple targets and eradicate several subpopulations of tumor cells. This results in improving their therapeutic efficacy, limiting their toxicity by lowering the effective therapeutic dose of each agent, preventing the development of resistance and consequently ensuring an effective eradication of the complex heterogeneous nature of the tumor[13]. Bioactive natural products are attracting considerable attention in cancer therapy because they are less toxic and more available and cost effective when compared to synthetic monotargeted drugs[14]. Natural therapeutics have been found to exert effective antineoplastic activity and to potentiate the anticancer effect of conventional therapeutics against CSCs and cancer cells[15,16]. Around 38% of the anticancer drugs approved during the last 40 years are either natural products *per se*, their derivatives or have a pharmacophore derived from a natural product[17].

Thymoquinone (TQ), the major bioactive compound extracted from *Nigella sativa* essential oil, has shown promising antitumor activity *in vitro* and *in vivo* against a wide range of cancer types[18]. What makes TQ an attractive therapeutic agent is its safe profile. It was found to be non-toxic to several normal cells including normal mouse kidney cells[19], normal human lung fibroblasts[20] and normal human intestinal cells[21]. TQ exerts its antineoplastic effects through several modes of action, and its exact molecular target is not known yet. It inhibits cancer cell proliferation and blocks the cell cycle progression. In addition, TQ induces apoptosis by generating reactive oxygen species (ROS), causing DNA damage, upregulating pro-apoptotic factors, activating caspases and causing poly (ADP-ribose) polymerases (PARP) cleavage, disrupting mitochondrial membrane integrity besides modulating several pathways such as p53, wingless/integrated (Wnt), mitogen-activated protein kinase, signal transducer and activator of transcription 3 (STAT3)[22]. It also interrupts metastasis by downregulating the epithelial to mesenchymal transition transcription factors twist-related protein 1 (TWIST1) and E-Cadherin, and inhibits angiogenesis by suppressing the nuclear factor kappa B (NFB) pathway[22]. Interestingly, TQ was found to inhibit the proliferation of several chemoresistant cancer cells and induce apoptosis in colon CSCs that are resistant to the conventional chemotherapeutic drug 5-fluorouracil (5-FU)[23,24].

These effective anticancer properties of TQ made it an interesting therapeutic candidate for combination therapy with standard therapeutic agents or other natural products to improve cancer treatment efficacy and safety (Figure 1). Here, we shed light on the combinatorial effects of TQ on the activity of these therapeutic agents used in treating CSCs and cancer cells.

**TQ EFFECTS AGAINST CANCER CELLS**

***TQ in combination with conventional chemotherapeutic agents***

The mode of action of each chemotherapeutic agent as well as the cellular and molecular mechanisms of action of the combination treatment are presented in Table 1.

***Alkylating agents***

**Cyclophosphamide[25]:** Cyclophosphamide has been used in treating a broad spectrum of cancers including leukemia, lymphoma, breast and ovarian cancers[26]. In a study conducted by Khan *et al*[27], TQ was found to amplify the growth inhibitory effects of low doses of cyclophosphamide in breast cancer cells. This combination upregulated the expression of phosphatase and tensin homolog (PTEN) and downregulated the phosphorylation of its downstream signaling molecule Akt in addition to decreasing the expression of cyclin D1. The PTEN/phosphatidylinositol-3-kinase (PI3K)/Akt pathway is known to be an important tumorigenic pathway responsible for cell cycle progression, survival, and migration of malignant cells[28].

**Temozolomide[29]:** Temozolomide (TMZ) has been approved by the Food and Drug Administration for the treatment of glioblastoma multiforme[30]. However, the anticancer efficacy of TMZ has been limited by cancer resistance[31]. TQ was found to be a potent enhancer of the anti-proliferative and apoptotic activity of TMZ in glioblastoma cells. The modulation of the apoptotic players including ROS generation, disruption of mitochondrial membrane potential, activation of p53, caspases 9 and 3 was more pronounced in combination treatment compared to separate treatments[32]. Moreover, combining TQ and TMZ caused a stronger inhibitory effect on glioblastoma cells migration, invasion and adhesion than each drug alone. This synergistic inhibitory effect was found to be associated with a decrease in the expression and secretion of matrix metalloproteinases MMP-2 and MMP-9[33] known to promote metastatic spread and to contribute to angiogenesis[34]. Interestingly, TQ was found to block TMZ-induced autophagy, which was suggested to be a prosurvival mechanism of cell resistance to TMZ. TQ suppressed TMZ-induced expression of key players in the autophagy pathway beclin-1 and autophagy-related 7[35].

**Cisplatin[36]:** Cisplatin (CDDP) is one of the most used chemotherapeutic drugs in the treatment of a wide range of cancer types[37]. The primary dose-limiting side effect of CDDP is the dose- dependent nephrotoxicity, which restricts the use of high doses of CDDP to increase its anticancer activity[38]. Numerous studies have demonstrated the anti-neoplastic efficacy of combining TQ with CDDP in different types of cancers as an alternative way to increase CDDP potency. In ovarian cancer, these two agents were found to synergize to induce apoptosis *in vitro* and in a mouse syngeneic model. The combination was more effective in increasing the levels of Bcl-2-associated X protein (Bax), phospho-histone 2AX on serine 139, cleaved caspase 3 and PARP and in downregulating proliferating cell nuclear antigen compared to CDDP alone[39]. A study conducted by Hu *et al*[40] found that TQ enhanced the apoptotic effect of CDDP in esophageal carcinoma *in vitro* and *in vivo* through downregulating JAK2/STAT3 pathway known to be involved in cancer cell proliferation, survival, angiogenesis and metastasis[41]. Another study showed the synergistic inhibitory effects of the combination of TQ and CDDP on the proliferation of non-small lung cancer cells and on the growth of lung cancer xenografts through the suppression of NFB[42]. The improvement of CDDP-induced apoptosis by TQ was also demonstrated in oral squamous carcinoma cells. The combination was more potent in upregulating p53 and caspase 9 and downregulating Bcl-2 than CDDP alone[43]. In addition, combining TQ with CDDP resulted in a superior anti-neoplastic activity in gastric cancer *in vitro* and *in vivo* by further upregulating PTEN expression compared to CDDP alone[44].

***Antimetabolites***

**5-FU[45]:** 5-FU is the third most frequently used chemotherapeutic drug in the treatment of a variety of solid cancers, but its clinical efficacy is hampered by drug resistance and treatment-associated toxicities[46,47]. It is the second most frequent chemotherapeutic agent that causes cardiotoxicity symptoms[46]. The potential chemomodulatory effects of TQ on 5-FU anticancer activity have been investigated in various cancer types. TQ was reported to chemosensitize gastric cancer cells to 5-FU-induced apoptosis by upregulating Bax, caspases 3 and 9 and downregulating Bcl-2[48]. Moreover, the combination of TQ with 5-FU synergistically suppressed azoxymethane-induced colorectal tumors initiation and development in rats without causing nephro- and hepato-toxicities. The dual combination enhanced the decrease in the expression level of pro-oncogenic genes [Wnt, β-catenin, NFB, cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), vascular endothelial growth factor(VEGF), and thiobarbituric acid reactive substances] and the increase in the expression level of anti-oncogenic genes [dickkopf-related protein-1 (DKK-1), cyclin-dependent kinase inhibitor 1A (CDNK-1A), transforming growth factor beta 1 (TGF-β1), transforming growth factor, beta receptor II (TGF-βRII), Smad4, and glutathione peroxidase] compared to separate treatments[49]. In another study, Ndreshkjana *et al*[50] linked 5-FU with TQ by esterification to form a new hybrid molecule SARB and tested it on colon cancer cells. Both combination and hybrid treatments enhanced the cytotoxic effects of single agents *in vitro*, while SARB was more effective in suppressing the growth of chorioallantoic membrane xenografts *in vivo*. The cytotoxic effects of 5-FU, TQ and the natural product epigallocatechin-3-gallate in triple and double combinations were evaluated in nasopharyngeal cancer cells. The results revealed that the triple combination had the most potent effect in reducing the total number of cancer cells, and the dual combination of TQ and 5-FU was more effective than the combination of TQ and epigallocatechin-3-gallate[51]. In addition, TQ augmented the apoptotic effects of each of 5-FU and the alkylating agent oxaliplatin in osteosarcoma cells. Interestingly, combining TQ with low doses of each of these drugs was found to produce the same anticancer efficacy as higher doses of these agents[52]. Therefore, this treatment strategy may help in alleviating 5-FU and oxaliplatin undesired adverse effects.

**Gemcitabine[53]:** Gemcitabine (GCB) has been approved for treating different types of cancer including pancreatic and breast cancers[54]. The therapeutic application of GCB was compromised by several drawbacks including its short half-life in the blood circulation, poor membrane permeability in addition to the development of chemoresistance[55]. TQ and GCB were found to induce synergistic apoptosis in GCB sensitive and resistant pancreatic cancer cells by downregulating pyruvate kinase M2 expression[56]. In another study, pretreatment of pancreatic cancer cells with TQ followed by low doses of GCB resulted in a synergistic apoptotic and growth inhibitory responses *in vitro* and *in vivo* by downregulating Notch1/PTEN, PI3K/Akt/mammalian target of rapamycin and NFB mediated signaling pathways[57]. In the context of breast cancer, TQ boosted the apoptotic activity of GCB against T47D cells. While in the apoptosis defective MCF-7 cells, the combination of TQ with GCB induced significant cell death by autophagy[58].

***Antimicrotubules***

**Paclitaxel[59]:** Paclitaxel (PAC) is widely used for the treatment of several cancer types including breast, ovary, colorectal and lung cancers[60]. The major challenges that restrict its curative effect are chemoresistance and adverse effects that are mainly caused by the polyethylated castor oil that is usually added to its formulation to increase its solubility[61,62]. Three studies have evaluated the potential of the combinatorial effect of TQ and PAC in breast cancer. TQ-PAC combination produced a synergistic anticancer activity through the modulation of genes involved in apoptosis, cytokine-cytokine receptor interaction, Fas signaling, p53 signaling and JAK/STAT signaling[63]. In another study, combining TQ with PAC augmented the necrotic and caspase dependent- apoptotic responses in T47D breast cancer cells compared to PAC alone. While in the apoptosis defective MCF-7 cells, both individual and combined treatments induced significant cell death by autophagy[64]. The co-encapsulation of TQ and PAC in polymeric biodegradable poly *(*lactide*-*co*-*glycolide) nanoparticles lowered PAC effective anticancer dose and reduced cancer cell viability more effectively than PAC loaded nanoparticles or its free counterpart[65]. Therefore, this therapeutic approach may help in hijacking the toxicities associated with the clinical use of PAC.

**Docetaxel:** Docetaxel (DTX) has been approved for the treatment of different type of tumors including prostate cancer and breast cancer[66]. However, low water solubility, treatment related toxicitiesand drug resistance limit its application in clinical practice[61,67,68]. TQ was found to potentiate the apoptotic activity of DTX in prostate cancer cells by inducing a more prominent suppression of the signaling pathway PI3K/Akt compared to DTX alone[69]. Co-treatment of prostate cancer cells with these two agents resulted in a greater upregulation of Bax, BH3 interacting-domain death agonist (Bid), caspase 3 and PARP and a higher downregulation of Bcl-xL compared to individual treatments[70]. To enhance drug solubility, increase their efficacy and reduce DTX toxicities, multiple nanoparticle drug delivery systems for the co-delivery of TQ and DTX have been developed and evaluated on breast cancer cells. Loading TQ and DTX into a borage nanoemulsion delivery system allowed the lowering of the required effective dose of DTX and enhanced cell death in cancer cells through simultaneous stimulation of apoptosis and autophagy[71]. In another study, co-encapsulating TQ and DTX in low-molecular-weight chitosan coated lipid nanocapsules was found to exhibit stronger cytotoxic and anti-angiogenic responses in cancer cells compared to the free single treatments[72]. The co-delivery of TQ and DTX in pegylated lipid nanocapsules produced more effective apoptotic and anti-migratory effects in cancer cells in addition to a higher tumor growth inhibition in mice bearing Ehrlich ascites carcinoma compared to free single treatments. Interestingly, these dual drugs loaded lipid nanocapsules prevented the development of DTX-induced hematological, hepato- and nephro- toxicities, an indicator of their protective potential[73]. Moreover, TQ and DTX were co-encapsulated into pegylated liposomes and tested against MCF-7 breast cancer cells. The half maximal inhibitor concentration of each of TQ and DTX co-loaded into liposomes were lower than those of the free individual drugs[74].

**Cabazitaxel:** Cabazitaxel (CBZ) was approved as the second line therapy for metastatic castration-resistant prostate cancer[75]. However, its low aqueous solubility, poor membrane permeability, and severe side effects like neutropenia and anemia are the challenging drawbacks for successful cancer management[76,77]. Combining TQ with CBZ caused synergistic apoptotic effects in breast cancer cells. To address the drug delivery challenge, TQ and CBZ were co-loaded in lipospheres. The combined drugs loaded lipospheres had enhanced apoptotic effects compared to the drug combination in solution[78].

***Cytotoxic antibiotics***

**Doxorubicin[79]:** Doxorubicin (DOX) is a primarily adopted chemotherapeutic agent for treating a wide spectrum of solid and liquid tumors[80]. Despite the robust anticancer activity of DOX, chemoresistance and severe side effects especially cardiotoxicity weakened its potency[81]. Nearly 11% of the patients treated with this agent develop acute cardiotoxicity[82]. Several studies demonstrated the powerful combinatorial effect of TQ on the anticancer efficacy of DOX. Combining TQ with DOX allowed the lowering of DOX dose by up to 2-fold while maintaining its anticancer potential against adult T cell leukemia (ATL). TQ and DOX synergized to induce caspases and ROS mediated apoptosis in human T-lymphotropic virus-1 positive and human T-lymphotropic virus-1 negative CD4+ malignant T cell lines *in vitro* in addition to suppressing the growth of an ATL xenograft in mice[83]. In addition, co-treatment of HL-60 acute myeloid leukemia cells with TQ and DOX induced two consecutives waves of caspase 3 activity in addition to more than 7-fold increase in ROS generation compared to DOX alone[84]. In breast cancer, TQ potentiated the anti-tumor activity of DOX *in vivo* by inducing apoptosis and inhibiting tumor cell proliferation to a larger extent than separate treatments[85]. Recently, TQ was shown to improve the apoptotic effect of subtoxic doses of DOX in hepatocarcinoma cells by further increasing the cleavage of caspase 3 and PARP in addition to reducing DOX-induced cytotoxicity to normal liver cells[86]. This synergistic inhibitory effect of TQ and DOX combination was also observed in chemoresistant cancer cells. TQ augmented DOX cell growth inhibitory effect by 2- and 1.2-fold in multi-drug resistant breast cancer cells and in DOX resistant colorectal cancer cells, respectively[84]. To enhance the synergistic effect of these two agents, two nanodrug delivery systems have been developed. Loading TQ and DOX in cockle shell-derived aragonite calcium carbonate nanoparticles (ACNP) showed higher efficacy in inducing apoptosis and reducing migration and invasion in breast cancer cells than the free drugs or the single drug loaded ACNP while being non-toxic to non-neoplastic cells[87]. In addition, incorporating TQ and DOX into F2 gel (poly-N-acetyl glucosamine) nanofibers exhibited superior cellular growth inhibition and apoptosis in breast and liver cancer cells compared to free drugs and single drug loaded nanoparticles. The anticancer potency of this nanodrug co-delivery system was further demonstrated in two *in vivo* cancer models. The dual loading TQ and DOX nanoparticles enhanced tumor suppression *via* apoptosis in mice bearing liver carcinoma by decreasing NFB level and increasing caspase 3 as well as in mice bearing solid Ehrlich carcinoma by attenuating Bcl-2 level and up-regulating p53. Interestingly, this treatment also reduced the nephro- and cardio-toxicities induced by DOX through the attenuation of the oxidative stress[88,89].

***Topoisomerase inhibitor***

**Topotecan[90]:** Topotecan (TP) was approved for the second-line treatment of small cell lung cancer and was recommended to treat platinum resistant ovarian cancer[91,92]. The instability of the chemical structure of TP in aqueous solutions and in the plasma reduces its anticancer efficacy and causes side effects[93,94]. TQ was found to boost the anti-proliferative and apoptotic effects of non-cytotoxic doses of TP in acute myelogenous leukemia and in colon cancer cells. This effect was exerted by upregulation of p53 and Bax, downregulation of Bcl-2, increase in the cleavage of caspases 9 and 3 in leukemia cells and through p53- and Bax/Bcl-2-independent mechanisms in colon cancer cells. In addition, pretreatment of leukemia cells with TQ followed by TP was found to be more effective than the simultaneous application of both therapeutic agents[95,96].

***Proteasome inhibitor***

**Bortezomib:** Bortezomib (BTZ) was approved for the treatment of multiple myeloma[97]. It acts by inhibiting NFB pathway known to be constitutively activated in multiple myeloma due to genetic aberrations in its components[98]. TQ was found to augment the apoptotic activity of BTZ in multiple myeloma cells *in vitro* by enhancing caspase 3 activation and PARP cleavage. In a xenograft multiple myeloma mouse model, TQ potentiated the anti-neoplastic effects of BTZ by further suppressing NFB and consequently downregulating the proliferative (Ki67), anti-apoptotic (Bcl-2), angiogenic (VEGF) and inflammatory (interleukin-6 and tumor necrosis factor-α) effectors. The authors further showed that TQ reduced the proliferation of BTZ resistant multiple myeloma cells[99].

***Tyrosine kinase inhibitor***

**Imatinib:** Imatinib (IM) is a potent tyrosine kinase inhibitor that was approved for treating chronic myeloid leukemia and gastrointestinal stromal tumors[100]. Resistance to IM was reported to develop in cancer patients through several mechanisms including the modulation of the expression of drug efflux and influx transporters[101,102]. In a study conducted by Thabet *et al*[103], TQ was found to improve the anti-proliferative and apoptotic effects of IM in colorectal cancer cells *in vitro*. Interestingly, this was accompanied by a significant decrease in the expression of the drug transporters ATP-binding cassette (ABC) subfamily B member (ABCB) 1, ABCG2 and human organic cation transporter 1 leading to a significant increase in IM uptake/efflux ratio compared to IM alone.

***Hormone receptor modulator***

**Tamoxifen[104]:** Tamoxifen (TAM) is one of the first-line therapies for hormone receptor-positive breast cancer patients[105]. A synergistic apoptotic effect was observed by combining TQ and TAM in breast cancer cells *in vitro* regardless of hormone receptor status[106]. Apoptosis was induced through synergistic inhibition of X-linked inhibitor of apoptosis protein (XIAP) resulting in caspase 9 activation and PARP cleavage along with PI3K/Akt pathway inhibition, which caused the downregulation of Bcl-xL, Bcl-2, and upregulation of Bax, apoptosis inducing factor, cytochrome c and p27. TQ was also found to enhance TAM anti-angiogenic, anti-migratory and anti-invasive effects in breast cancer[107]. In addition, treating breast cancer patients with a combination of TQ and TAM resulted in greater increase in 5-year survival rate and decrease in relapse rate of patients compared to single treatments. At the molecular level, the dual treatment induced a higher increase in tumor tissue antioxidant enzymes (catalase and superoxide dismutase) and increased caspase 3 expression compared to individual treatments. Moreover, the combination of TQ and TAM enhanced the decrease in tumor tissue Bcl-2, TGF-β1, lipid peroxidation product malondialdehyde and pro-inflammatory cytokines tumor necrosis factor-α andinterleukin-6 compared to each treatment alone[108].

***Biphosphonate***

**Zoledronic acid:** Zoledronic acid is a nitrogen-containing bisphosphonate that inhibits osteoclast*-*mediated [bone resorption](https://www.sciencedirect.com/topics/medicine-and-dentistry/osteolysis). It was approved to prevent and reduce the progression of skeletal complications associated with bone metastasis from solid tumors including prostate cancer[109]. Besides its anti-resorption activity, preclinical and clinical data demonstrated its anti-tumor effects in different types of cancer[110,111]. TQ intensified the apoptotic activity of zoledronic acid in PC-3 (hor­mone resistant and chemotherapy sensitive) and DU-145 (hormone and chemotherapy resistant) prostate cancer cell lines through a synergistic increase in DNA fragmentation in both cell lines and a synergistic activation of caspases 3 and 7 in PC-3 cells[112].

***Arsenic trioxide***

Arsenic trioxide was approved for the treatment of acute promyelocytic leukemia[113]. The combination of arsenic trioxide (As) with interferon alpha(IFN-α) was found to have an effective anti-neoplastic activity in ATL. As and IFN-α synergistically induced apoptosis in ATL leukemia cells *in vitro* and cured murine ATL[114,115]. A phase II trial involving patients with relapsed/refractory adult T-cell leukemia/lymphoma showed that the combination of As and IFN-α exhibited anticancer effects but caused significant toxicity[116]. Combining TQ with As and IFN-α induced synergistic apoptotic activity *in vitro* and *in vivo* and allowed the reduction of the toxic doses of As. TQ alone or TQ/As/IFN-α combination downregulated XIAP and Bcl-2, upregulated Bax and induced cleavage of PARP and caspase 3[117], ultimately leading to enhanced apoptosis.

***TQ in combination with ionizing radiation***

Radiotherapy is a mainstay therapeutic modality for the treatment of early and advanced solid cancers. Nearly 50% of cancer patients receive radiotherapy during their treatment course[118]. However, its therapeutic potency was found to be compromised by the damage of the surrounding healthy tissue in addition to the development of radioresistance[119]. To overcome these challenges and enhance radiotherapy efficacy, exploring radiosensitizers, molecules that make cancer cells more susceptible to radiations, has attracted great attention[120]. Several studies demonstrated the radiosensitizing role of TQ on cancer cells *in vitro*. TQ augmented the anti-proliferative and apoptotic effects of ionizing radiation and further enriched the sub-G1 population in breast cancer cells[121]. In addition, sensitization with TQ prevented the radiation-induced metastatic progression of breast cancer cells through the restoration of the levels of TGF-β and its downstream effectors in addition to epithelial and mesenchymal markers[122]. In melanoma, TQ enhanced the apoptotic responses of low doses of gamma knife irradiation by further inhibiting the phosphorylation of STAT3, which is known to play a key role in cancer cell proliferation, survival, angiogenesis and metastasis[41]. It also improved the gamma knife irradiation-induced immune response by further attenuating the secretion of tumor-related inflammatory cytokines[123]. The cellular and molecular mechanisms of action of TQ in combination with radiation and other therapeutic agents discussed in this review are presented in Table 2.

***TQ in combination with non-coding RNA***

Gene therapy is a modern therapeutic approach that demonstrated immense and impressive potential against cancer. It consists of delivering therapeutic genetic materials such as small interfering RNA (siRNA), microRNA, and anti-sense oligonucleotides into cancer cells to restore target gene expression, which is modulated and associated with tumorigenesis[124]. miR-34a is a tumor-suppressive microRNA found to be downregulated in numerous human cancers including breast cancer[125]. Re-introducing miR-34a in metastatic breast cancer cells targeted and inhibited the expression of epithelial to mesenchymal transition-associated proteins TWIST1, zinc finger E-box binding homeobox 1 and NOTCH1 and suppressed breast cancer cell migration and invasion. Moreover, combining TQ with miR-34a synergistically downregulated TWIST1 and zinc finger E-box binding homeobox 1, suggesting the promising therapeutic potential of this combination against breast cancer metastasis[126]. In another study, multilamellar gold niosomes were developed for the co-delivery of therapeutic Akt-siRNA and TQ to overcome chemotherapeutic resistance induced by Akt overexpression in breast cancer. TQ-siRNA dual loaded niosomes produced stronger anti-proliferative and apoptotic effects in breast cancer *in vitro* and *in vivo* compared to free TQ and TQ loaded niosomes. The mechanism of the combination treatment involved an effective decrease of the cellular level of Akt which sensitized breast cancer cells to TQ toxicity leading to inhibition of mouse double minute 2 and therefore induction of p53-dependent apoptosis[127].

***TQ in combination with natural molecules***

**Vitamins:** Vitamin D3, the active metabolite of vitamin D, was reported to have potent chemopreventive effects against colorectal cancer *in vitro* and *in vivo*[128,129]. In addition, vitamin D supplementation was demonstrated to have clinically positive effects on survival outcomes in patients with colorectal cancer[130]. TQ was found to enhance the chemopreventive effect of vitamin D3 in suppressing the initiation and progression of colon tumors in an azoxymethane-induced rat model of colon cancer. The combination treatment significantly attenuated the number of grown tumors and large aberrant crypts foci. In addition, it decreased the level of pro-oncogenic (Wnt, β-catenin, NFB, heat shock protein 90 HSP-90) and angiogenic (VEGF, iNOS and COX2) biomarkers and increased the expression of anti-oncogenic (DKK-1, CDNK-1A, TGF-β1, TGF-β/RII and Smad4) biomarkers compared with individual treatments[131].

***Hormones***

**Melatonin****:** Melatonin is a natural hormone involved in different biological activities including regulating the circadian rhythm[132]. Ample evidence revealed that melatonin exerts powerful anti-tumor effects through different modes of action including the activation of anticancer immune responses[133]. The combination of TQ with melatonin in breast cancer bearing mice resulted in 60% of cure in treated mice and produced a stronger apoptotic, necrotic and anti-angiogenetic response in addition to a more potent activation of T helper 1 mediated anticancer immune response compared to separate treatments[134].

***Plant-derived molecules***

Numerous studies have tested the anti-neoplastic efficacy of combining TQ with other plant-derived molecules in different types of cancer. Artemisinin is a sesquiterpene lactone extracted from the Chinese medicinal plant *Artemisia annua*[135]. Fröhlich *et al*[136,137] linked each of Artemisinin and its semisynthetic derivative artesunic acid with TQ *via* covalent bonds and tested the anticancer efficacy of the formed hybrid molecules *in vitro*. They found that the ether-linked artemisinin-TQ hybrid exhibited a potent and selective anti-proliferative activity that was superior to that of the conventional drug DOX against sensitive and multidrug-resistant leukemia cells without being toxic to normal human foreskin fibroblasts[136]. They also found that the ester-linked artesunic acid-TQ hybrid promoted apoptosis mediated by ROS-induced DNA damage in colon cancer cells while being non-toxic to normal colon epithelial cells. The hybrid’s effect was found superior to each of the conventional drug 5-FU, the dual and individual treatments[137]. In another study, Das *et al*[138] demonstrated the synergistic anti-proliferative and apoptotic potential of combining TQ with diosgenin, a natural steroidal saponin isolated from several plants such as *Trigonella foenum-graecum*[139], in squamous cell carcinoma *in vitro* and in a sarcoma 180-induced mouse model. Recently, [Bhattacharjee](https://www.sciencedirect.com/science/article/pii/S0304416520302075%22%20%5Cl%20%22%21) *et al*[140] investigated the combined effect of TQ with emodin, which is a natural anthraquinone obtained from various herbs including *Rheum palmatum*[141]. The results revealed that the dual treatment triggered a synergistic apoptotic response in breast cancer cells and enhanced the reduction of cancer cell migration compared to monotreatment by downregulating two important molecular players, namely focal adhesion kinase and integrin β1. In an *ex ovo* chorioallantoic membrane xenograft model, TQ and emodin were found to suppress the tumor growth and limit the migration of tumor cells to the liver and lung of the chick embryo[140]. Combining low doses of TQ and ferulic acid, obtained from *Ferula asafetida* plant[142], potently inhibited the proliferation of breast cancer cells, while single treatments did not exhibit any inhibitory effects[143]. Moreover, it has been found that the combination of TQ and genistein, a flavonoid found in soybeans[144], resulted in a higher induction of apoptosis in thyroid cancer cells than treatment with either agent alone[145]. In lung cancer, the combination of TQ and indirubin-3-monoxime, a drug derived from the traditional Chinese herbal remedy Danggui Longhui Wan[146], resulted in synergistic apoptotic and anti-migratory effects *in vitro* and synergistic tumor growth suppression *in vivo*[147]. At the molecular level, the dual treatment decreased the phosphorylation of survival-regulatory proteins Akt, mammalian target of rapamycin and NFB and activated caspase 3 and p53 in animal tumors[147]. Furthermore, combining TQ and piperine, the major alkaloid found in *Piper nigrum L*[148], resulted in a synergistic inhibition of breast cancer *in vitro* and *in vivo*[149]. It induced a high degree of apoptosis and extensive necrosis, inhibited angiogenesis, and stimulated T helper 1 anticancer immune response with no liver and kidney toxicities. Interestingly, TQ was found to play the major role in inducing the caspase-mediated apoptosis[149]. In another study, the encapsulation of TQ and piperine in micro-vehicles made of a natural polymer guar gum extracted from the seeds of *Cymompsis tetraganolobus* plant[150] synergistically reduced the viability of hepatocellular carcinoma cells[151]. This was associated with ROS generation as indicated by an enhanced decrease in the level of intracellular antioxidant glutathione and nicotinamide-adenine dinucleotide phosphate[151]. Two studies assessed the anticancer effectiveness of combining TQ with resveratrol, a stilbene polyphenolic compound extracted from over 70 plants including Polygonum cuspidatum[152]. TQ and resveratrol combination resulted in a greater cytotoxic effect on hepatocellular carcinoma cells compared to single treatments[153]. In addition, TQ and resveratrol synergized to effectively inhibit breast cancer *in vitro* and *in vivo*. The combined drugs induced apoptosis and necrosis, inhibited angiogenesis and stimulated the anticancer immune response without causing liver and kidney toxicities[154]. Co-treating osteosarcoma cells with TQ and selenium, a micronutrient/trace element found abundantly in Astragalus bisulcatus[155], was found to be effective in decreasing cell viability, inducing cellular damage, and attenuating the levels of alkaline phosphatase and glutathione[156].

**TQ EFFECTS AGAINST CANCER STEM CELLS**

***TQ in combination with chemotherapeutic agents***

TQ was found to potentiate the effects of each of GCB and PAC in depleting the CD44+/CD24- CSCs population within MCF-7 and T47D breast cancer cells[58,64]. In another study, the co-delivery of DOX and TQ in ACNP effectively eradicated breast CSCs enriched from MDA-MB-231 cells cultured in 3D compared to single drug loaded ACNP and drug combinations in solution. The combined drugs loaded ACNP efficiently attenuated the self-renewal potential of breast CSCs as evidenced by the decrease of their mammospheres forming efficiency. This was accompanied by the reduction of breast CSCs markers CD44 and CD24 expression and aldehyde dehydrogenase 1 activity. In addition, the dual drugs loaded ACNP suppressed breast CSCs migration and invasion[157]. In colorectal cancer, combination of TQ and 5-FU as well as their hybrid SARB downregulated two major stem cell regulatory pathways Wnt/β-catenin and PI3K/Akt. In addition, they were found to effectively reduce the self-renewal potential of colorectal CSCs and eradicate CD133+ colorectal CSC population[50].

***TQ in combination with natural products***

The combined treatment of TQ and emodin improved the elimination of breast CSCs as demonstrated by the enhanced reduction in mammospheres forming efficiency and in CD44+/CD24- CSCS population compared to single treatments. Moreover, it downregulated the stemness promoting transcription factors Oct4 and SOX2[140].

**CONCLUSION**

We have emphasized the tremendous potential of TQ in augmenting the anti-neoplastic effects of different therapeutic modalities against a wide range of cancer cells. TQ sensitized cancer cells to radiotherapy and improved outcomes of cancer resistance to conventional chemotherapeutic agents. The use of TQ in combination therapy also lowered the effective doses of standard chemotherapies which helped reduce their associated toxicities while maintaining their therapeutic effectiveness. The combination of TQ with other plant-derived molecules has shown interesting results and merits further investigation to introduce them as potential candidates for treating cancer. Although the studies investigating TQ potency in eliminating CSC in combination therapy are scarce, their results demonstrated great promise. Involving TQ in combination therapy could possibly further eliminate CSCs from tumors and prevent regrowth of neoplasms.

Despite its remarkable anticancer activity, studies reporting TQ anticancer therapeutic potential in clinical settings are still limited due mainly to its hydrophobicity and poor bioavailability. Few studies have supported combined therapies of TQ with nanoparticle formulations to circumvent the drug delivery challenges. These nanoparticles further enhanced the inhibitory effects of the combined agents against cancer or CSC in preclinical studies. Future efforts should be devoted to developing and testing these effective targeted nanoformulations of the combined agents including TQ for potential clinical translation.

**REFERENCES**

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Tang L**, Wei F, Wu Y, He Y, Shi L, Xiong F, Gong Z, Guo C, Li X, Deng H, Cao K, Zhou M, Xiang B, Li X, Li Y, Li G, Xiong W, Zeng Z. Role of metabolism in cancer cell radioresistance and radiosensitization methods. *J Exp Clin Cancer Res* 2018; **37**: 87 [PMID: 29688867 DOI: 10.1186/s13046-018-0758-7]

3 **Alfarouk KO**, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, Bashir AH, Mohammed OY, Elhassan GO, Harguindey S, Reshkin SJ, Ibrahim ME, Rauch C. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. *Cancer Cell Int* 2015; **15**: 71 [PMID: 26180516 DOI: 10.1186/s12935-015-0221-1]

4 **Haque MM**, Desai KV. Pathways to Endocrine Therapy Resistance in Breast Cancer. *Front Endocrinol (Lausanne)* 2019; **10**: 573 [PMID: 31496995 DOI: 10.3389/fendo.2019.00573]

5 **Sabnis AJ**, Bivona TG. Principles of Resistance to Targeted Cancer Therapy: Lessons from Basic and Translational Cancer Biology. *Trends Mol Med* 2019; **25**: 185-197 [PMID: 30686761 DOI: 10.1016/j.molmed.2018.12.009]

6 **Mao JJ**, Chung A, Benton A, Hill S, Ungar L, Leonard CE, Hennessy S, Holmes JH. Online discussion of drug side effects and discontinuation among breast cancer survivors. *Pharmacoepidemiol Drug Saf* 2013; **22**: 256-262 [PMID: 23322591 DOI: 10.1002/pds.3365]

7 **Dagogo-Jack I**, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* 2018; **15**: 81-94 [PMID: 29115304 DOI: 10.1038/nrclinonc.2017.166]

8 **Park SY**, Gönen M, Kim HJ, Michor F, Polyak K. Cellular and genetic diversity in the progression of in situ human breast carcinomas to an invasive phenotype. *J Clin Invest* 2010; **120**: 636-644 [PMID: 20101094 DOI: 10.1172/JCI40724]

9 **Assenov Y**, Brocks D, Gerhäuser C. Intratumor heterogeneity in epigenetic patterns. *Semin Cancer Biol* 2018; **51**: 12-21 [PMID: 29366906 DOI: 10.1016/j.semcancer.2018.01.010]

10 **Jolly MK**, Celià-Terrassa T. Dynamics of Phenotypic Heterogeneity Associated with EMT and Stemness during Cancer Progression *J Clin Med* 2019; **8** [PMID: 31557977 DOI: 10.3390/jcm8101542]

11 **Pribluda A**, de la Cruz CC, Jackson EL. Intratumoral Heterogeneity: From Diversity Comes Resistance. *Clin Cancer Res* 2015; **21**: 2916-2923 [PMID: 25838394 DOI: 10.1158/1078-0432.CCR-14-1213]

12 **Prieto-Vila M**, Takahashi RU, Usuba W, Kohama I, Ochiya T. Drug Resistance Driven by Cancer Stem Cells and Their Niche. *Int J Mol Sci* 2017; **18** [PMID: 29194401 DOI: 10.3390/ijms18122574]

13 **Bayat Mokhtari R**, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, Yeger H. Combination therapy in combating cancer. *Oncotarget* 2017; **8**: 38022-38043 [PMID: 28410237 DOI: 10.18632/oncotarget.16723]

14 **Newman DJ**, Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Nat Prod* 2007; **70**: 461-477 [PMID: 17309302 DOI: 10.1021/np068054v]

15 **Taylor WF**, Jabbarzadeh E. The use of natural products to target cancer stem cells. *Am J Cancer Res* 2017; **7**: 1588-1605 [PMID: 28744407]

16 **Sauter ER**. Cancer prevention and treatment using combination therapy with natural compounds. *Expert Rev Clin Pharmacol* 2020; **13**: 265-285 [PMID: 32154753 DOI: 10.1080/17512433.2020.1738218]

17 **Newman DJ**, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J Nat Prod* 2020; **83**: 770-803 [PMID: 32162523 DOI: 10.1021/acs.jnatprod.9b01285]

18 **Majdalawieh AF**, Fayyad MW, Nasrallah GK. Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of Nigella sativa. *Crit Rev Food Sci Nutr* 2017; **57**: 3911-3928 [PMID: 28140613 DOI: 10.1080/10408398.2016.1277971]

19 **Park EJ**, Chauhan AK, Min KJ, Park DC, Kwon TK. Thymoquinone induces apoptosis through downregulation of c-FLIP and Bcl-2 in renal carcinoma Caki cells. *Oncol Rep* 2016; **36**: 2261-2267 [PMID: 27573448 DOI: 10.3892/or.2016.5019]

20 **Gurung RL**, Lim SN, Khaw AK, Soon JF, Shenoy K, Mohamed Ali S, Jayapal M, Sethu S, Baskar R, Hande MP. Thymoquinone induces telomere shortening, DNA damage and apoptosis in human glioblastoma cells. *PLoS One* 2010; **5**: e12124 [PMID: 20711342 DOI: 10.1371/journal.pone.0012124]

21 **El-Najjar N**, Chatila M, Moukadem H, Vuorela H, Ocker M, Gandesiri M, Schneider-Stock R, Gali-Muhtasib H. Reactive oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. *Apoptosis* 2010; **15**: 183-195 [PMID: 19882352 DOI: 10.1007/s10495-009-0421-z]

22 **Mahmoud YK**, Abdelrazek HMA. Cancer: Thymoquinone antioxidant/pro-oxidant effect as potential anticancer remedy. *Biomed Pharmacother* 2019; **115**: 108783 [PMID: 31060003 DOI: 10.1016/j.biopha.2019.108783]

23 **Schneider-Stock R**, Fakhoury IH, Zaki AM, El-Baba CO, Gali-Muhtasib HU. Thymoquinone: fifty years of success in the battle against cancer models. *Drug Discov Today* 2014; **19**: 18-30 [PMID: 24001594 DOI: 10.1016/j.drudis.2013.08.021]

24 **Ballout F**, Monzer A, Fatfat M, Ouweini HE, Jaffa MA, Abdel-Samad R, Darwiche N, Abou-Kheir W, Gali-Muhtasib H. Thymoquinone induces apoptosis and DNA damage in 5-Fluorouracil-resistant colorectal cancer stem/progenitor cells. *Oncotarget* 2020; **11**: 2959-2972 [PMID: 32821342 DOI: 10.18632/oncotarget.27426]

25 **Emadi A**, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. *Nat Rev Clin Oncol* 2009; **6**: 638-647 [PMID: 19786984 DOI: 10.1038/nrclinonc.2009.146]

26 **Falzone L**, Salomone S, Libra M. Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium. *Front Pharmacol* 2018; **9**: 1300 [PMID: 30483135 DOI: 10.3389/fphar.2018.01300]

27 **Khan A**, Aldebasi YH, Alsuhaibani SA, Khan MA. Thymoquinone Augments Cyclophosphamide-Mediated Inhibition of Cell Proliferation in Breast Cancer Cells *Asian Pac J Cancer Prev* 2019; **20**: 1153-1160 [PMID: 31030489 DOI: 10.31557/APJCP.2019.20.4.1153]

28 **Blanco-Aparicio C**, Renner O, Leal JF, Carnero A. PTEN, more than the AKT pathway. *Carcinogenesis* 2007; **28**: 1379-1386 [PMID: 17341655 DOI: 10.1093/carcin/bgm052]

29 **Stupp R**, Gander M, Leyvraz S, Newlands E. Current and future developments in the use of temozolomide for the treatment of brain tumours. *Lancet Oncol* 2001; **2**: 552-560 [PMID: 11905710 DOI: 10.1016/S1470-2045(01)00489-2]

30 **Dehdashti AR**, Hegi ME, Regli L, Pica A, Stupp R. New trends in the medical management of glioblastoma multiforme: the role of temozolomide chemotherapy. *Neurosurg Focus* 2006; **20**: E6 [PMID: 16709037 DOI: 10.3171/foc.2006.20.4.3]

31 **Lee SY**. Temozolomide resistance in glioblastoma multiforme. *Genes Dis* 2016; **3**: 198-210 [PMID: 30258889 DOI: 10.1016/j.gendis.2016.04.007]

32 **Khazaei M**, Pazhouhi M. Temozolomide-Mediated Apoptotic Death Is Improved by Thymoquinone in U87MG Cell Line. *Cancer Invest* 2017; **35**: 225-236 [PMID: 28355088 DOI: 10.1080/07357907.2017.1289383]

33 **Pazhouhi M**, Sariri R, Khazaei MR, Moradi MT, Khazaei M. Synergistic effect of temozolomide and thymoquinone on human glioblastoma multiforme cell line (U87MG). *J Cancer Res Ther* 2018; **14**: 1023-1028 [PMID: 30197342 DOI: 10.4103/0973-1482.187241]

34 **Winer A**, Adams S, Mignatti P. Matrix Metalloproteinase Inhibitors in Cancer Therapy: Turning Past Failures Into Future Successes. *Mol Cancer Ther* 2018; **17**: 1147-1155 [PMID: 29735645 DOI: 10.1158/1535-7163.MCT-17-0646]

35 **Pazhouhi M**, Sariri R, Rabzia A, Khazaei M. Thymoquinone synergistically potentiates temozolomide cytotoxicity through the inhibition of autophagy in U87MG cell line. *Iran J Basic Med Sci* 2016; **19**: 890-898 [PMID: 27746872]

36 **Siddik ZH**. Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene* 2003; **22**: 7265-7279 [PMID: 14576837 DOI: 10.1038/sj.onc.1206933]

37 **van den Berg JH**, Beijnen JH, Balm AJ, Schellens JH. Future opportunities in preventing cisplatin induced ototoxicity. *Cancer Treat Rev* 2006; **32**: 390-397 [PMID: 16781082 DOI: 10.1016/j.ctrv.2006.04.011]

38 **Volarevic V**, Djokovic B, Jankovic MG, Harrell CR, Fellabaum C, Djonov V, Arsenijevic N. Molecular mechanisms of cisplatin-induced nephrotoxicity: a balance on the knife edge between renoprotection and tumor toxicity. *J Biomed Sci* 2019; **26**: 25 [PMID: 30866950 DOI: 10.1186/s12929-019-0518-9]

39 **Wilson AJ**, Saskowski J, Barham W, Yull F, Khabele D. Thymoquinone enhances cisplatin-response through direct tumor effects in a syngeneic mouse model of ovarian cancer. *J Ovarian Res* 2015; **8**: 46 [PMID: 26215403 DOI: 10.1186/s13048-015-0177-8]

40 **Hu X**, Ma J, Vikash V, Li J, Wu D, Liu Y, Zhang J, Dong W. Thymoquinone Augments Cisplatin-Induced Apoptosis on Esophageal Carcinoma Through Mitigating the Activation of JAK2/STAT3 Pathway. *Dig Dis Sci* 2018; **63**: 126-134 [PMID: 29197940 DOI: 10.1007/s10620-017-4856-8]

41 **Yu H**, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. *Nat Rev Cancer* 2014; **14**: 736-746 [PMID: 25342631 DOI: 10.1038/nrc3818]

42 **Jafri SH**, Glass J, Shi R, Zhang S, Prince M, Kleiner-Hancock H. Thymoquinone and cisplatin as a therapeutic combination in lung cancer: In vitro and in vivo. *J Exp Clin Cancer Res* 2010; **29**: 87 [PMID: 20594324 DOI: 10.1186/1756-9966-29-87]

43 **Alaufi OM**, Noorwali A, Zahran F, Al-Abd AM, Al-Attas S. Cytotoxicity of thymoquinone alone or in combination with cisplatin (CDDP) against oral squamous cell carcinoma in vitro. *Sci Rep* 2017; **7**: 13131 [PMID: 29030590 DOI: 10.1038/s41598-017-13357-5]

44 **Ma J**, Hu X, Li J, Wu D, Lan Q, Wang Q, Tian S, Dong W. Enhancing conventional chemotherapy drug cisplatin-induced anti-tumor effects on human gastric cancer cells both *in vitro* and *in vivo* by Thymoquinone targeting PTEN gene. *Oncotarget* 2017; **8**: 85926-85939 [PMID: 29156767 DOI: 10.18632/oncotarget.20721]

45 **Wilson PM**, Danenberg PV, Johnston PG, Lenz HJ, Ladner RD. Standing the test of time: targeting thymidylate biosynthesis in cancer therapy. *Nat Rev Clin Oncol* 2014; **11**: 282-298 [PMID: 24732946 DOI: 10.1038/nrclinonc.2014.51]

46 **Sara JD**, Kaur J, Khodadadi R, Rehman M, Lobo R, Chakrabarti S, Herrmann J, Lerman A, Grothey A. 5-fluorouracil and cardiotoxicity: a review. *Ther Adv Med Oncol* 2018; **10**: 1758835918780140 [PMID: 29977352 DOI: 10.1177/1758835918780140]

47 **Zhang N**, Yin Y, Xu SJ, Chen WS. 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules* 2008; **13**: 1551-1569 [PMID: 18794772 DOI: 10.3390/molecules13081551]

48 **Lei X**, Lv X, Liu M, Yang Z, Ji M, Guo X, Dong W. Thymoquinone inhibits growth and augments 5-fluorouracil-induced apoptosis in gastric cancer cells both in vitro and in vivo. *Biochem Biophys Res Commun* 2012; **417**: 864-868 [PMID: 22206670 DOI: 10.1016/j.bbrc.2011.12.063]

49 **Kensara OA**, El-Shemi AG, Mohamed AM, Refaat B, Idris S, Ahmad J. Thymoquinone subdues tumor growth and potentiates the chemopreventive effect of 5-fluorouracil on the early stages of colorectal carcinogenesis in rats. *Drug Des Devel Ther* 2016; **10**: 2239-2253 [PMID: 27468227 DOI: 10.2147/DDDT.S109721]

50 **Ndreshkjana B**, Çapci A, Klein V, Chanvorachote P, Muenzner JK, Huebner K, Steinmann S, Erlenbach-Wuensch K, Geppert CI, Agaimy A, Ballout F, El-Baba C, Gali-Muhtasib H, Roehe AV, Hartmann A, Tsogoeva SB, Schneider-Stock R. Combination of 5-fluorouracil and thymoquinone targets stem cell gene signature in colorectal cancer cells. *Cell Death Dis* 2019; **10**: 379 [PMID: 31097715 DOI: 10.1038/s41419-019-1611-4]

51 **Williams S**, Tucci MA, Benghuzzi HA. The effect of combination treatments of epigallocatechin-3-gallate, thymoquinone, and 5-Fluorouracil on fadu nasopharyngeal carcinoma cells. *Biomed Sci Instrum* 2014; **50**: 361-366 [PMID: 25405445]

52 **Sarman H**, Bayram R, Benek SB. Anticancer drugs with chemotherapeutic interactions with thymoquinone in osteosarcoma cells. *Eur Rev Med Pharmacol Sci* 2016; **20**: 1263-1270 [PMID: 27097945]

53 **Moysan E**, Bastiat G, Benoit JP. Gemcitabine versus Modified Gemcitabine: a review of several promising chemical modifications. *Mol Pharm* 2013; **10**: 430-444 [PMID: 22978251 DOI: 10.1021/mp300370t]

54 **Toschi L**, Finocchiaro G, Bartolini S, Gioia V, Cappuzzo F. Role of gemcitabine in cancer therapy. *Future Oncol* 2005; **1**: 7-17 [PMID: 16555971 DOI: 10.1517/14796694.1.1.7]

55 **Amrutkar M**, Gladhaug IP. Pancreatic Cancer Chemoresistance to Gemcitabine. *Cancers (Basel)* 2017; **9** [PMID: 29144412 DOI: 10.3390/cancers9110157]

56 **Pandita A**, Kumar B, Manvati S, Vaishnavi S, Singh SK, Bamezai RN. Synergistic combination of gemcitabine and dietary molecule induces apoptosis in pancreatic cancer cells and down regulates PKM2 expression. *PLoS One* 2014; **9**: e107154 [PMID: 25197966 DOI: 10.1371/journal.pone.0107154]

57 **Mu GG**, Zhang LL, Li HY, Liao Y, Yu HG. Thymoquinone Pretreatment Overcomes the Insensitivity and Potentiates the Antitumor Effect of Gemcitabine Through Abrogation of Notch1, PI3K/Akt/mTOR Regulated Signaling Pathways in Pancreatic Cancer. *Dig Dis Sci* 2015; **60**: 1067-1080 [PMID: 25344906 DOI: 10.1007/s10620-014-3394-x]

58 **Bashmail HA**, Alamoudi AA, Noorwali A, Hegazy GA, AJabnoor G, Choudhry H, Al-Abd AM. Thymoquinone synergizes gemcitabine anti-breast cancer activity via modulating its apoptotic and autophagic activities. *Sci Rep* 2018; **8**: 11674 [PMID: 30076320 DOI: 10.1038/s41598-018-30046-z]

59 **Ojima I**, Lichtenthal B, Lee S, Wang C, Wang X. Taxane anticancer agents: a patent perspective. *Expert Opin Ther Pat* 2016; **26**: 1-20 [PMID: 26651178 DOI: 10.1517/13543776.2016.1111872]

60 **Zhu L**, Chen L. Progress in research on paclitaxel and tumor immunotherapy. *Cell Mol Biol Lett* 2019; **24**: 40 [PMID: 31223315 DOI: 10.1186/s11658-019-0164-y]

61 **Galletti E**, Magnani M, Renzulli ML, Botta M. Paclitaxel and docetaxel resistance: molecular mechanisms and development of new generation taxanes. *ChemMedChem* 2007; **2**: 920-942 [PMID: 17530726 DOI: 10.1002/cmdc.200600308]

62 **Gelderblom H**, Verweij J, Nooter K, Sparreboom A. Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *Eur J Cancer* 2001; **37**: 1590-1598 [PMID: 11527683 DOI: 10.1016/s0959-8049(01)00171-x]

63 **Şakalar Ç**, İzgi K, İskender B, Sezen S, Aksu H, Çakır M, Kurt B, Turan A, Canatan H. The combination of thymoquinone and paclitaxel shows anti-tumor activity through the interplay with apoptosis network in triple-negative breast cancer. *Tumour Biol* 2016; **37**: 4467-4477 [PMID: 26500095 DOI: 10.1007/s13277-015-4307-0]

64 **Bashmail HA**, Alamoudi AA, Noorwali A, Hegazy GA, Ajabnoor GM, Al-Abd AM. Thymoquinone Enhances Paclitaxel Anti-Breast Cancer Activity via Inhibiting Tumor-Associated Stem Cells Despite Apparent Mathematical Antagonism. *Molecules* 2020; **25** [PMID: 31968657 DOI: 10.3390/molecules25020426]

65 **Soni P**, Kaur J, Tikoo K. Dual drug-loaded paclitaxel–thymoquinone nanoparticles for effective breast cancer therapy. *J Nanopart Res* 2015; **17**: 1-12 [DOI 10.1007/s11051-014-2821-4]

66 **Ramaswamy B**, Puhalla S. Docetaxel: a tubulin-stabilizing agent approved for the management of several solid tumors. *Drugs Today (Barc)* 2006; **42**: 265-279 [PMID: 16703123 DOI: 10.1358/dot.2006.42.4.968648]

67 **Akhtartavan S**, Karimi M, Karimian K, Azarpira N, Khatami M, Heli H. Evaluation of a self-nanoemulsifying docetaxel delivery system. *Biomed Pharmacother* 2019; **109**: 2427-2433 [PMID: 30551502 DOI: 10.1016/j.biopha.2018.11.110]

68 **Baker J**, Ajani J, Scotté F, Winther D, Martin M, Aapro MS, von Minckwitz G. Docetaxel-related side effects and their management. *Eur J Oncol Nurs* 2009; **13**: 49-59 [PMID: 19201649 DOI: 10.1016/j.ejon.2008.10.003]

69 **Dirican A**, Atmaca H, Bozkurt E, Erten C, Karaca B, Uslu R. Novel combination of docetaxel and thymoquinone induces synergistic cytotoxicity and apoptosis in DU-145 human prostate cancer cells by modulating PI3K-AKT pathway. *Clin Transl Oncol* 2015; **17**: 145-151 [PMID: 25060568 DOI: 10.1007/s12094-014-1206-6]

70 **Singh SK**, Apata T, Gordetsky JB, Singh R. Docetaxel Combined with Thymoquinone Induces Apoptosis in Prostate Cancer Cells via Inhibition of the PI3K/AKT Signaling Pathway. *Cancers (Basel)* 2019; **11** [PMID: 31540423 DOI: 10.3390/cancers11091390]

71 **Alkhatib MH**, Bawadud RS, Gashlan HM. Incorporation of docetaxel and thymoquinone in borage nanoemulsion potentiates their antineoplastic activity in breast cancer cells. *Sci Rep* 2020; **10**: 18124 [PMID: 33093596 DOI: 10.1038/s41598-020-75017-5]

72 **Zafar S**, Akhter S, Ahmad I, Hafeez Z, Alam Rizvi MM, Jain GK, Ahmad FJ. Improved chemotherapeutic efficacy against resistant human breast cancer cells with co-delivery of Docetaxel and Thymoquinone by Chitosan grafted lipid nanocapsules: Formulation optimization, in vitro and in vivo studies. *Colloids Surf B Biointerfaces* 2020; **186**: 110603 [PMID: 31846892 DOI: 10.1016/j.colsurfb.2019.110603]

73 **Zafar S**, Akhter S, Garg N, Selvapandiyan A, Kumar Jain G, Ahmad FJ. Co-encapsulation of docetaxel and thymoquinone in mPEG-DSPE-vitamin E TPGS-lipid nanocapsules for breast cancer therapy: Formulation optimization and implications on cellular and in vivo toxicity. *Eur J Pharm Biopharm* 2020; **148**: 10-26 [PMID: 31923585 DOI: 10.1016/j.ejpb.2019.12.016]

74 **Odeh F**, Naffa R, Azzam H, Mahmoud IS, Alshaer W, Al Bawab A, Ismail S. Co-encapsulation of thymoquinone with docetaxel enhances the encapsulation efficiency into PEGylated liposomes and the chemosensitivity of MCF7 breast cancer cells to docetaxel. *Heliyon* 2019; **5**: e02919 [PMID: 31844767 DOI: 10.1016/j.heliyon.2019.e02919]

75 **Paller CJ**, Antonarakis ES. Cabazitaxel: a novel second-line treatment for metastatic castration-resistant prostate cancer. *Drug Des Devel Ther* 2011; **5**: 117-124 [PMID: 21448449 DOI: 10.2147/DDDT.S13029]

76 **Mahajan M**, Khurana RK, Sahajpal NS, Utreja P, Sankar R, Singh B, Jain SK. Emerging Strategies and Challenges for Controlled Delivery of Taxanes: A Comprehensive Review. *Curr Drug Metab* 2015; **16**: 453-473 [PMID: 26264203 DOI: 10.2174/1389200216666150812123414]

77 **Kommineni N**, Mahira S, Domb AJ, Khan W. Cabazitaxel-Loaded Nanocarriers for Cancer Therapy with Reduced Side Effects. *Pharmaceutics* 2019; **11** [PMID: 30934535 DOI: 10.3390/pharmaceutics11030141]

78 **Kommineni N**, Saka R, Bulbake U, Khan W. Cabazitaxel and thymoquinone co-loaded lipospheres as a synergistic combination for breast cancer. *Chem Phys Lipids* 2019; **224**: 104707 [PMID: 30521787 DOI: 10.1016/j.chemphyslip.2018.11.009]

79 **Meredith AM**, Dass CR. Increasing role of the cancer chemotherapeutic doxorubicin in cellular metabolism. *J Pharm Pharmacol* 2016; **68**: 729-741 [PMID: 26989862 DOI: 10.1111/jphp.12539]

80 **Carvalho C**, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI. Doxorubicin: the good, the bad and the ugly effect. *Curr Med Chem* 2009; **16**: 3267-3285 [PMID: 19548866 DOI: 10.2174/092986709788803312]

81 **Al-Malky HS**, Al Harthi SE, Osman AM. Major obstacles to doxorubicin therapy: Cardiotoxicity and drug resistance. *J Oncol Pharm Pract* 2020; **26**: 434-444 [PMID: 31594518 DOI: 10.1177/1078155219877931]

82 **Takemura G**, Fujiwara H. Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. *Prog Cardiovasc Dis* 2007; **49**: 330-352 [PMID: 17329180 DOI: 10.1016/j.pcad.2006.10.002]

83 **Fatfat M**, Fakhoury I, Habli Z, Mismar R, Gali-Muhtasib H. Thymoquinone enhances the anticancer activity of doxorubicin against adult T-cell leukemia in vitro and in vivo through ROS-dependent mechanisms. *Life Sci* 2019; **232**: 116628 [PMID: 31278946 DOI: 10.1016/j.lfs.2019.116628]

84 **Effenberger-Neidnicht K**, Schobert R. Combinatorial effects of thymoquinone on the anti-cancer activity of doxorubicin. *Cancer Chemother Pharmacol* 2011; **67**: 867-874 [PMID: 20582416 DOI: 10.1007/s00280-010-1386-x]

85 **Woo CC**, Hsu A, Kumar AP, Sethi G, Tan KH. Thymoquinone inhibits tumor growth and induces apoptosis in a breast cancer xenograft mouse model: the role of p38 MAPK and ROS. *PLoS One* 2013; **8**: e75356 [PMID: 24098377 DOI: 10.1371/journal.pone.0075356]

86 **Jehan S**, Zhong C, Li G, Zulqarnain Bakhtiar S, Li D, Sui G. Thymoquinone Selectively Induces Hepatocellular Carcinoma Cell Apoptosis in Synergism With Clinical Therapeutics and Dependence of p53 Status. *Front Pharmacol* 2020; **11**: 555283 [PMID: 33041795 DOI: 10.3389/fphar.2020.555283]

87 **Ibiyeye KM**, Nordin N, Ajat M, Zuki ABZ. Ultrastructural Changes and Antitumor Effects of Doxorubicin/Thymoquinone-Loaded CaCO3 Nanoparticles on Breast Cancer Cell Line. *Front Oncol* 2019; **9**: 599 [PMID: 31334120 DOI: 10.3389/fonc.2019.00599]

88 **Zidan AA**, El-Ashmawy NE, Khedr EG, Ebeid EM, Salem ML, Mosalam EM. Loading of doxorubicin and thymoquinone with F2 gel nanofibers improves the antitumor activity and ameliorates doxorubicin-associated nephrotoxicity. *Life Sci* 2018; **207**: 461-470 [PMID: 29885348 DOI: 10.1016/j.lfs.2018.06.008]

89 **El-Ashmawy NE**, Khedr EG, Ebeid EM, Salem ML, Zidan AA, Mosalam EM. Enhanced anticancer effect and reduced toxicity of doxorubicin in combination with thymoquinone released from poly-N-acetyl glucosamine nanomatrix in mice bearing solid Ehrlish carcinoma. *Eur J Pharm Sci* 2017; **109**: 525-532 [PMID: 28890201 DOI: 10.1016/j.ejps.2017.09.012]

90 **Robati M**, Holtz D, Dunton CJ. A review of topotecan in combination chemotherapy for advanced cervical cancer. *Ther Clin Risk Manag* 2008; **4**: 213-218 [PMID: 18728710 DOI: 10.2147/tcrm.s1771]

91 **Kim YH**, Mishima M. Second-line chemotherapy for small-cell lung cancer (SCLC). *Cancer Treat Rev* 2011; **37**: 143-150 [PMID: 20580163 DOI: 10.1016/j.ctrv.2010.05.004]

92 **Abushahin F**, Singh DK, Lurain JR, Grendys EC, Rademaker AW, Schink JC. Weekly topotecan for recurrent platinum resistant ovarian cancer. *Gynecol Oncol* 2008; **108**: 53-57 [PMID: 17904208 DOI: 10.1016/j.ygyno.2007.08.062]

93 **Vali AM**, Toliyat T, Shafaghi B, Dadashzadeh S. Preparation, optimization, and characterization of topotecan loaded PEGylated liposomes using factorial design. *Drug Dev Ind Pharm* 2008; **34**: 10-23 [PMID: 18214751 DOI: 10.1080/03639040701385055]

94 **Kollmannsberger C**, Mross K, Jakob A, Kanz L, Bokemeyer C. Topotecan - A novel topoisomerase I inhibitor: pharmacology and clinical experience. *Oncology* 1999; **56**: 1-12 [PMID: 9885371 DOI: 10.1159/000011923]

95 **Khalife R**, El-Hayek S, Tarras O, Hodroj MH, Rizk S. Antiproliferative and proapoptotic effects of topotecan in combination with thymoquinone on acute myelogenous leukemia. *Clin Lymphoma Myeloma Leuk* 2014; **14 Suppl**: S46-S55 [PMID: 25486955 DOI: 10.1016/j.clml.2014.04.014]

96 **Khalife R**, Hodroj MH, Fakhoury R, Rizk S. Thymoquinone from Nigella sativa Seeds Promotes the Antitumor Activity of Noncytotoxic Doses of Topotecan in Human Colorectal Cancer Cells in Vitro. *Planta Med* 2016; **82**: 312-321 [PMID: 26848703 DOI: 10.1055/s-0035-1558289]

97 **Kane RC**, Bross PF, Farrell AT, Pazdur R. Velcade: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. *Oncologist* 2003; **8**: 508-513 [PMID: 14657528 DOI: 10.1634/theoncologist.8-6-508]

98 **Annunziata CM**, Davis RE, Demchenko Y, Bellamy W, Gabrea A, Zhan F, Lenz G, Hanamura I, Wright G, Xiao W, Dave S, Hurt EM, Tan B, Zhao H, Stephens O, Santra M, Williams DR, Dang L, Barlogie B, Shaughnessy JD Jr, Kuehl WM, Staudt LM. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* 2007; **12**: 115-130 [PMID: 17692804 DOI: 10.1016/j.ccr.2007.07.004]

99 **Siveen KS**, Mustafa N, Li F, Kannaiyan R, Ahn KS, Kumar AP, Chng WJ, Sethi G. Thymoquinone overcomes chemoresistance and enhances the anticancer effects of bortezomib through abrogation of NF-κB regulated gene products in multiple myeloma xenograft mouse model. *Oncotarget* 2014; **5**: 634-648 [PMID: 24504138 DOI: 10.18632/oncotarget.1596]

100 **Westerdijk K**, Desar IME, Steeghs N, van der Graaf WTA, van Erp NP; Dutch Pharmacology and Oncology Group (DPOG). Imatinib, sunitinib and pazopanib: From flat-fixed dosing towards a pharmacokinetically guided personalized dose. *Br J Clin Pharmacol* 2020; **86**: 258-273 [PMID: 31782166 DOI: 10.1111/bcp.14185]

101 **Nestal de Moraes G**, Souza PS, Costas FC, Vasconcelos FC, Reis FR, Maia RC. The Interface between BCR-ABL-Dependent and -Independent Resistance Signaling Pathways in Chronic Myeloid Leukemia. *Leuk Res Treatment* 2012; **2012**: 671702 [PMID: 23259070 DOI: 10.1155/2012/671702]

102 **Zhang Q**, Li Z, Xu K, Qian Y, Chen M, Sun L, Song S, Huang X, He Z, Li F, Zhang D, Yang L, Wang Y, Xu H, Xu Z. Intracellular concentration and transporters in imatinib resistance of gastrointestinal stromal tumor. *Scand J Gastroenterol* 2019; **54**: 220-226 [PMID: 30879345 DOI: 10.1080/00365521.2019.1577488]

103 **Thabet NA**, El-Khouly D, Sayed-Ahmed MM, Omran MM. Thymoquinone chemosensitizes human colorectal cancer cells to imatinib via uptake/efflux genes modulation. *Clin Exp Pharmacol Physiol* 2021; **48**: 911-920 [PMID: 33783002 DOI: 10.1111/1440-1681.13476]

104 **Day CM**, Hickey SM, Song Y, Plush SE, Garg S. Novel Tamoxifen Nanoformulations for Improving Breast Cancer Treatment: Old Wine in New Bottles. *Molecules* 2020; **25** [PMID: 32151063 DOI: 10.3390/molecules25051182]

105 **Zhang T**, Feng F, Zhao W, Tian J, Yao Y, Zhou C, Dong S, Wang C, Zang C, Lv Q, Sun C. Effect of first-line endocrine therapy in patients with hormone-sensitive advanced breast cancer: a network meta-analysis. *Onco Targets Ther* 2018; **11**: 2647-2656 [PMID: 29780257 DOI: 10.2147/OTT.S165681]

106 **Ganji-Harsini S**, Khazaei M, Rashidi Z, Ghanbari A. Thymoquinone Could Increase The Efficacy of Tamoxifen Induced Apoptosis in Human Breast Cancer Cells: An In Vitro Study. *Cell J* 2016; **18**: 245-254 [PMID: 27540530 DOI: 10.22074/cellj.2016.4320]

107 **Rajput S**, Kumar BN, Sarkar S, Das S, Azab B, Santhekadur PK, Das SK, Emdad L, Sarkar D, Fisher PB, Mandal M. Targeted apoptotic effects of thymoquinone and tamoxifen on XIAP mediated Akt regulation in breast cancer. *PLoS One* 2013; **8**: e61342 [PMID: 23613836 DOI: 10.1371/journal.pone.0061342]

108 **Kabel AM**, Rashidy MAE, Omar MS. Ameliorative Potential of Tamoxifen/Thymoquinone Combination in Patients with Breast Cancer: A Biochemical and Immunohistochemical. *J Can Sci Res* 2016; **1**: 102 [DOI:10.4172/2576-1447.1000102]

109 **Polascik TJ**, Mouraviev V. Zoledronic acid in the management of metastatic bone disease. *Ther Clin Risk Manag* 2008; **4**: 261-268 [PMID: 18728715 DOI: 10.2147/tcrm.s2707]

110 **Green J**, Lipton A. Anticancer properties of zoledronic acid. *Cancer Invest* 2010; **28**: 944-957 [PMID: 20879838 DOI: 10.3109/07357907.2010.512598]

111 **Avilés A**, Nambo MJ, Neri N, Castañeda C, Cleto S, Huerta-Guzmán J. Antitumor effect of zoledronic acid in previously untreated patients with multiple myeloma. *Med Oncol* 2007; **24**: 227-230 [PMID: 17848748 DOI: 10.1007/BF02698044]

112 **Dirican A**, Erten C, Atmaca H, Bozkurt E, Kucukzeybek Y, Varol U, Oktay Tarhan M, Karaca B, Uslu R. Enhanced cytotoxicity and apoptosis by thymoquinone in combination with zoledronic acid in hormone- and drug-resistant prostate cancer cell lines. *J BUON* 2014; **19**: 1055-1061 [PMID: 25536616]

113 **Wang QQ**, Jiang Y, Naranmandura H. Therapeutic strategy of arsenic trioxide in the fight against cancers and other diseases. *Metallomics* 2020; **12**: 326-336 [PMID: 32163072 DOI: 10.1039/c9mt00308h]

114 **El-Sabban ME**, Nasr R, Dbaibo G, Hermine O, Abboushi N, Quignon F, Ameisen JC, Bex F, de Thé H, Bazarbachi A. Arsenic-interferon-alpha-triggered apoptosis in HTLV-I transformed cells is associated with tax down-regulation and reversal of NF-kappa B activation. *Blood* 2000; **96**: 2849-2855 [PMID: 11023521]

115 **El Hajj H**, El-Sabban M, Hasegawa H, Zaatari G, Ablain J, Saab ST, Janin A, Mahfouz R, Nasr R, Kfoury Y, Nicot C, Hermine O, Hall W, de Thé H, Bazarbachi A. Therapy-induced selective loss of leukemia-initiating activity in murine adult T cell leukemia. *J Exp Med* 2010; **207**: 2785-2792 [PMID: 21135137 DOI: 10.1084/jem.20101095]

116 **Hermine O**, Dombret H, Poupon J, Arnulf B, Lefrère F, Rousselot P, Damaj G, Delarue R, Fermand JP, Brouet JC, Degos L, Varet B, de Thé H, Bazarbachi A. Phase II trial of arsenic trioxide and alpha interferon in patients with relapsed/refractory adult T-cell leukemia/lymphoma. *Hematol J* 2004; **5**: 130-134 [PMID: 15048063 DOI: 10.1038/sj.thj.6200374]

117 **Houssein M**, Fatfat M, Habli Z, Ghazal N, Moodad S, Khalife H, Khalil M, Gali-Muhtasib H. Thymoquinone synergizes with arsenic and interferon alpha to target human T-cell leukemia/lymphoma. *Life Sci* 2020; **251**: 117639 [PMID: 32272181 DOI: 10.1016/j.lfs.2020.117639]

118 **Delaney G**, Jacob S, Featherstone C, Barton M. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. *Cancer* 2005; **104**: 1129-1137 [PMID: 16080176 DOI: 10.1002/cncr.21324]

119 **Barker HE**, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nat Rev Cancer* 2015; **15**: 409-425 [PMID: 26105538 DOI: 10.1038/nrc3958]

120 **Linam J**, Yang LX. Recent developments in radiosensitization. *Anticancer Res* 2015; **35**: 2479-2485 [PMID: 25964520]

121 **Velho-Pereira R**, Kumar A, Pandey BN, Jagtap AG, Mishra KP. Radiosensitization in human breast carcinoma cells by thymoquinone: role of cell cycle and apoptosis. *Cell Biol Int* 2011; **35**: 1025-1029 [PMID: 21557727 DOI: 10.1042/CBI20100701]

122 **Rajput S**, Kumar BN, Banik P, Parida S, Mandal M. Thymoquinone restores radiation-induced TGF-β expression and abrogates EMT in chemoradiotherapy of breast cancer cells. *J Cell Physiol* 2015; **230**: 620-629 [PMID: 25164250 DOI: 10.1002/jcp.24780]

123 **Hatiboglu MA**, Kocyigit A, Guler EM, Akdur K, Khan I, Nalli A, Karatas E, Tuzgen S. Thymoquinone Enhances the Effect of Gamma Knife in B16-F10 Melanoma Through Inhibition of Phosphorylated STAT3. *World Neurosurg* 2019; **128**: e570-e581 [PMID: 31054338 DOI: 10.1016/j.wneu.2019.04.205]

124 **Goswami R**, Subramanian G, Silayeva L, Newkirk I, Doctor D, Chawla K, Chattopadhyay S, Chandra D, Chilukuri N, Betapudi V. Gene Therapy Leaves a Vicious Cycle. *Front Oncol* 2019; **9**: 297 [PMID: 31069169 DOI: 10.3389/fonc.2019.00297]

125 **Misso G**, Di Martino MT, De Rosa G, Farooqi AA, Lombardi A, Campani V, Zarone MR, Gullà A, Tagliaferri P, Tassone P, Caraglia M. Mir-34: a new weapon against cancer? *Mol Ther Nucleic Acids* 2014; **3**: e194 [PMID: 25247240 DOI: 10.1038/mtna.2014.47]

126 **Imani S**, Wei C, Cheng J, Khan MA, Fu S, Yang L, Tania M, Zhang X, Xiao X, Zhang X, Fu J. MicroRNA-34a targets epithelial to mesenchymal transition-inducing transcription factors (EMT-TFs) and inhibits breast cancer cell migration and invasion. *Oncotarget* 2017; **8**: 21362-21379 [PMID: 28423483 DOI: 10.18632/oncotarget.15214]

127 **Rajput S**, Puvvada N, Kumar BN, Sarkar S, Konar S, Bharti R, Dey G, Mazumdar A, Pathak A, Fisher PB, Mandal M. Overcoming Akt Induced Therapeutic Resistance in Breast Cancer through siRNA and Thymoquinone Encapsulated Multilamellar Gold Niosomes. *Mol Pharm* 2015; **12**: 4214-4225 [PMID: 26505213 DOI: 10.1021/acs.molpharmaceut.5b00692]

128 **Murillo G**, Matusiak D, Benya RV, Mehta RG. Chemopreventive efficacy of 25-hydroxyvitamin D3 in colon cancer. *J Steroid Biochem Mol Biol* 2007; **103**: 763-767 [PMID: 17257827 DOI: 10.1016/j.jsbmb.2006.12.074]

129 **Tangpricha V**, Spina C, Yao M, Chen TC, Wolfe MM, Holick MF. Vitamin D deficiency enhances the growth of MC-26 colon cancer xenografts in Balb/c mice. *J Nutr* 2005; **135**: 2350-2354 [PMID: 16177194 DOI: 10.1093/jn/135.10.2350]

130 **Vaughan-Shaw PG**, Buijs LF, Blackmur JP, Theodoratou E, Zgaga L, Din FVN, Farrington SM, Dunlop MG. The effect of vitamin D supplementation on survival in patients with colorectal cancer: systematic review and meta-analysis of randomised controlled trials. *Br J Cancer* 2020; **123**: 1705-1712 [PMID: 32929196 DOI: 10.1038/s41416-020-01060-8]

131 **Mohamed AM**, Refaat BA, El-Shemi AG, Kensara OA, Ahmad J, Idris S. Thymoquinone potentiates chemoprotective effect of Vitamin D3 against colon cancer: a pre-clinical finding. *Am J Transl Res* 2017; **9**: 774-790 [PMID: 28337306]

132 **Luchetti F**, Canonico B, Betti M, Arcangeletti M, Pilolli F, Piroddi M, Canesi L, Papa S, Galli F. Melatonin signaling and cell protection function. *FASEB J* 2010; **24**: 3603-3624 [PMID: 20534884 DOI: 10.1096/fj.10-154450]

133 **Vinther AG**, Claësson MH. [The influence of melatonin on the immune system and cancer]. *Ugeskr Laeger* 2015; **177**: V10140568 [PMID: 26027592]

134 **Odeh LH**, Talib WH, Basheti IA. Synergistic effect of thymoquinone and melatonin against breast cancer implanted in mice. *J Cancer Res Ther* 2018; **14**: S324-S330 [PMID: 29970684 DOI: 10.4103/0973-1482.235349]

135 **Tu Y**. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nat Med* 2011; **17**: 1217-1220 [PMID: 21989013 DOI: 10.1038/nm.2471]

136 **Fröhlich T**, Reiter C, Saeed MEM, Hutterer C, Hahn F, Leidenberger M, Friedrich O, Kappes B, Marschall M, Efferth T, Tsogoeva SB. Synthesis of Thymoquinone-Artemisinin Hybrids: New Potent Antileukemia, Antiviral, and Antimalarial Agents. *ACS Med Chem Lett* 2018; **9**: 534-539 [PMID: 29937978 DOI: 10.1021/acsmedchemlett.7b00412]

137 **Fröhlich T**, Ndreshkjana B, Muenzner JK, Reiter C, Hofmeister E, Mederer S, Fatfat M, El-Baba C, Gali-Muhtasib H, Schneider-Stock R, Tsogoeva SB. Synthesis of Novel Hybrids of Thymoquinone and Artemisinin with High Activity and Selectivity Against Colon Cancer. *ChemMedChem* 2017; **12**: 226-234 [PMID: 27973725 DOI: 10.1002/cmdc.201600594]

138 **Das S**, Dey KK, Dey G, Pal I, Majumder A, MaitiChoudhury S, kundu SC, Mandal M. Antineoplastic and apoptotic potential of traditional medicines thymoquinone and diosgenin in squamous cell carcinoma. *PLoS One* 2012; **7**: e46641 [PMID: 23077516 DOI: 10.1371/journal.pone.0046641]

139 **Sethi G**, Shanmugam MK, Warrier S, Merarchi M, Arfuso F, Kumar AP, Bishayee A. Pro-Apoptotic and Anti-Cancer Properties of Diosgenin: A Comprehensive and Critical Review. *Nutrients* 2018; **10** [PMID: 29783752 DOI: 10.3390/nu10050645]

140 **Bhattacharjee M**, Upadhyay P, Sarker S, Basu A, Das S, Ghosh A, Ghosh S, Adhikary A. Combinatorial therapy of Thymoquinone and Emodin synergistically enhances apoptosis, attenuates cell migration and reduces stemness efficiently in breast cancer. *Biochim Biophys Acta Gen Subj* 2020; **1864**: 129695 [PMID: 32735937 DOI: 10.1016/j.bbagen.2020.129695]

141 **Dong X**, Fu J, Yin X, Cao S, Li X, Lin L; Huyiligeqi, Ni J. Emodin: A Review of its Pharmacology, Toxicity and Pharmacokinetics. *Phytother Res* 2016; **30**: 1207-1218 [PMID: 27188216 DOI: 10.1002/ptr.5631]

142 **Mahendra P**, Bisht S. Ferula asafoetida: Traditional uses and pharmacological activity. *Pharmacogn Rev* 2012; **6**: 141-146 [PMID: 23055640 DOI: 10.4103/0973-7847.99948]

143 **Al-Mutairi A**, Rahman A, Rao MS. Low Doses of Thymoquinone and Ferulic Acid in Combination Effectively Inhibit Proliferation of Cultured MDA-MB 231 Breast Adenocarcinoma Cells. *Nutr Cancer* 2021; **73**: 282-289 [PMID: 32223348 DOI: 10.1080/01635581.2020.1743869]

144 **Dixon RA**, Ferreira D. Genistein. *Phytochemistry* 2002; **60**: 205-211 [PMID: 12031439 DOI: 10.1016/s0031-9422(02)00116-4]

145 **Ozturk SA**, Alp E, Yar Saglam AS, Konac E, Menevse ES. The effects of thymoquinone and genistein treatment on telomerase activity, apoptosis, angiogenesis, and survival in thyroid cancer cell lines. *J Cancer Res Ther* 2018; **14**: 328-334 [PMID: 29516914 DOI: 10.4103/0973-1482.202886]

146 **Damiens E**, Baratte B, Marie D, Eisenbrand G, Meijer L. Anti-mitotic properties of indirubin-3'-monoxime, a CDK/GSK-3 inhibitor: induction of endoreplication following prophase arrest. *Oncogene* 2001; **20**: 3786-3797 [PMID: 11439342 DOI: 10.1038/sj.onc.1204503]

147 **Dera AA**, Rajagopalan P, Al Fayi M, Ahmed I, Chandramoorthy HC. Indirubin-3-monoxime and thymoquinone exhibit synergistic efficacy as therapeutic combination in in-vitro and in-vivo models of Lung cancer. *Arch Pharm Res* 2020; **43**: 655-665 [PMID: 32588331 DOI: 10.1007/s12272-020-01241-2]

148 **Meghwal M**, Goswami TK. Piper nigrum and piperine: an update. *Phytother Res* 2013; **27**: 1121-1130 [PMID: 23625885 DOI: 10.1002/ptr.4972]

149 **Talib WH**. Regressions of Breast Carcinoma Syngraft Following Treatment with Piperine in Combination with Thymoquinone. *Sci Pharm* 2017; **85** [PMID: 28671634 DOI: 10.3390/scipharm85030027]

150 **Thombare N**, Jha U, Mishra S, Siddiqui MZ. Guar gum as a promising starting material for diverse applications: A review. *Int J Biol Macromol* 2016; **88**: 361-372 [PMID: 27044346 DOI: 10.1016/j.ijbiomac.2016.04.001]

151 **Das S**, Bera D, Pal K, Mondal D, Karmakar P, Das S, et al. Guar gum micro-vehicle mediated delivery strategy and synergistic activity of thymoquinone and piperine: An in vitro study on bacterial and hepatocellular carcinoma cells. *J Drug Deliv Sci Technol* 2020; **60**: 101994 [DOI: 10.1016/j.jddst.2020.101994]

152 **Perrone D**, Fuggetta MP, Ardito F, Cottarelli A, De Filippis A, Ravagnan G, De Maria S, Lo Muzio L. Resveratrol (3,5,4'-trihydroxystilbene) and its properties in oral diseases. *Exp Ther Med* 2017; **14**: 3-9 [PMID: 28672886 DOI: 10.3892/etm.2017.4472]

153 **Ismail N**, Abdel–Mottaleb Y, Eissa Ahmed AA, El-Maraghy NN. Novel combination of thymoquinone and resveratrol enhances anticancer effect on hepatocellular carcinoma cell line. *Futur J Pharm Sci* 2018; **4**: 41-46 [DOI: 10.1016/j.fjps.2017.08.001]

154 **Alobaedi OH**, Talib WH, Basheti IA. Antitumor effect of thymoquinone combined with resveratrol on mice transplanted with breast cancer. *Asian Pac J Trop Med* 2017; **10**: 400-408 [PMID: 28552110 DOI: 10.1016/j.apjtm.2017.03.026]

155 **Kieliszek M**. Selenium⁻Fascinating Microelement, Properties and Sources in Food. *Molecules* 2019; **24** [PMID: 30987088 DOI: 10.3390/molecules24071298]

156 **Barron J**, Benghuzzi H, Tucci M. Effects of thymoquinone and selenium on the proliferation of mg 63 cells in tissue culture. *Biomed Sci Instrum* 2008; **44**: 434-440 [PMID: 19141954]

157 **Ibiyeye KM**, Zuki ABZ. Cockle Shell-Derived Aragonite CaCO3 Nanoparticles for Co-Delivery of Doxorubicin and Thymoquinone Eliminates Cancer Stem Cells. *Int J Mol Sci* 2020; **21** [PMID: 32164352 DOI: 10.3390/ijms21051900]

**Footnotes**

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Peer-review started:** February 27, 2021

**First decision:** March 31, 2021

**Article in press:** June 18, 2021

**Specialty type:** Oncology

**Country/Territory of origin:** Lebanon

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Jeong KY **S-Editor:** Zhang H **L-Editor:** Filipodia **P-Editor:** Yuan YY

**Figure Legends**

****

****

**Figure 1 Thymoquinone in combination therapy against different types of cancer.** A: Thymoquinone in combination with conventional chemotherapeutic drugs; B: Thymoquinone in combination with natural products. TQ: Thymoquinone; CYC: Cyclophosphamide; TMZ: Temozolomide; CDDP: Cisplatin; BTZ: Bortezomib; 5-FU: 5-Fluorouracil; GCB: Gemcitabine; PAC: Paclitaxel; DTX: Docetaxel; CBZ: Cabazitaxel; TP: Topotecan; DOX: Doxorubicin; ZA: Zoledronic acid; TAM: Tamoxifen; As: Arsenic trioxide; IM: Imatinib; Vit D3: Vitamin D3; Mel: Melatonin; Res: Resveratrol; Pip: Piperine; Ams: Artemisinin; Art: Artesunic acid; Dio: Diosgenin; Gen: Genistein; I3M: Indirubin‑3‑monoxime; FA: Ferulic acid; Emo: Emodin; Sel: Selenium.

**Table 1 Mode of action of the chemotherapeutic agents and cellular and molecular mechanism of action of the combination treatment in preclinical and clinical studies**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Chemotherapeutic agent** | **Mode of action** | **Patients or animal model or cell lines** | **Cellular and molecular mechanism of action of the combination treatment** | **Ref.** |
| Cyclophosphamide | Alkylates guanine base and causes the formation of DNA crosslinks leading to cell death | SKBR-3 and MDA-231 breast cancer cells | Increases the percentage of cells in G1 and sub- G1 phases. Downregulates the phosphorylation of Akt and the expression of cyclin D1 and upregulates PTEN | Emadi *et al*[25], Khan *et al*[27] |
| Temozolomide | Methylates DNA at specific sites on guanine and adenine bases causing cell demise | U87MG human glioblastoma multiforme cells | Increases the mitochondrial membrane potential disruption, cytochrome c release, ROS generation, DNA fragmentation and Bax/Bcl-2 ratio. Activates p53, caspases 9 and 3 and reduces NO and GSH levels. Reduces the expression and secretion of MMP-2 and MMP-9. Downregulates beclin-1 and ATG-7 | Stupp *et al*[29], Khazaei *et al*[32], Pazhouhi *et al*[33], Pazhouhi *et al*[35] |
| Cisplatin | Interacts with purine bases and forms DNA crosslinks resulting in cell death | ID8-NGL mouse ovarian cancer cells. OVCAR3 and NCI/ADR-RES human ovarian cancer cells. BL/6 mice injected with ID8-NGL cells | Increases the level of Bax, pH2AX (ser139), cleaved caspase 3 and PARP. Decreases the level of PCNA and Ki67 | Siddik *et al*[36], Wilson *et al*[39] |
|  |  | Eca-109 human esophageal cancer cells. BALB/c nude mice inoculated with Eca-109 cells | Decreases the expression of p-STAT3, p-JAK2, Bcl-2, survivin and cyclin D1. Increases the expression of Bax and activates caspases 3, 7 and 9. Induces chromatin condensation and nuclear fragmentation | Hu *et al*[40] |
|  |  | NCI-H460 non-small lung cancer cells. SCID mice injected with NCI-H460 cancer cells | Reduces the ratio of phosphor-Ser529 NFB/NFB | Jafri *et al*[42] |
|  |  | UMSCC-14C head and neck squamous cancer cells and normal oral epithelial cells | Increases p53 and caspase 9 expression. Decreases Bcl-2 expression | Alaufi *et al*[43] |
|  |  | SGC-7901 human gastric cancer cells. BALB/c mice implanted with gastric cancer cells | Increases the level of Bax, AIF, cytochrome c, cleaved caspases 9 and 3. Decreases the level of cyclin D1, Bcl-2, procaspases 9 and 3. Inhibits PI3K/Akt signaling pathway and downregulates P-gp by upregulating PTEN | Ma *et al*[44] |
| 5-Fluorouracil | A pyrimidine analogue inhibiting the activity of thymidylate synthase enzyme causing the disruption of DNA synthesis and cell death | BGC-823, SGC-7901, MGC-803 and HGC-27 human gastric cancer cells. BALB/c athymic nude mice inoculated with gastric cancer cells | Increases the release of mitochondrial cytochrome c and the level of Bax, caspases 3 and 9. Decreases the level of Bcl-2 and induces nuclear fragmentation and chromatin condensation | Wilson *et al*[45], Lei *et al*[48] |
|  |  | Azoxymethane-induced colorectal tumors in Wistar rats | Increases the expression of DKK-1, CDNK-1A, TGF-β1, TGF-βRII, Smad4 and GPx. Decreases the expression of Wnt, β-catenin, NFκB, COX-2, iNOS, VEGF and TBRAS | Kensara *et al*[49] |
|  |  | HCT116, HT29 and SW620 human colon cancer cellsSW837 rectal cancer cells. Normal human intestinal epithelial cells. CAM tumors derived from HCT116 cells | Downregulates Wnt/β-catenin and PI3K/Akt pathways | Ndreshkjana *et al*[50] |
|  |  | FADU nasopharyngeal cancer cells | Decreases the level of GSH | Williams *et al***[**51] |
|  |  | MG63 human osteosarcoma cells |  | Sarman *et al*[52] |
| Gemcitabine | A [deoxycytidine](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/deoxycytidine) analog preventing chain elongation during [DNA synthesis](https://www.sciencedirect.com/topics/neuroscience/dna-synthesis) causing cell death | PANC-1 and MIA PaCa-2 human pancreatic cancer cells | Downregulates PKM2 and decreases the expression of procaspase 3 and PARP | Moysan *et al*[53], Pandita *et al*[56] |
|  |  | PANC-1, BxPC-3, and AsPC-1 human pancreatic cancer cell lines. BALB/c nude mice injected with PANC-1 cells | Downregulates Notch1, NICD, Bcl-2, Bcl-xL and XIAP. Inactivates Akt/mTOR/S6 signaling pathway and decreases the phosphorylation and nuclear translocation of p65. Upregulates PTEN, caspases 3 and 9 and Bax and increases cytochrome c release | Mu *et al*[57] |
|  |  | MCF-7 and T47D human breast cancer cells | Increases pre-G1 cell population | Bashmail *et al*[58] |
| Paclitaxel | Inhibits microtubules disassembly and induces mitotic arrest | 4T1 mouse breast cancer cells. Ehrlich tumor cells. Balb/c mice injected with Ehrlich tumor ascites cells | Increases the level of full length and cleaved caspases 3, 7 and 12 and PARP. Reduces phosphorylated p65 and Akt1. Modulates genes involved in apoptosis, cytokine -cytokine receptor interaction, Fas signaling, p53 signaling and JAK/STAT signaling | Ojima *et al*[59], Şakalar *et al*[63] |
|  |  | MCF-7 and T47D human breast cancer cells | Increases pre-G1 cell population. Increases the level of cleaved caspase 3 and PARP and the expression of beclin-1 and LC3-II | Bashmail *et al*[64] |
|  |  | MCF-7 human breast cancer cells |  | Soni *et al*[65] |
| Docetaxel | Inhibits microtubules disassembly and induces mitotic arrest | DU-145 human prostate cancer cells | Blocks PI3K/Akt signaling pathway and induces DNA fragmentation | Ojima *et al*[59], Dirican *et al*[69] |
|  |  | DU-145 and C4-2B human prostate cancer cells | Inhibits PI3K/Akt signaling pathway. Increases the expression of Bax, Bid, caspase 3 and PARP and decreases the expression of Bcl-xL | Singh *et al*[70] |
|  |  | MCF-7 and MDA-MB-231 human breast cancer cells | Induces DNA damage, cells shrinkage, nuclear fragments, apoptotic bodies and cytoplasmic vacuolation | Alkhatib *et al*[71] |
|  |  | MCF-7 and MDA-MB-231 human breast cancer cells |  | Zafar *et al*[72] |
|  |  | MCF-7 and MDA-MB-231 human breast cancer cells. Balb/c mice healthy or injected with Ehrlich ascites carcinoma cells | Induces nuclear fragmentation and restores the levels of oxidative stress parameters MDA, SOD and GSH. Prevents the alteration of blood cell count and serum biochemical parameters AST, ALT, creatinine and BUN | Zafar *et al*[73] |
|  |  | MCF-7 breast cancer cells |  | Odeh *et al*[74] |
| Cabazitaxel | Inhibits microtubules disassembly and induces mitotic arrest | MCF-7 and MDA-MB-231 human breast cancer cells | Induces DNA fragmentation and increases the sub-G1 population | Ojima *et al*[59], Kommineni *et al*[78] |
| Doxorubicin  | Intercalates DNA, inhibits topoisomerase II, forms free radicals when reduced leading to cell cycle arrest and cell death | Human HTLV-1 positive (HuT-102) and HTLV-1 negative (Jurkat) CD4+ malignant T-cell lines. NOD/SCID mice inoculated with HuT-102 tumor cells | Increases the sub-G1 population and induces ROS production. Disrupts the mitochondrial membrane potential. Downregulates the expression of NFΒ and Ki67 and increases the phosphorylation of p53 | Meredith *et al*[79],Fatfat *et al*[83] |
|  |  | HL-60 acute myeloid leukemia cells. Dox resistant HT-29 colon carcinoma cells. MCF-7/TOPO multi-drug resistant breast cancer cells | Induces caspases 3 and 8 activity and ROS generation. Disrupts the mitochondrial membrane potential | Effenberger-Neidnicht*et al*[84] |
|  |  | BALB/c OlaHsd-foxn1 nude mice injected with MDA-MB-231 breast cancer cells | Induces p38 MAPK phosphorylation and inhibit the expression of XIAP, survivin, Bcl-xL and Bcl-2 | Woo *et al*[85] |
|  |  | SMMC-7721 and HepG2 hepatocarcinoma cells and human normal liver cells HL-7702 | Increases caspase 3 and PARP cleavage | Jehan *et al*[86] |
|  |  | MDA-MB-231 human breast cancer cells. MCF-10A and 3T3 non-neoplastic cells | Induces cell shrinkage, membrane blebbing and apoptotic bodies and disrupts the cell membrane. Increases the Sub-G0 population | Ibiyeye *et al*[87] |
|  |  | MCF-7 human breast adenocarcinoma and HEPG2 human hepatocellular carcinoma. Albino mice implanted with Heps murine liver cancer cells | Decreases NFB level and increases that of caspase 3. Increases the level of renal antioxidant enzymes SOD and catalase. Modulates the level of renal oxidative stress biomarkers GSH and MDA. Decreases the level of nephrotoxicity biomarkers BUN and serum creatinine | Zidan *et al*[88] |
|  |  | [Albino mice](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/albino-mouse) transplanted with Ehrlich carcinoma cells | Upregulates [p53](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/protein-p53) and reduces the level of Bcl-2. Decreases the level of cardiac MDA. Decreases the serum level of cardiac markers [lactate dehydrogenase](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/lactate-dehydrogenase) and [creatine kinase](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/creatine-kinase) | El-Ashmawy *et al*[89] |
| Topotecan | Inhibits DNA topoisomerase I and causes the formation of irreversible DNA double stranded breaks resulting in cell death. Inhibits hypoxia-inducible factor 1α | U937 acute myelogenous leukemia cells | Increases the sub-G1 population. Increases the expression level of Bax/Bcl-2, p53 and p21 and the cleavage of caspases 3 and 9 | Robati *et al*[90],Khalife *et al*[95] |
|  |  | HT-29 human colon cancer cells | Increases the sub-G1 population. Has no effect on p53, Bax and Bcl-2 expression | Khalife *et al*[96] |
| Bortezomib | Inhibits the proteasome | U266, H929, KMS, RPMI-8226, RPMI-8226-Dox-6 (doxorubicin-resistant clone), RPMI-8226-LR-5 (a melphalan-resistant clone) human multiple myeloma cells. Balb/c mice implanted with U266 cells | Increases the sub-G1 population and the cleavage of caspase 3 and PARP. Reduces the phosphorylation of NFB (p65) and the expression of Ki67, VEGF, Bcl-2 and the serum levels of IL-6 and TNF-α | Siveen *et al*[99] |
| Imatinib | Inhibits tyrosine kinase | HCT116 human colorectal cancer cells | Decreases the expression of ABCB1, ABCG2 and hOCT1. Increases the uptake/efflux ratio of imatinib | Thabet *et al*[103] |
| Tamoxifen | Competes with estrogen and estradiol for the binding to their receptors and modulates their signaling pathway | MCF-7 and MDA-MB-231 human breast cancer cells |  | Day *et al*[104], Ganji-Harsini *et al*[106] |
|  |  | MCF-7, MDA-MB-231, MDA-MB-468, T47D, NIH/3T3 and HaCaT human breast cancer cells. Athymic BALB/c mice injected with MDA-MB-231 cells | Decreases the expression of XIAP and the level of p-Akt, p-Bad, p-MAPK and p-GSK-3β and downregulates the expression of Bcl-xL, Bcl-2 and Ki67. Increases the cleavage of caspase 9 and PARP and induces the expression of Bax, AIF, cytochrome c and p27. Increases the percentage of cells in sub-G1 phase and the fragmentation of DNA | Rajput *et al*[107] |
|  |  | Breast cancer patients | Increases the tumor tissue catalase, SOD and caspase 3. Decreases the tumor tissue Bcl-2, TGF-β1, MDA, TNF-α and IL-6 | Kabel *et al*[108] |
| Zoledronic acid | Inhibits osteoclast-mediated [bone resorption](https://www.sciencedirect.com/topics/medicine-and-dentistry/osteolysis) | PC-3 and DU- 145 human prostate cancer cells | Increases DNA fragmentation and activates caspases 3 and 7 | Polascik *et al*[109], Dirican *et al*[112] |
| Arsenic trioxide |  | Human HTLV-I positive (HuT-102 and C91) and HTLV-I negative (CEM and Jurkat) malignant T-cell lines. NOD SCID mice inoculated with HuT-102 cells | Increases the percentage of cells in Pre-G1 phase, the disruption of the mitochondrial membrane potential and the cleavage of PARP and caspase 3. Upregulates p53, Bax and downregulates XIAP and Bcl- 2 | Houssein *et al*[117] |

PTEN: Phosphatase and tensin homolog; ROS: Reactive oxygen species; Bax: Bcl-2-associated X protein; NO: Nitric oxide; GSH: Glutathione; MMP: Matrix metalloproteinase; ATG-7: Autophagy-related 7; pH2AX: Phospho-histone 2AX; PCNA: Proliferating cell nuclear antigen; JAK2: Janus kinase 2; STAT3: Signal transducer and activator of transcription 3; NFB: Nuclear factor kappa B; PI3K: Phosphatidylinositol-3-kinase; AIF: Apoptosis inducing factor; PARP: Poly (ADP-ribose) polymerases; CAM: Chorioallantoic membrane; MAPK: Mitogen-activated protein kinase; COX-2: Cyclooxygenase 2; iNOS: Inducible nitric oxide synthase; VEGF: Vascular endothelial growth factor; TBRAS: Thiobarbituric acid reactive substances; DKK-1: Dickkopf-related protein-1; CDNK-1A: Cyclin-dependent kinase inhibitor 1A*;* TGF-β1: Tak transforming growth factor beta 1; TGF-βRII: Transforming growth factor, beta receptor II; GPx: Glutathione peroxidase; GSH: Glutathione; XIAP: X-linked inhibitor of apoptosis protein; mTOR: Mammalian target of rapamycin; PKM2: Pyruvate kinase M2; Bid: BH3 interacting-domain death agonist; AST: Aspartate transaminase; ALT: Alanine transaminase; MDA: Malondialdehyde; SOD: Superoxide dismutase; BUN: Blood urea nitrogen; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; GSK-3β: Glycogen synthase kinase 3 beta; ABCB1A: ATP-binding cassette subfamily B member 1; ABCG2: ATP-binding cassette subfamily G member 2; hOCT1: Human organic cation transporter 1.

**Table 2 Cellular and molecular mechanism of action of the combination treatment in preclinical studies**

|  |  |  |  |
| --- | --- | --- | --- |
| **Therapeutic agent** | **Animal model or cell line** | **Cellular and molecular mechanism of action of the combination treatment** | **Ref.** |
| Radiation | MCF-7 and T47D human breast cancer cells | Increases the percentage of cells in sub-G1 phase | Velho-Pereira *et al*[121] |
|  | MCF-7 and MDA-MB-231 human breast cancer cells | Restores the expression levels of TGF-β and its downstream molecules NFB, Smad2, Snail and Twist, adhesion molecules E-cadherin and cytokeratin 19, mesenchymal markers integrin αV, MMP-9, and MMP-2 | Rajput *et al*[122] |
|  | B16-F10 melanoma cells | Inhibits the phosphorylation of JAK2 and STAT3. Increases the expression of caspase 3 and Bax. Reduce the expression of Bcl-2 and survivin and the level of VEGF-A, MCP-1, TGF-β1, RANTES and IL-1β. Induces DNA damage | Hatiboglu *et al*[123] |
| microRNA-34a | BT-549 metastatic breast cancer cells | Targets and downregulates TWIST1 and ZEB1 | Imani *et al*[126] |
| Akt-siRNA | Akt-overexpressing MCF-7 and T47D. Tamoxifen resistant MCF-7 and T47D breast cancer cells. BALB/c mice injected with MCF-7/TAM cells | Reduces Akt expression and MDM-2 activation. Activates p53, increases the level of Bax and Bim and decreases the level of Bcl-2 and Ki67 | Rajput *et al*[127] |
| Vitamin D3 | Azoxymethane-induced colorectal tumors in Wistar rats | Reduces the level of Wnt, β-catenin, NFB, COX-2, iNOS, VEGF and HSP-90 and increases that of DKK-1, CDNK-1A, TGF-β1, TGF-β/RII and Smad4 | Mohamed *et al*[131] |
| Melatonin | EMT6/P mouse breast cancer cells. Balb/C mice transplanted with EMT6/P cells | Reduces the expression of VEGF and the serum level of AST and ALT. Increases the serum level of IFN-α and decreases that of IL-4 | Odeh *et al*[134] |
| Artemisinin | CCRF-CEM and multidrug-resistant CEM/ADR5000 human leukemia cells. Healthy human foreskin fibroblasts |  | Fröhlich *et al*[136] |
| Artesunic acid | HCT116, HT29, Caco-2, DLD-1 colon cancer cells. HCEC nonmalignant colon epithelial cells | Induces ROS generation, DNA damage, PARP and caspase 9 cleavage. Increases the level of ɣ-H2AX | Fröhlich *et al*[137] |
| Diosgenin | A431 and Hep2 human squamous cell carcinoma. Swiss albino mice injected with sarcoma 180 cells | Induces DNA fragmentation and cytoskeletal changes. Decreases the expression of CD31 and Ki67 | Das *et al*[138] |
| Emodin | MCF-7, MDA-MB-231, MDA-MB-468 and T47D human breast cancer cells. CAM inoculated with MCF-7 cells | Increases the percentage of cells in sub-G1 phase. Increases ROS generation, cytochrome c release, expression levels of p53, Bax and cleaved caspase 3. Reduces Bcl-2, pFAK and integrinβ1 expression level. Induces nuclear fragmentation, shrinkage, apoptotic body formation, chromatin condensation and membrane blebbing | Bhattacharjee *et al*[140] |
| Ferulic acid | MDA-MB-231 human breast cancer cells |  | Al-Mutairi *et al*[143] |
| Genistein | CALC-62 and ACC448 human thyroid cells derived from anaplastic carcinoma CGTH-W1, ACC360 derived from follicular carcinoma | Reduces the expression level of human telomerase reverse transcriptase, VEGF-A and NFB. Increases the expression level of PTEN and p21 and activates caspase 3 | Ozturk *et al*[145] |
| Indirubin-3-monoxime | A549 human lung cancer cells. HFL-1 human fetal lung fibroblast. CD1-nude mice injected with A549 cells | Increases the percentage of cells in Sub-G0 phase. Reduces Bcl-2/Bax ratio, TNF-α release and p-Akt (s473), p-mTOR, NFB/p65, caspase3 and p53 expression level | Dera *et al*[147] |
| Piperine | EMT6/P mouse mammary cancer cells. Balb/C female mice injected with EMT6/P cancer cells | Reduces VEGF expression. Increases IFN-γ and IL-2 level and caspase 3 activity | Talib *et al*[149] |
|  | HepG2 human hepatocellular cancer cells | Increase ROS generation and decreases GSH and NADPH level | Das *et al*[151] |
| Resveratrol | HepG2 human hepatocellular cancer cells | Increases caspase 3 activity. Decreases GSH and MDA level | Ismail *et al*[153] |
|  | EMT6/p mouse epithelial breast cancer cells. MCF-7 and T47D human epithelial breast cancer cells kidney epithelial cells. Balb/C mice injected with EMT6/p cancer cells | Induces DNA fragmentation and increases IFN-γ and IL-4 level. Reduces VEGF expression | Alobaedi *et al*[154] |
| Selenium | MG-63 human osteosarcoma cell line | Increases cellular damage, and decreases the level of alkaline phosphatase and GSH | Barron *et al*[156] |

PTEN: Phosphatase and tensin homolog; ROS: Reactive oxygen species; Bax: Bcl-2-associated X protein; GSH: Glutathione; MMP: Matrix metalloproteinases; ɣ-H2AX: Gamma-histone 2AX; JAK2: Janus kinase 2; STAT3: Signal transducer and activator of transcription 3; NFB: Nuclear factor kappa B; COX-2: Cyclooxygenase 2; iNOS:Inducible nitric oxide synthase*;* VEGF:Vascular endothelial growth factor;DKK-1: Dickkopf-related protein-1; CDNK-1A: Cyclin-dependent kinase inhibitor 1A*;* TGF-β1: Transforming growth factor beta 1; TGF-βRII: Transforming growth factor beta receptor II; GSH: Glutathione; mTOR: Mammalian target of rapamycin; AST: Aspartate transaminase; ALT: Alanine transaminase; MDA: Malondialdehyde; IL: Interleukin; INF: Interferon; TNF-α: Tumor necrosis factor alpha; MCP-1: Monocyte chemoattractant protein-1; RANTES: Regulated on activation normal T cell expressed sequence; TWIST1: Twist-related protein 1; ZEB1: Zinc finger E-box binding homeobox 1; MDM-2: Mouse double minute 2; NADPH: Nicotinamide-adenine dinucleotide phosphate; CAM: Chorioallantoic membrane.



Published by **Baishideng Publishing Group Inc**

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** bpgoffice@wjgnet.com

**Help Desk:** https://www.f6publishing.com/helpdesk

https://www.wjgnet.com



**© 2021 Baishideng Publishing Group Inc. All rights reserved.**