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**Review Article**

# The Anticancer Potential of Ivermectin: Mechanisms of Action and Therapeutic Implications

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**ABSTRACT**

Ivermectin is a well-known antiparasitic drug in the macrolide class with a 16-membered ring. Its use in treating various parasitic diseases, including onchocerciasis, lymphatic filariasis, and strongyloidiasis, is well established. The present study aimed to review the mechanisms of action and therapeutic implications of Ivermectin as an anticancer agent. Recently, the potential use of ivermectin in cancer treatment has emerged. A growing body of evidence suggests that ivermectin has anticancer properties, making it an attractive candidate for treating various types of cancer. Studies have shown that ivermectin targets multiple signaling pathways, including the Wnt/β-catenin, PI3K/Akt/mTOR, and STAT3 pathways, to inhibit cancer cell proliferation and induce apoptosis. Inhibition of these pathways by ivermectin leads to suppression of cancer cell growth, making it an effective antitumor agent. Additionally, ivermectin has been shown to induce autophagy, which can lead to programmed cell death in cancer cells. One of the significant advantages of ivermectin as an anticancer drug is its safety profile. It has been used for over three decades, and its safety has been well-established in humans. Furthermore, it is easily available and affordable, making it a promising alternative to conventional chemotherapy. Several preclinical studies have demonstrated the efficacy of ivermectin against various types of cancer, including breast, lung, and colon cancer. However, further research is needed to evaluate its clinical effectiveness in humans. Clinical trials are underway to investigate ivermectin's safety and efficacy in cancer treatment. In conclusion, using ivermectin as an anticancer drug is a promising area of research. Its ability to target multiple signaling pathways and induce programmed cell death in cancer cells makes it an attractive candidate for the treatment of various types of cancer. Its safety profile and low cost make it a feasible alternative to conventional chemotherapy.

## 1. Introduction

Ivermectin (IVM), a broad-spectrum antiparasitic drug, has been used for several decades to treat various parasitic diseases[1]. Its effectiveness is due to its ability to activate glutamate-gated chloride channels, which results in excessive chloride influx and hyperpolarization of neurons, leading to somatic muscle paralysis and the eventual death of the parasites. Ivermectin is effective against several parasitic diseases, including scabies, elephantiasis, river blindness, trypanosomiasis, trichinosis, malaria, leishmaniasis, and schistosomiasis[2]. In addition to its antiparasitic properties, IVM has shown potential as an antiviral agent[3]. It has been found to inhibit the replication of flaviviruses by blocking the NS3 helicase activity and interfering with the transport of viral proteins to the nucleus via the α/β-mediated mechanism[3]. Ivermectin also displays antiviral activity against viruses such as dengue and HIV-1[4]. Recent studies have shown that IVM may also be effective against SARS-CoV-2, which caused the COVID-19 pandemic[5]. Recent studies have highlighted the potential of IVM as innovative cancer treatment[6,7]. Ivermectin has been shown to

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overcome tumor multidrug resistance (MDR), making it a promising candidate for combination therapy with conventional chemotherapy drugs[8]. Furthermore, *in vitro* and *in vivo* studies have demonstrated that IVM directly inhibits the proliferation of several types of cancer cells, including breast, lung, colon, and prostate cancer[9,10].

One proposed mechanism for IVM's anticancer activity is its ability to disrupt multiple signaling pathways that regulate cell growth and survival. For example, IVM has been shown to inhibit the Akt/mTOR pathway, which is frequently activated in cancer cells and promotes cell growth and proliferation[11,12]. Ivermectin has also been reported to suppress the Wnt/β-catenin signaling pathway, which is critical in tumor initiation and progression[13]. In addition to its effects on signaling pathways, IVM has been shown to induce programmed cell death (apoptosis) in cancer cells[14]. This effect has been observed in multiple cancer cell lines, including breast, colon, and ovarian cancer cells. IVM-induced apoptosis is thought to occur through several mechanisms, including mitochondrial dysfunction and activation of caspase-mediated cell death pathways. Another potential mechanism for IVM's anticancer activity is its ability to target cancer stem cells (CSCs), which are thought to be responsible for tumor initiation, progression, and recurrence[15]. Ivermectin has been shown to selectively kill CSCs in several types of cancer, including breast, colon, and ovarian cancer. This effect is thought to occur by inhibiting the Hedgehog signaling pathway, which is critical for maintaining CSCs[16,17].

The evidence suggests that IVM has significant potential as an anticancer medication. However, more research is needed to fully understand the mechanisms underlying its anticancer activity and optimize its use with other therapies. If further studies confirm its efficacy, IVM could offer a cost-effective and readily available alternative to current cancer treatments. The present study aimed to review the mechanisms of action and therapeutic implications of IVM as an anticancer agent.

## 2. The role of Ivermectin in different cancers

### *2.1. Breast cancer*

Breast cancer is one of the most prevalent malignant tumors among women worldwide. It is a complex disease that results from the accumulation of genetic and epigenetic alterations that lead to the abnormal proliferation of breast epithelial cells[18]. Traditional treatment options for breast cancer include surgery, chemotherapy, radiation therapy, and hormone therapy[19]. However, these treatments have significant side effects and limited effectiveness, especially for triple-negative breast cancer (TNBC).

Ivermectin is a well-known anthelmintic drug used to treat parasitic infections in humans and animals for decades. In recent years, IVM has gained attention as a potential anticancer agent due to its ability to inhibit the proliferation of various cancer cell lines, including breast cancer. In particular, IVM has shown promising results in inhibiting the growth and survival of TNBC cells[20].

One of the mechanisms through which IVM exerts its anticancer effects is inducing autophagy in breast cancer cells[21]. Autophagy is a cellular process that involves the degradation of damaged or dysfunctional organelles and proteins to maintain cellular homeostasis. The Akt/mTOR pathway is a critical autophagy regulator. Ivermectin has been shown to block this pathway, leading to autophagy induction and subsequent inhibition of breast cancer cell proliferation[11].

In addition to autophagy induction, IVM has been found to inhibit P-21-activated kinase 1 (PAK1), a protein crucial in breast cancer cell migration, invasion, and metastasis [22]. IVM's inhibition of PAK1 leads to a reduction in breast cancer cell proliferation and invasiveness. Another promising mechanism of IVM's anticancer activity is its ability to act as an epigenetic regulator[20]. Epigenetic alterations, such as DNA methylation and histone modifications, are crucial in cancer development and progression. The IVM has been shown to regulate the expression of E-cadherin, an EMT-related gene, to restore TNBC cells' sensitivity to tamoxifen. The IVM inhibits the interaction between the SIN3-interaction domain and paired a-helix2, which could make it a potential therapeutic candidate for TNBC. Moreover, IVM has been shown to regulate the tumor microenvironment in breast cancer treatment. The tumor microenvironment comprises various cells, including immune cells, and plays a crucial role in tumor growth and progression. When the tumor microenvironment contains high levels of ATP, IVM can increase the release of HMGB1 via the P2 × 4/P2 × 7/Pannexin-1 pathway. HMGB1 is a damage-associated molecular pattern molecule that triggers immune cell-mediated immunogenic death and inflammation, inhibiting tumor growth[23].

Therefore, IVM shows promise as a potential treatment for breast cancer, particularly for TNBC, through its various mechanisms of action. Its ability to regulate the tumor microenvironment and mediate immunogenic cell death is a novel and promising direction for future anticancer research. The findings from preclinical studies are promising, and clinical trials are warranted to evaluate IVM's efficacy and safety as a breast cancer treatment.

### *2.2. Digestive system cancer*

Gastric cancer is a highly prevalent malignant tumor affecting millions worldwide. While chemotherapy and other treatment options have been developed, there is still a need for more effective treatments. Ivermectin has shown promising results in inhibiting the proliferation of gastric cancer cells both *in vitro* and *in vivo*. Nambara's study found that IVM could inhibit the growth of gastric cancer cells by blocking the activity of YAP1 (Yes-associated protein 1)[24]. This is an important finding, as YAP1 is known to play a role as an oncogene in tumorigenesis, and inhibiting its activity could be an effective strategy for treating cancer[25]. Furthermore, IVM

has been shown to inhibit the Wnt pathway, which is known to play a role in tumorigenesis in multiple cancers. A study using colorectal cancer cell lines CC36, Ls174 T, CC14, and DLD1 found that IVM inhibited proliferation and induced apoptosis by blocking the Wnt pathway. In addition, caspase-3 expression in Ls174 T and DLD1 cells increased following IVM treatment, indicating that IVM can induce apoptosis in cancer cells [26].

Another area where IVM shows promise as a potential treatment for cancer is in the treatment of hepatocellular carcinoma (HCC). The HCC is the fourth most common cause of cancer death worldwide and is often associated with a combined infection with both hepatitis B and C viruses. A Mob1b-/- mouse model of spontaneous liver cancer showed that IVM could inhibit the growth of liver cancer cells by blocking YAP1 function[27]. This is an important finding, as YAP1 is known to play a role in the development and progression of liver cancer. Finally, IVM has also been effective in treating cholangiocarcinoma, a malignant condition affecting the bile ducts inside and outside the liver. Intuyod's trials found that IVM could inhibit the growth of KKU214 cholangiocarcinoma cells dose-dependently[28]. Additionally, IVM was able to induce apoptosis in these cells and was effective in treating cells that were resistant to traditional chemotherapy.

Therefore, IVM efficiently treats gastric cancer, hepatocellular carcinoma, and cholangiocarcinoma. Its ability to inhibit YAP1, block the Wnt pathway, and induce apoptosis in cancer cells make it a novel and promising direction for future anticancer research. However, further research is needed to fully understand the molecular mechanisms of IVM's anticancer effects and develop effective treatment strategies.

### *2.3. Urinary system cancer*

Urinary or urologic cancer encompasses cancers that arise from the urinary tract, including the bladder, kidneys, ureters, and urethra[29]. Among them, bladder cancer is the most common, with over 400,000 new cases and 170,000 deaths globally each year[30]. Recently, researchers have been investigating the potential of IVM in treating urinary cancers, with promising results[31]. In a study by Kato et al., IVM was found to inhibit the proliferation of bladder cancer cells in a dose-dependent manner. The inhibition was due to the downregulation of the protein Akt, essential for cell growth and survival. In addition, IVM induced apoptosis in the bladder cancer cells by activating caspase-3 and caspase-7, two proteins involved in the programmed cell death pathway [32]. These findings suggest that IVM could be a potential therapeutic option for bladder cancer. Similarly, IVM was found to be effective in inhibiting the growth of renal cell carcinoma cells. Renal cell carcinoma is the most common type of kidney cancer, accounting for 2-3% of all adult malignancies. In a study by Wan et al., IVM suppresses the proliferation and invasion of renal cell carcinoma cells by inhibiting the Wnt/β-catenin pathway. The study also showed that IVM induced apoptosis in renal cell carcinoma cells through the mitochondrial pathway.

Furthermore, IVM has also shown potential in treating prostate cancer. Prostate cancer is the second most common cancer in men worldwide, with over 1.4 million new cases and 375,000 deaths each year[33]. In a recent study, IVM was found to inhibit the growth of prostate cancer cells by downregulating the expression of the androgen receptor. This protein is critical for the growth and survival of prostate cancer cells[34]. Furthermore, IVM could enhance the sensitivity of prostate cancer cells to enzalutamide, an anti-androgen drug used to treat advanced prostate cancer.

These studies suggest IVM could be a promising candidate for treating urinary cancers. However, further research is needed to determine its efficacy and safety in clinical trials.

### *2.4. Hematological cancer*

Hematological malignancies are a diverse group of cancers that affect the blood, bone marrow, and lymphatic system[35]. Ivermectin has shown potential as a treatment for hematological cancer, particularly leukemia.

Leukemia is a clonal malignancy arising from the hematopoietic system's abnormal stem cells. In a study aimed at screening drug candidates for leukemia, IVM was found to selectively kill leukemia cells at low concentrations without disrupting normal hematopoietic cells[36]. The mechanism behind this selective toxicity was due to a high influx of chloride ions into the cell, which resulted in the production of reactive oxygen species and hyperpolarization of the plasma membrane. These effects ultimately lead to the death of leukemia cells. Further studies have shown that IVM can synergize with other chemotherapy drugs commonly used to treat leukemia. In particular, a synergistic interaction is observed with daunorubicin and cytarabine, two drugs often used to treat acute myeloid leukemia. This combination increases apoptosis in leukemia cells and reduces toxicity in normal hematopoietic cells.

Ivermectin is also shown to induce mitochondrial dysfunction and oxidative stress selectively in cancerous cells[37]. In a study by Wang and colleagues, IVM boosted caspase-dependent apoptosis in chronic myeloid leukemia K562 cells compared to normal bone marrow cells. In addition, when IVM was combined with the chemotherapy drug dasatinib, a higher efficiency was observed, and tumor growth was impeded dose-dependently.

These findings suggest that IVM may be a promising candidate for treating hematological malignancies, particularly leukemia. Its ability to selectively target cancerous cells and synergize with other chemotherapy drugs could make it a valuable addition to current treatment regimens. However, further studies are needed to fully understand the mechanism behind its anticancer effects and optimize its use in the clinic.

### *2.5. Respiratory system cancer*

In a recent study, IVM was found to have potential as

a therapeutic agent for treating nasopharyngeal carcinoma[38]. This is significant because the current treatments for this type of cancer, such as radiation therapy and chemotherapy, can have significant side effects and may not be effective for all patients. The study showed that IVM could selectively kill nasopharyngeal carcinoma cells while leaving normal thymocytes unharmed, indicating that IVM has a high level of specificity and low toxicity.

The MAPK pathway plays a crucial role in cell proliferation, differentiation, and survival, and it has been implicated in various types of cancer, including nasopharyngeal carcinoma. The study demonstrated that IVM inhibits the MAPK pathway by reducing the function of PAK1 kinase, a key player in this pathway. The IVM's inhibition of the MAPK pathway suppresses nasopharyngeal carcinoma cell growth, leading to its potential as a therapeutic agent for this type of cancer. The IVM has also shown promising results in lung cancer as a potential treatment. YAP1 is a transcriptional co-activator that is upregulated in many types of cancer and has been linked to poor prognosis. In the study by Nishio et al., IVM inhibited the activity of YAP1, leading to the profound inhibition of H1299 lung cancer cell growth[27]. Furthermore, combining IVM with erlotinib, an EGFR inhibitor, showed a potent killing effect on HCC827 lung cancer cells by modulating the activity of EGFR. This combination therapy could potentially provide a new approach to treating lung cancer, especially for patients resistant to EGFR inhibitors alone. In addition to its effects on cancer cell growth, IVM has also been found to inhibit EMT, an important process in the metastasis of cancer cells. By inhibiting EMT, IVM reduces the ability of lung cancer cells to spread to other parts of the body, potentially improving patient outcomes.

Overall, the findings of these studies demonstrate the potential of IVM as a therapeutic agent for treating different types of cancer, including nasopharyngeal and lung cancer. Further research is needed to fully understand the mechanisms of IVM's effects on cancer cells and to optimize its use in clinical settings.

## 3. Ivermectin-induced programmed cell death in tumor cells and related mechanisms

### *3.1. Apoptosis*

Apoptosis is a highly regulated form of programmed cell death critical in maintaining tissue homeostasis and eliminating damaged or abnormal cells[39]. It is characterized by distinct morphological and biochemical features, including chromatin condensation, DNA fragmentation, and the formation of apoptotic bodies[40]. IVM has been shown to induce apoptosis in various tumor cells, including Hela cells, through both extrinsic and intrinsic pathways[32,41,42].

The activation of death receptors on the cell surface, such as Fas, TNFα, and TRAIL receptors, initiates the extrinsic apoptosis pathway. This leads to the activation of caspase-8, which activates downstream caspases, including caspase-3, leading to cell death. In contrast, the intrinsic pathway is initiated by releasing cytochrome C from the mitochondria into the cytoplasm. This activates caspase-9, which then activates caspase-3, resulting in apoptosis. Studies have demonstrated that IVM primarily induces apoptosis through the intrinsic pathway by decreasing the mitochondrial membrane potential and increasing the efflux of cytochrome C into the cytoplasm, activating caspase-9 and caspase-3[31,37]. This is consistent with the observed morphological changes, including chromatin condensation, DNA fragmentation, and apoptotic body formation. Furthermore, IVM has been shown to regulate the expression of Bcl-2 family proteins, which play a crucial role in regulating mitochondrial permeabilization and initiating apoptosis[43]. IVM treatment results in a decrease in anti-apoptotic protein Bcl-2 levels and an increase in pro-apoptotic protein Bax levels. This shift in the Bax/Bcl-2 ratio favors the activation of caspase-9 and caspase-3, leading to apoptosis.

In summary, IVM induces apoptosis in tumor cells primarily through the intrinsic pathway by decreasing mitochondrial membrane potential, increasing cytochrome C efflux, regulating the expression of Bcl-2 family proteins, and activating caspase-9 and caspase-3. These findings suggest that IVM has the potential as a therapeutic agent for treating various cancers by inducing programmed cell death in tumor cells (Figure 1).

### *3.2. Autophagy*

Autophagy is a programmed cell death mechanism that relies on lysosomes to remove excess or damaged cellular components, maintaining cellular homeostasis[44]. It is characterized by the formation of autophagosomes, which contain cytoplasmic components and are double-layered or multilayered vacuolar structures[45]. Recent studies have demonstrated that autophagy plays a dual role in tumor development[46,47]. On the one hand, autophagy can help tumor cells adapt to nutrient-deficient microenvironments and protect them from chemotherapy- or radiotherapy-induced damage [48]. On the other hand, excessive autophagy activation can lead to tumor cell death, and some autophagy activators have been shown to increase tumor sensitivity to chemotherapy and radiotherapy[49]. Therefore, the effects of autophagy on tumor development depend on the specific tumor microenvironment. Enhancing autophagy activity has become a promising approach in cancer therapy.

One interesting area of research is the role of autophagy in programmed cell death after intervention with IVM, an antiparasitic drug with potential anticancer properties. In breast cancer cell lines, IVM increased intracellular autophagic flux and the expression of key autophagy proteins[21]. However, when autophagy

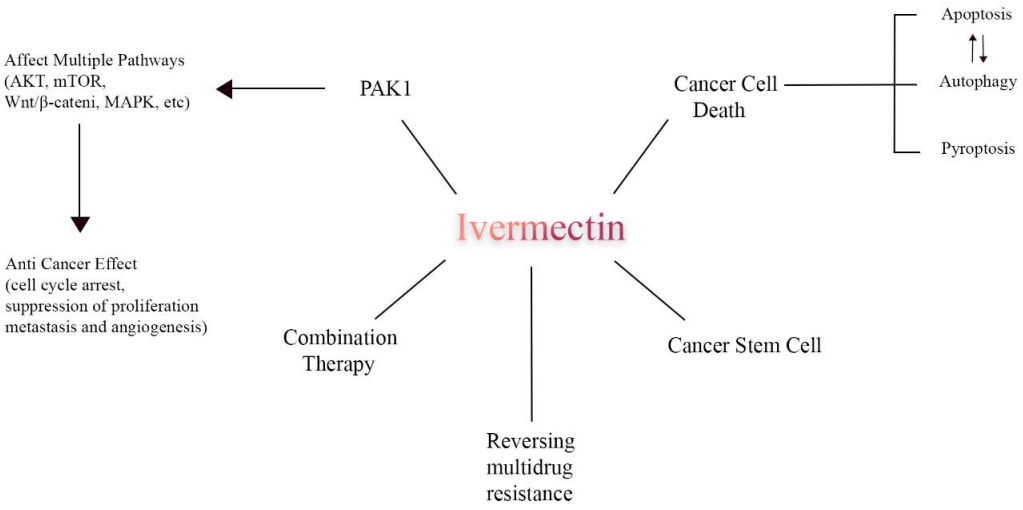


**Figure 1.** Ivermectin mechanism of action against tumors

was inhibited using chloroquine, wortmannin, or siRNA, the anticancer activity of IVM was significantly reduced. This suggests that IVM exerts its anticancer effects primarily through the autophagy pathway. Additionally, researchers found that IVM induces autophagy by inhibiting the phosphorylation of Akt and mTOR, as demonstrated in glioma and melanoma[50-52]. These findings suggest that IVM may be a promising autophagy activator for inducing autophagy-dependent death in tumor cells.

### *3.3. Pyroptosis*

Pyroptosis is a form of cell death initiated by inflammasomes, multi-molecular complexes composed of a pattern recognition receptor (PRR), apoptosis-associated speck-like protein containing a CARD (ASC), and pro-caspase-1[53]. The PRR can recognize both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns. Activating inflammasomes cleavage pro-caspase-1 into activated caspase-1, which leads to the secretion of pro-IL-1β and pro-IL-18 cytokines[54]. Gasdermin D (GSDMD) is a crucial protein involved in pyroptosis[55]. Upon cleavage by activated caspase-1, GSDMD causes swelling and rupture of the cell.

A study by Draganov demonstrated that IVM intervention increased the release of lactate dehydrogenase and activated caspase-1 in breast cancer cells, indicating the occurrence of pyroptosis[23]. Moreover, the study observed cell swelling and rupturing, characteristic pyroptosis phenomena. Although the authors speculated that IVM might mediate pyroptosis via the P2 × 4/P2 × 7/NLRP3 pathway, no conclusive evidence supports this.

Interestingly, IVM exacerbates renal ischemia-reperfusion injury via the P2 × 7/NLRP3 pathway and increases the release of pro-inflammatory cytokines in human proximal tubular cells in ischemia-reperfusion experiments[56]. It is important to note that IVM may induce different types of programmed cell death in different types of cancer. Further studies are needed to investigate the role of IVM in causing pyroptosis in other cancers (Figure 2).

## 4. Antitumor effects of ivermectin *in vivo*

The antitumor effects of IVM have been studied *in vivo* in immune-deficient mice using various cancer cells, including human acute myeloblastic leukemia, glioblastoma, and breast and colon carcinoma well as in the murine lymphosarcoma cell line MDAY-D2 [21,41,57]. Treatment with IVM for 10 to 42 days through oral, intraperitoneal, or intratumoral routes (more commonly intraperitoneal) resulted in a more than 50% reduction in tumor volumes. The median dose used in these studies was 5 mg/kg (ranging from 2.4 to 40 mg/kg), equivalent to 0.40 mg/kg in humans. This dose is lower than the highest safe dose evaluated in humans so far (2 mg/kg). Thus, the results of these *in vivo* studies suggest that IVM may have antitumor effects in cancer patients at feasible doses[58,59]. The *in vivo* summary of the antitumor effects of IVM is presented in Table 1.

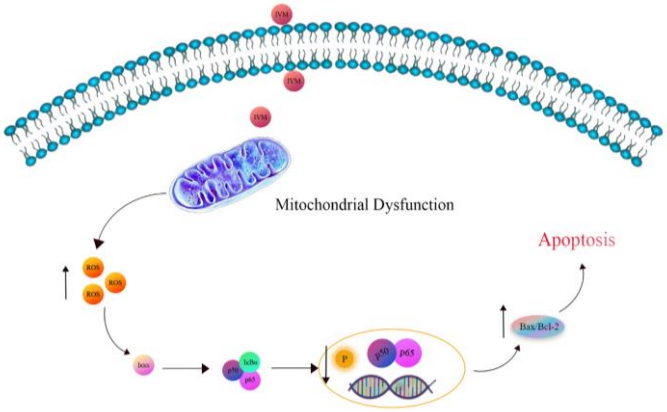


**Figure 2.** Ivermectin induce apoptosis in tumor cell

**Table 1.** summary of antitumor effects of ivermectin *in vivo*

| Cancer type | Tumor cell line | Days of treat. | Dose mg/kg | Mice | Result | Ref. |
|---|---|---|---|---|---|---|
| Murine leukemia | MDAY-D2<br>OCI-AML2 | 10 | 3,<br>5,<br>6<br>i.p. | NOD/SCID mice | In all 3 dosages reduces tumor volume up to 70% | 36 |
| Human glioblastoma | U87<br>T98G | 21 | 40<br>i.p. | SCID mice | Decreases body weight of the mice were not observed but significantly inhibited growth of tumors. | 43 |
| Breast cancer | MDA-MB-231-GFP | 10 | 2.4<br>i.p. | NOD/SCID mice | Tumor weight and size were reduced. | 21 |
| Human glioma | U87MG | 42 | 3,<br>10<br>i.t. | Balb/c nude mice | Reduces tumor volume up to 50% at 3 mg/kg.<br>At 10 mg/kg tumors were not detectable | 59 |
| Human colon cancer | LDL1<br>CC14<br>HT29 | 21 | 10<br>i.p. | NMRI nude mice | Reduces tumor volume up to 85% (LDL1 cell line). No effect is observed in the tumor TCF-independent cell line (CC14) | 26 |

## 5. Conclusion

Ivermectin also affects various signaling pathways in tumor cells, including the PI3K/Akt/mTOR pathway and the MAPK/ERK pathway, both of which are important for cancer cell survival and growth. Additionally, IVM has been shown to stimulate antitumor activity when it targets specific ways, such as the Wnt/β-catenin pathway in colorectal cancer cells. Ivermectin has been found to modify the tumor microenvironment by inhibiting the release of pro-inflammatory cytokines and reducing the activity of tumor-associated macrophages, which can promote tumor growth. Ivermectin has also been shown to decrease tumor stem cell activity and inhibit angiogenesis, which is the process through which tumors develop their blood supply. However, the molecular mechanisms underlying these effects are not yet fully understood. Further research is needed to identify the exact molecular targets of IVM and how it affects the tumor microenvironment and angiogenesis.

In addition to apoptosis, IVM has been found to induce other forms of programmed cell death, including pyroptosis and autophagy. The type of cell death induced by IVM appears to depend on the type of cancer cells and the environment in which they are growing. Understanding the different types of cell death induced by IVM and how they are regulated will be essential for developing effective cancer treatments. Importantly, the *in vivo* antitumor activity of IVM is achieved at concentrations that can be clinically reachable based on the human pharmacokinetic studies done in healthy and parasite patients. Thus, existing information on IVM could allow its rapid move into clinical trials for cancer patients.

## Declarations

### *Competing interests*

The authors have declared no conflicts of interest.

### *Authors' contributions*

Narges Lotfalizadeh, Arian Gharib, Ashkan Hajjafari, and Hassan Borji wrote the draft of the manuscript. Zeynab Bayat revised the draft of the manuscript and checked the final version of the article. All authors have read and approved the final version of the manuscript for publication in the present journal.

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### *Ethical considerations*

The authors declare that this manuscript is original and has not been submitted elsewhere for possible publication. The authors also declare that the data used/presented in this manuscript has not been fabricated.

### *Availability of data and materials*

The authors will provide the data from the present study in case of request.

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