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Research on Drug Repurposing of Benzimidazole Anthelmintics as Anti-cancer Agents

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벤지미다졸 구충제의 항암 효능 약물 재창출 연구

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ABBREVIATIONS

ABZ	Albendazole
ABZSO	albendazole sulfoxide
$ABZSO_2$	albendazole sulfone
AML	acute myeloid leukemia
BBB	blood-brain barrier
b.i.d	twice daily
BCSC	breast cancer stem cell
C_{max}	maximum concentration
CBZ	Carbendazim
ERK	extracellular signal-regulated kinases
FBZ	Fenbendazole
FBZSO	fenbendazole sulfoxide
$FBZSO_2$	fenbendazole sulfone
FDA	food and drug administration
FLZ	Flubendazole
HER	human epidermal growth factor receptor

HNSCC	head and neck squamous cell carcinoma
HSF	heat shock factor
IC_{50}	half maximal IC50 inhibitory concentration
i.p.	Intraperitoneal
KRAS	Kirsten rat sarcoma virus
MBZ	Mebendazole
MDR	multiple drug resistance
MEK	mitogen-activated protein kinase
MTZ	Methiazole
NCZ	Nocodazole
NSCLC	non-small cell lung cancer
OBZ	Oxibendazole
OFZ	Oxfendazole
PBZ	Parbendazole
p.o.	oral administration
RBZ	Ricobendazole
$T_{1/2}$	half-life
T-ALL	T cell acute lymphoblastic leukemia

TNBC	Triple-negative breast cancer
TNIK	TRAF2- and NCK-interacting kinase
VEGF	vascular endothelial growth factor

ABSTRACT

Research on Drug Repurposing of Benzimidazole Anthelmintics as Anti-cancer Agents

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In recent decades, a chemical group of benzimidazoles has shown significant promise as repurposing cancer therapy, among other drugs. Besides, repurposing of anthelmintics for cancer treatment caught attention of cancer patients in 2019 in South Korea because of a huge controversy triggered by successful experiences of Joe Tippens, and anthelmintics are used by many patients in cancer treatment in South Korea. Nevertheless, there have been few studies about developing status of benzimidazoles as anti-cancer agents. For that reason, an extensive review on the preclinical and clinical studies for benzimidazoles was conducted in this thesis, and additionally, a perception study for cancer treatments among cancer patients was also performed.

In the first part, the current thesis aimed to reveal the possibilities and

limitations of the anti-cancer effects of benzimidazole anthelmintics, by exploring a variety of studies, and suggested ways to overcome the limitations of benzimidazle anthelmintics, for possible application as repurposed drugs. The review included studies on anti-cancer effects of 11 benzimidazoles. In three sections for preclinical anti-cancer effects, clinical anti-cancer effects, and pharmacokinetic properties, the properties of each benzimidazole were examined and key properties were elucidated. Notably, although many preclinical studies have demonstrated the anti-cancer effects of benzimidazoles, there is limited evidence regarding their prominent effects in clinical settings. It was presumed that this was because the clinical trials conducted on benzimidazoles, failed to restrict their participants with specific criteria including cancer entities and cancer stages, due to the reason that main targets and the multiple anti-cancer properties of them have not clearly clarified yet. In addition, these drugs face the limitation of low bioavailability, resulting in insufficient concentration levels. In conclusion, additional efforts in the form of further studies on whole anti-cancer pathways and development strategies, including formulations, are required to repurpose benzimidazoles as anti-cancer agents.

In the second part, a cross-sectional survey was conducted because adequate data on their experiences or perceptions is lacking although anthelmintics are used by many patients with cancers. The survey investigated the repercussions of anthelmintics for cancer treatment and evaluated their effectiveness and adverse

effects. It included 86 cancer patients, aged 19 years and above, who underwent anthelmintic therapy for cancer. They were recruited from two online communities in South Korea, and a structural questionnaire was provided online. The survey results showed that cancer patients under anthelmintics therapy for cancer in South Korea were mostly in their advanced stages and had started the treatment in 2019. More than one-third of cancer patients had taken anthelmintics during their chemotherapy, and 97% of them did not inform clinicians. These participants had a positive perception towards the effectiveness of anthelmintics, as it improved their physical condition (42.9%). Examination of adverse effects of anthelmintics showed that more than two-thirds of the subjects did not experience adverse effect, and gastrointestinal side effects were mostly reported. In conclusion, it might be worth evaluating the benefits and risks of anthelmintics in cancer treatment through further clinical trials considering perceptions among the patients. Communication between the clinicians and cancer patients needs to be enhanced regarding the use of anthelmintics to prevent adverse effects.

Through these two parts, it was concluded that although benzimidazoles had *in vitro* and *in vivo* anti-cancer effects, data for anti-cancer effects and safety in clinical settings are still not enough. Therefore, for drug repurposing of benzimidazoles as anti-cancer agents, more rigorous studies need to be performed. In addition, although many cancer patients had a positive perception

towards the effectiveness of anthelmintics, it needs careful attention because diverse adverse effects might occur from taking drugs without consultation with doctors. Furthermore, it is required the more active involvement of government in order to present evidence-based guidelines for social issues regarding drug uses.

국문초록

벤지미다졸 구충제의 항암 효능 약물 재창출 연구

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구충제로 사용되고 있는 벤지미다졸 계열의 약물 성분은 최근 수십 년 동안 항암 치료 목적의 약물 재창출 연구 분야에서도 활발히 연구가 진행되어 왔다. 특히, 2019년 한국에서는 조 티펜스의 성공적인 암 치료 경험담이 알려짐으로써 암 치료를 위한 구충제 복용에 암 환자들이 관심을 갖게 되었고, 실제로 많은 환자들이 구충제로 암 치료를 하는 상황이 초래되었다. 그럼에도 불구하고 벤지미다졸 계열 구충제를 항암제로 개발하고자 하는 현황 및 사용실태에 대해 조사가 부족했다. 따라서 본 연구에서는 벤지미다졸 계열 구충제의 비임상 및 임상 연구에 대한 문헌 조사를 수행하였으며, 추가적으로 벤지미다졸 계열의 구충제를 복용한 암환자를 대상으로 인식도 조사를 실시하였다.

첫 번째 연구는 문헌 고찰 연구로 벤지미다졸 성분의 구충제가 가진 항암 효능 및 항암제로서 약물재창출 과정 시 관찰되는 한계를 분석하고, 이의 극복방법을 고찰하고자 하였다. 주로 사용되는 11개의 벤지미다졸 약물의 비임상 및 임상 항암 효능, 약동학적 특성을 검토하였다. 여러 비임상 연구에서 벤지미다졸 계열의 항암 효과가 다수 보고되었지만, 실제 임상 연구에서는 현저한 효과가 관찰된 사례가 없었다. 그 이유 중 첫 번째로, 벤지미다졸의 약물 표적 및 항암 특성이 완전하게 규명되지 않았기 때문에 임상 시험에서 참여자 모집 시 암종 및 병기가 구체화되지 못했기 때문인 것으로 사료된다. 두 번째, 벤지미다졸의 낮은 생체이용률로 인해 충분한 약물 농도에 도달하지 못하는 한계를 갖고 있다. 결론적으로, 벤지미다졸 구충제의 항암제로 약물 재창출을 위해서는 벤지미다졸 약물의 항암효과 기전의 구체화와 함께 물리적 특성으로 인한 한계를 극복하기 위해 투여 경로 및 제형 등에 대한 추가적인 연구가 필요할 것으로 보인다.

두 번째 연구는 단면조사연구로 진행되었다. 다수의 암 환자들이 구충제를 항암 목적으로 사용하고 있음에도 이들의 경험이나 인식에 대한 조사가 수행된 바가 없다. 따라서 본 연구에서는 항암 목적으로 구충제를 사용한 방법과 치료 효과 및 이상반응에 대한 인식을

평가하였다. 설문은 항암 목적으로 약물 사용 경험이 있는 만 19세 이상의 암 환자 86명을 대상으로 실시하였다. 참여자는 한국의 온라인 커뮤니티 두 곳에서 모집하였고, 구조화된 설문지를 온라인으로 제공하여 시행하였다. 설문 결과, 한국에서 구충제를 이용하여 암을 치료한 환자 다수는 대부분 말기 환자였으며, 2019년에 구충제 복용을 시작한 것으로 나타났다. 3분의 1 이상의 암 환자는 기존 항암요법 기간에 구충제를 복용하였고, 참여자의 97%가 구충제 복용에 대해 주치의와 상의하지 않은 것으로 밝혀졌다. 참여자의 대부분은 구충제의 치료 효과에 대해 긍정적인 인식을 가지고 있었고, 이는 컨디션 향상(42.9%)을 주된 이유로 꼽았다. 구충제 복용 후 나타난 이상반응은 참여자의 약 3분의 1에서 관찰되었으며 나머지 3분의 2 이상은 이상반응을 경험하지 않았고 가장 주된 이상반응은 위장관 장해였다. 결론적으로, 구충제 사용에 대한 환자들의 긍정적인 인식이 큰 것으로 조사되었으나 참여자의 3분의 1 이상이 이상반응을 경험한 것을 볼 때, 암 치료의 이익과 위험을 보다 정확히 평가할 필요가 있으며 이를 위해 추가적인 임상시험을 시행할 필요가 있을 것으로 보인다. 그리고 구충제 사용에 따른 이상 반응을 방지할 수 있도록 주치의와 환자 간 소통도 향상될 필요가 있다.

이 두 개의 연구를 종합하면, 벤지미다졸은 비임상 시험에서 항암 효과를 나타내는 것으로 보고되고 있으나, 임상연구를 통한 항암 효과 및 안전성에 관한 자료는 부족한 실정이다. 따라서 벤지미다졸 약물의 항암제로의 약물 재창출을 위해서는 추가적인 비임상 및 임상 연구가 수행되어야 한다. 또한 다수의 암환자들이 약물사용에 긍정적 인식을 가지고 있음에도 전문가와 상담없이 약물을 복용할 경우 여러 이상 반응들이 나타날 수 있기 때문에 보다 세밀한 주의가 필요하다. 마지막으로 약물 사용에 대한 사회적 이슈가 있는 경우에 보건 당국의 보다 적극적이고 선제적인 대응을 통하여 증거 기반의 사용 가이드라인을 제시할 필요가 있다고 판단된다.

Part I. Review on Preclinical and Clinical Evidences of Anti-cancer Effects of Benzimidazole Anthelmintics

I. Introduction

According to several cross-sectional surveys, more than one-third of cancer patients receive unconventional therapies to support their cancer treatments or replace the conventional therapies, in Japan, Poland, and Wales [1-3]. This may be explained as follows. As shown in the statistics reported in studies by Jung et al. [4] and American Cancer Society [5], the 5-year relative survival rates for cancer patients were the 41.2% reported between 1993 and 1995 in South Korea, and 63% between 1995 and 1997 the United States, respectively. Even though it is a sharp improvement over the survival rates in those periods, the rates have been revealed to be low, at 70.6% and 68%, respectively, for those diagnosed in South Korea between 2012 and 2016, and for those between 2011 and 2017 in the United States.

First, cancer remains a fatal disease that ranks high as a cause of death globally [6] despite several decades of efforts to develop medicines for its treatment. According to the American Cancer Society, three cancers, including pancreatic cancer, liver cancer, and esophageal cancer, have been reported to have the lowest 5-year relative survival rates [5]. Erlotinib has recently been

introduced to treat pancreatic cancer, while atezolizumab and bevacizumab also have recently been used for the treatment of liver cancer. Erlotinib inhibits the epidermal growth factor receptor tyrosine kinase, and the use of erlotinib in combination with standard chemotherapy led to a prominent increase in the survival rate of patients with pancreatic cancer, from 17% to 24% [7], although the rate was still very low, far below 50%. Atezolizumab targets programmed death-ligand 1, thereby increasing the attack of T cells on cancer cells. Bevacizumab targets the vascular endothelial growth factor (VEGF), thereby inhibiting angiogenesis of cancer cells. Upon the combined use of atezolizumab and bevacizumab for treatment of liver cancer, the resultant overall survival at 12 months was reported to be 67.2% [8]. Therefore, many cancer patients are still in dire need of alternative medicines for cancer treatment.

Second, the development of new anti-cancer drugs is becoming increasingly difficult. Many pharmaceutical companies have faced several challenges in the 2000s, such as patent cliffs and intense generic competition, in addition to a stagnated success rate for new drug approval by the food and drug administration (FDA), due to cost increase and strengthened approval requirements [9]. A recent study estimated that the mean research and development cost for a new drug was $985.3 million, with the cost for anti-cancer drugs especially reaching $2771.6 million [10].

Third, many cancer patients are likely to be under financial pressure, due to

the high cost of cancer treatment. According to Iragorri et al., the portion of cost of cancer care paid by patients could account for approximately 40% of their annual income, in low- and middle-income countries [11]. Thus, the consumption of alternative drugs might be attributed to the fact that there are still limited remedies to effectively suppress various types of cancers, and no complete and cost-effective medicine that offers a perfect cure for cancers.

Therefore, repurposing drugs with anti-cancer efficacy can be considered a novel strategy for cancer therapy. As part of repurposing drugs for cancer treatment, a number of medicines, including metformin, itraconazole, and indomethacin [12], which have been developed or approved for other diseases, have been attracting interest and investigation. Among other drugs, benzimidazoles have shown significant promise, with various studies revealing their anti-cancer effects and relatively safe properties over a long period of use [13]. In addition, they have also attracted public attention in South Korea owing to a talk by Joe Tippens declaring a complete recovery from his lung cancer upon using a benzimidazole [14].

Several chemical groups are classified as anthelmintic drugs, including benzimidazoles (e.g., albendazole), halogenated salicylanilides (e.g., niclosamide), imidazothiazole derivatives (e.g., levamisole), thiazolides (e.g., nitazoxanide), macrocycliclactones (e.g., ivermectin), antitrematodals (e.g., praziquantel), quinolines (e.g., pyrvinium), and piperazine [15]. This review

focuses on the anti-cancer activities of benzimidazoles and includes the following 11 drugs: albendazole (ABZ), fenbendazole (FBZ), flubendazole (FLZ), mebendazole (MBZ), carbendazim (CBZ), methiazole (MTZ), nocodazole (NCZ), oxfendazole (OFZ), oxibendazole (OBZ), ricobendazole (RBZ), and parbendazole (PBZ). Among these, ABZ and MBZ have been approved by the FDA for fighting parasitic infections in humans [16], while FBZ, OFZ, and OBZ have been approved for veterinary parasite treatment [16]. Like other microtubule-targeting agents that have been widely used for cancer treatment [17], benzimidazole anthelmintics that exert anti-parasitic effects by inhibiting microtubule polymerization [18, 19] have also been regarded as having anti-cancer effects, and in practice, the chemical group has exhibited tumor suppression in many studies. Various research studies have revealed that the anti-cancer activities of benzimidazoles can be attributed to underlying mechanisms such as disruption of microtubule polymerization [20-25], induction of apoptosis [18, 25-30], or inhibition of angiogenesis [31, 32], metastasis [18, 25, 32, 33], etc. The anti-cancer mechanisms of benzimidazoles are illustrated in Figure 1.

This review aims to better understand the comprehensive anti-cancer efficacies of benzimidazole anthelmintics in terms of the *in vitro* and *in vivo*, and clinical evidence available till date, and summarizes their pharmacokinetic properties as well.

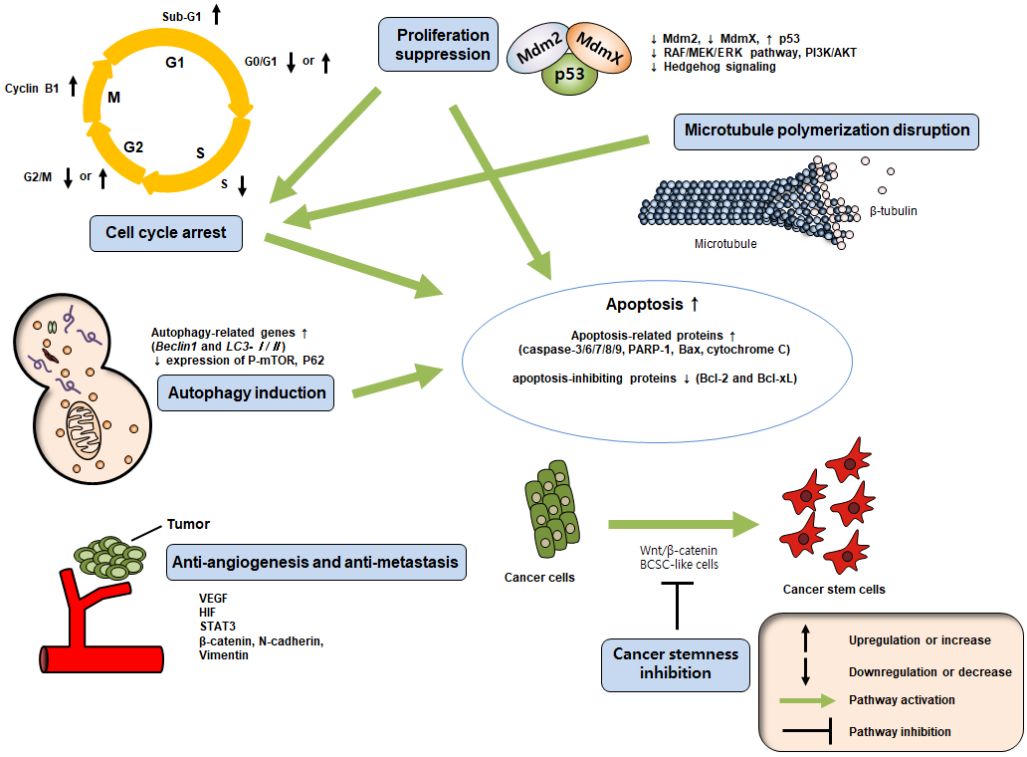

Figure1. Various anti-cancer mechanisms of benzimidazoles

Abbreviations: Mdm2: mouse double minute 2 homolog; MdmX: mouse double minute 4; RAF: rapidly accelerated fibrosarcoma; MEK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinases; PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B; LC3: microtubule-associated protein 1 light chain 3; mTOR: mammalian target of rapamycin; PARP: poly(ADP-ribose) polymerase; Bax: B-cell lymphoma 2 (Bcl-2)-associated X protein; Bcl-2: B-cell lymphoma 2; Bcl-xL: B-cell lymphoma-extra large; VEGF: vascular endothelial growth factor; HIF: hypoxia-inducible factor; STAT3: signal transducer and activator of transcription 3; BCSC: breast cancer stem cell

II. Preclinical Anti-cancer Effects of Benzimidazole Anthelmintics

II-1. Albendazole (ABZ)

II-1-1. *In Vitro* Anti-cancer Effects

ABZ offers various possibilities for its utilization in cancer therapies, with diverse advantages beyond its effects in microtubule inhibition, such as its effectiveness in suppression of growth of a wide-range of cancer cells, for example, those of the brain [19], breast [18, 34], lung [35], etc., including some cancer cells that are tricky to deal with. For example, it is noteworthy that ABZ exerted cytotoxicity against a human glioblastoma multiforme stem-like neurosphere cell line, at a low half maximal inhibitory concentration (IC_{50}) of 0.1 μM [19]. As drug delivery through the blood-brain barrier (BBB) is critical in the treatment of brain cancer, the fact that ABZ has been used as a medicine for central nervous system parasitic infections, because of its physicochemical ability to penetrate the BBB [19], suggests its potential for brain cancer therapy. Triple-negative breast cancer (TNBC) and Kirsten rat sarcoma virus (KRAS)-mutant lung cancer have limited therapeutic options, but ABZ has been reported

to suppress TNBC- [18, 34] and KRAS-mutant [35] cells. In addition, ABZ inhibits VEGF secretion in ovarian cancer cells [36].

Moreover, several studies have shown that ABZ enhances the anti-cancer efficacy of other microtubule-binding agents. When ABZ was used in combination with each of the drugs, paclitaxel [37], colchicine [38], or 2-methoxyestradiol [38], it exerted enhanced cytotoxicity against intestinal cancer cell lines (Caco2 and HCT8) with paclitaxel, while with the latter two, it did the same against both colorectal (HCT-116) and prostate (DU145) cancer cells. In addition, use of ABZ in combination with radiation resulted in a synergistic increase in the sensitivity of lung and skin cancer cells to radiation [39, 40].

II-1-2. *In Vivo* Anti-cancer Effects

In line with the *in vitro* results, ABZ showed *in vivo* inhibitory effects in brain [41], lung [31], and breast cancer [18, 34], etc. There is evidence that ABZ has *in vivo* anti-tumor effects in glioma (GL261 syngeneic mouse model) [19], non-small cell lung cancer (NSCLC) (A549 xenografts) [31], and TNBC cells (MDA-MB-231 xenografts and orthotopically injected 4T1 cells) [18, 34]. In addition, ascites formation is known to be associated with mortality in patients with peritoneal cancers caused by several cancers, including ovarian cancer [36].

Interestingly, ABZ did not show distinct anti-tumor effects in ovarian cancers, but reduced ascites formation by inhibiting VEGF expression, thus eventually inhibiting angiogenesis, which comprises factors of ascites formation [36, 42, 43]. Many *in vivo* tests have been conducted using mice, where the doses of ABZ were 30–300 mg/kg upon administration by means of oral administration (p.o.) [34, 44], 1.5–450 mg/kg by means of Intraperitoneal (i.p.) injection [43, 45], and 1.5 mg/kg by means of tail vein injection [41]. A dose of 50 mg/kg through i.p. injection [38, 46] was the most frequently used dose for the tests.

The *in vivo* combination therapy revealed that administration of paclitaxel with ABZ did not show any synergistic effects in ovarian cancer cell (OVCAR-3)-bearing mice [43]. Since the application of ABZ alone also did not show any tumor growth inhibitory effect in the same study, the reduced anti-tumor effect due to the low bioavailability of ABZ might be the reason for the lack of synergistic effects. On the other hand, administration of 2-methoxyestradiol at a low concentration (25 mg/kg) of ABZ prolonged the survival of colorectal cancer cells (HCT-116)-bearing mice in a dose-dependent manner, compared to the lack of survival benefit when combined with a high concentration (50 mg/kg) of ABZ [38]. This was in line with the *in vitro* results shown in the interaction analysis between ABZ and 2-methoxyestradiol in the same study, which

exhibited synergism at low concentrations of ABZ, as compared to the antagonism at high concentrations of ABZ, when used in combination with 2-methoxyestradiol. The synergistic effect was not due to synergistic tubulin depolymerization of the two drugs, but induced apoptosis upon the combined use of the drugs. The detailed mechanism of antagonism at high ABZ concentrations has not been clarified.

II-2. Fenbendazole (FBZ)

II-2-1. *In Vitro* Anti-cancer Effects

FBZ has been reported to suppress cancer cells of the brain [47], breast [21], colorectal [48], lung [35, 49], pancreatic [25], and skin [21]. Like ABZ, it has been used to treat intracranial parasites in dogs [47]; thus, it can be utilized for treating brain cancer owing to its BBB-penetrating characteristics [47]. In case of lung cancer, Shimomura et al. observed that FBZ significantly suppressed KRAS-mutant lung cancer cells to a greater extent as compared to other benzimidazole derivatives, by suppressing RAS-related signaling pathways [35]. As effective medicines have not yet been developed for mutant KRAS, this finding might provide a valuable option for treating lung cancer with mutant

KRAS. In addition, FBZ showed enhanced apoptosis when it was tested in H460 and A549 human NSCLC cell lines having wild-type p53, compared to those with mutant p53, thus suggesting its important role in FBZ-induced apoptosis [49].

With respect to its use in combination with other medicines, FBZ did not show any effect on cellular radiosensitivity in EMT6 mouse mammary tumor cells, even though it shares a similar chemical structure to that of benzimidazoles having an effect as hypoxic cell radiosensitizers [50], such as ABZ, which shows radiosensitizing activity in lung and skin cancer cells [39, 40]. However, in the same study, it was revealed that FBZ, in combination with docetaxel, produced additive cytotoxicity in EMT6 cells.

II-2-2. *In Vivo* Anti-cancer Effects

Although the inhibitory effects of FBZ in cancer cells have been reported for several types of cancers in *in vitro* tests, its cancer inhibitory effects *in vivo* have been reported only in case of lung cancer (A549 adenocarcinoma cell xenografts) in mice. When it was administered p.o. at a dose of 1 mg/mouse every other day for 12 days, tumor growth and vascularity were reduced and apoptosis was induced in tumor cells [49]. In contrast, feeding a diet with FBZ during

monitoring of tumor growth from one week before inoculation of tumors to the time they reached 1000 mm^3 [51], or injecting the maximal concentration of FBZ, based on solubility, at a dose of 50 mg/kg/day i.p. on three consecutive days [50], did not influence breast cancer (EMT6) tumor growth in mice. Furthermore, FBZ did not affect the growth inhibitory effects of radiation when added in the abovementioned administrations [50, 51]. The reason that there have been few studies *in vivo* might be attributed to the restricted possibilities of FBZ for humans, owing to its regulatory approval only for veterinary application by the FDA. Thus, more studies are required to understand its anti-cancer effects.

II-3. Flubendazole (FLZ)

II-3-1. *In Vitro* Anti-cancer Effects

Among benzimidazole derivatives, FLZ has shown a suppressive effect on cell viability in a broad spectrum of cancer cell lines. In a study conducted by Michaelis et al., FLZ was evaluated for its inhibitory effect on a wide range of 321 cell lines of various cancer types [52]. In that screening study, FLZ showed a remarkable tumor cell inhibition effect on three kinds of cancers, including multiple myeloma, neuroblastoma, and leukemia/lymphoma, while the effect on

the other cancers was somewhat modest. The mechanism of the inhibitory effect on the three cancer entities was not reported in that study, but p53-mediated apoptosis has been reported to play an important role during tumor cell inhibition of FLZ in UKF-NB-3 neuroblastoma cells.

Notably, it has shown potential for use in breast cancer therapy. First, it is difficult to treat TNBC, because sufficient targeted therapies for it have not yet been found; however, FLZ elicited anti-tumor effects in TNBC, by suppressing cell migration [27, 53], inducing autophagy [54, 55], and influencing a number of mechanisms that inhibit breast cancer stem cell (BCSC)-like properties [27, 53] which are related to metastasis, recurrence, and drug resistance in breast cancer. Second, FLZ treatment significantly downregulated human epidermal growth factor receptor (HER) 2-related signaling in HER2-positive breast cancer and induced apoptosis in trastuzumab-resistant cell lines as well as in sensitive cell lines [56], which indicates its capability as a substitute or supplement for trastuzumab. Third, FLZ also suppressed BCSC-like properties in non-TNBC [53, 56]. Thus, these findings may shed light on further investigations on the application of FLZ as a potent drug for breast cancer treatment.

FLZ administration can enhance anti-cancer effects through a combination of several approved anti-cancer medicines. FLZ combined with conventional

chemotherapy drugs, fluorouracil or doxorubicin, exerted a more enhanced cytotoxic effect, in both cell viability and colony formation tests, in breast cancer cells (MDA-MB-231 and BT-549) [53]. Furthermore, FLZ also exhibited synergistic cytotoxic effects in cell viability tests conducted on three colorectal cancer cell lines (HCT116, RKO, and SW480), when combined with 5-fluorouracil [57], and in addition, potentiated the anti-tumor efficacy of paclitaxel in an HCT8 intestinal cancer cell line [37]. Lastly, Spagnuolo et al. observed that FLZ inhibited tubulin polymerization, similar to vinblastine, but bound to a binding site different from that of vinblastine, thereby showing a cytotoxic effect in synergism with vinblastine in an OCI-AML2 leukemia cell line; in addition, the cells resistant to vinblastine were suppressed by FLZ [58].

II-3-2. *In Vivo* Anti-cancer Effects

Some cancer cells in the brain [52], breast [27], colorectal [57], hematological [58], and skin [59] are susceptible to FLZ *in vivo*. Among the *in vivo* studies, one study used the chick chorioallantoic membrane assay [52], while most of them used mice as test animals, which were administered doses of FLZ ranging from 10 mg/kg to 200 mg/kg i.p [27, 57-59]. In concordance with the *in vitro* results, FLZ showed a remarkable anti-tumor effect on neuroblastoma [52], via

inhibition of tumor growth and vessel formation in a chorioallantoic membrane assay for brain cancer cells (neuroblastoma xenograft) [52]. Furthermore, consistent with the *in vitro* results, it was also observed that FLZ can be effective for breast cancers encompassing TNBC [27, 53, 55] showing delayed tumor growth or anti-migration activity, such as decrease in matrix metalloproteinase-2, and trastuzumab-resistant xenografts in HER2-positive breast cancer [56]. With respect to combination therapy, one study showed that use of FLZ in combination with vinblastine or vincristine caused more effective suppression, as compared to the administration of either drug alone, in a leukemia xenograft test [58].

II-4. Mebendazole (MBZ)

II-4-1. *In Vitro* Anti-cancer Effects

Similar to FLZ, MBZ showed extensive inhibitory effects on a wide range of cancer cell lines. FBZ has also been suggested to be a useful therapy for brain cancer, based on its BBB-penetrating characteristics [19, 47, 60-63]. It was observed that MBZ could efficiently reduce BCSC-like cells in TNBC and also interfere with the reprogramming of breast cancer cells into BCSCs, which are

known to be induced after radiation therapy [64]. In head and neck squamous cell carcinoma (HNSCC) and acute myeloid leukemia (AML) [65], which are both aggressive types of cancers, MBZ showed a potent inhibitory effect. Especially, the anti-tumor effect of MBZ on HNSCC (CAL27 and SCC15) was more potent than that of cisplatin [66]. Proliferation of cancer cells was prominently suppressed at lower concentrations of MBZ than those of cisplatin, in both the HNSCC cell lines. Anti-tumor effects of MBZ are also related to inhibition of drug resistance. MBZ downregulated the expression of multiple drug resistance (MDR) genes (*ABCB1*, *ABCC1*, and *SLC47A1*) in malignant ascites cells [67]. In T cell acute lymphoblastic leukemia (T-ALL), MBZ was effective in suppressing the growth of cancer cell lines despite their chemoresistance, as shown in the test results that it inhibited camptothecin-resistant and MDR-1-overexpressing CEM/C1 cells [68]. Based on these activities, MBZ may be a potential adjuvant therapy for conventional anti-cancer treatments, to prevent drug efflux.

MBZ has been identified as a leading anti-cancer compound by screening established libraries in several studies. In a study conducted by Tan et al., upon screening 1,448 molecules using comparative modeling studies, MBZ was discovered to be a TRAF2- and NCK-interacting kinase (TNIK) inhibitor. Since

TNIK activates the Wnt/β-catenin/T-cell factor 4 pathway and its activation contributes to the transformation of cells to cancer cells, particularly colorectal cancer, it can be applied to Wnt-activated colorectal cancer [69]. In another study by Li et al., who used their own computational tool, MBZ was also identified as one of the top 20 molecules inducing differentiation of HL-60 leukemia cells, upon analyzing gene expression profiles, including myeloid markers of leukemia cells, after exposure to 1,235 molecules [70]. Moreover, in several screenings, MBZ showed a potent inhibitory effect on AML cell lines [65], melanoma cells [71], and crucial kinases in both types of cancers, $BRAF^{WT}$ and $BRAF^{V600E}$ [72]. The dominant anti-tumor effects of MBZ, which were revealed in various screening tests, suggest its potential for various uses in anti-cancer therapy.

Finally, there are more number of studies on combination therapies of MBZ with already in-use conventional drugs than on any other benzimidazole groups. The first case was a combination of MBZ with temozolomide, which is a standard therapy for glioblastoma multiforme, or with temozolomide and vinblastine as a triple combination; both combinations showed enhanced cytotoxicity than that for temozolomide alone [19, 60]. Three studies reported that MBZ sensitized cancer cells to ionizing radiation, through the mechanism of

inhibiting DNA damage response proteins in glioma cells [17] and promoting cancer cell apoptosis in meningioma [62] or TNBC cells [64]. In addition, the combination use of MBZ with gemcitabine, 5-fluorouracil, cisplatin, and docetaxel displays enhanced anti-cancer effects than those seen upon the use of the drug alone, in breast cancer [73], gastric cancer [74], HNSCC [66], and prostate cancer [75], respectively. In addition, MBZ showed potent anti-cancer effects in combination with trametinib, an mitogen-activated protein kinase (MEK) inhibitor, in $NRAS^{Q61K}$ melanoma, thereby highlighting its potential to be used in combination with trametinib [72].

II-4-2. *In Vivo* Anti-cancer Effects

Evidence of tumor-suppressive effects of MBZ have also been found consistently in *in vivo* tests for challenging cancers such as brain cancer [19, 61-63, 76, 77], TNBC [64], HNSCC [66], chemoresistant T-ALL [68] and AML [65]. In murine hepatocellular carcinoma, MBZ treatment resulted in outstanding effects encompassing not only inhibition of tumor growth and angiogenesis but also improved liver function and histology [78]. Moreover, MBZ showed the possibility of a new strategy for chemoprevention in a familial adenomatous polyposis model using $APC^{Min/+}$ mice, by reducing the number of

polyps and tumor formation, which can eventually contribute to suppressing the initiation of colorectal cancer [79]. Most of the *in vivo* tests were conducted using mice, where the doses of MBZ were 1–2 mg/mouse upon administration by means of p.o. [80, 81], 25–100 mg/kg by means of p.o. [65, 76], 7.5–100 mg/kg by means of i.p. injection [66, 68], and 180 mg/kg by means of tail vein injection [75]. The most frequently selected administration was 50 mg/kg by means of p.o. [19, 70, 77]. It should be noted that MBZ was administered orally in most *in vivo* studies, whereas the other benzimidazoles, except OBZ, were administered as injections. Considering that the most used doses in the *in vivo* studies are decided on the basis of previous *in vivo* results and *in vitro* data, it can be assumed that these doses and the use of oral application in these *in vivo* tests were regarded as sufficient to reach the required concentrations for the anti-cancer effects of MBZ *in vivo*. Although the data regarding this is limited, the reported bioavailability for MBZ in humans has been given as '5–10%' and '17-22%', which are higher than the reported bioavailability for ABZ in humans, which is '1–5%' [82]. It is assumed that the higher bioavailability of MBZ than that of the other benzimidazoles might be the reason for its availability for oral application *in vivo*.

Combination therapies tested for MBZ *in vivo* are described below. When

MBZ was used with radiation, enhanced inhibition of tumor growth was observed, compared to the use of radiation alone in TNBC [64] or MBZ alone in a rodent model of meningioma [62]. When MBZ was applied in combination with sorafenib [78], docetaxel [75], and trametinib [72], the effects of enhanced anti-cancer efficacy and prolonged survival of tumor-bearing mice were observed, as compared to the use of MBZ alone, in hepatocellular carcinoma, prostate cancer, and melanoma, respectively. Finally, MBZ can also be used to develop a new strategy for cancer therapy. MBZ suggests a new modality for chemoprevention in a familial adenomatous polyposis model, by improving the cancer-preventive effects above those exhibited by sulindac alone, when used in combination with it, resulting in a reduction in the number and size of polyps and microadenoma formation, through its anti-angiogenic activities and heightened anti-inflammatory effects [79]. This highlights the possibility of using this combination to prevent polyps from transforming into colorectal cancers. It seems that MBZ is in a better position for drug development than other benzimidazoles, owing to its advantages, such as relatively extensive preclinical studies and more useful application routes.

II-5. The Others

II-5-1. *In Vitro* Anti-cancer Effects

CBZ is a metabolite of benomyl that is used as a fungicide, unlike other benzimidazoles. Several studies have shown its anti-tumor activities against breast [24, 83, 84], colorectal [85], and liver [29] cancer cell lines; however, most of the studies on CBZ have been conducted in breast cancer cells. CBZ exerted more enhanced tumor cell inhibitory effects in MCF-7 breast cancer cells when it was used in combination with astaxanthin, a potent anti-oxidant, than when it was used alone, despite the controversy surrounding the combination of anti-oxidants with chemotherapeutics [83].

In case of MTZ, one study showed its anti-tumor effects in lung cancer cells, where it was identified to be selectively effective against KRAS-mutant lung cancer cells, as compared to wild-type cells, in screening tests carried out using 1271 small molecules; the selectivity of MTZ was more obvious than that of other benzimidazoles [35]. When MTZ was used in combination with trametinib, a MEK inhibitor, a synergistic cytotoxic effect was observed in KRAS-mutant lung cancer cells [35].

To date, NCZ has shown inhibitory effects against two types of cancer cell lines: colorectal [85] and lung [23, 35] cancer. Although the number of studies was insufficient, NCZ showed potent anti-tumor effects among the

benzimidazoles in two studies. In a study on colorectal cancer cell lines (RKO and HCT-116), NCZ was shown to be one of the two compounds with the lowest IC_{50} values among the seven benzimidazoles tested [85]. In another study on NSCLC, the depolymerization of tubulin and abnormal spindle formation, which are assumed to be the key factors determining the progress of apoptosis, were greater with NCZ than with MBZ [23]. When NCZ was treated with an inhibitor of heat shock factor (HSF) 1 or the MEK/extracellular signal-regulated kinases (ERK) pathway, it exerted higher cytotoxicity than NCZ alone, thereby lessening the chemotherapeutic resistance promoted by ERK-1/2-dependent HSF1 in colorectal cancer cells [85].

The anti-tumor effects of OFZ in colorectal [85] and lung [86] cancer cells have been reported. In A549 and H1299 NSCLC cell lines [86], OFZ inhibited cancer cell proliferation, and this inhibitory effect was related to the suppression of c-Src signaling, which is known to mediate cell proliferation. OFZ repressed cancer cell viability against NSCLC cell lines more effectively in combination with cisplatin, by enhancing inhibition of c-Src activation and upregulation of p53 [86].

OBZ has shown anti-proliferative effects in lung [35], pancreatic [25], colorectal [48], skin [71], and prostate [30] cancers. Shimomura et al. showed

that benzimidazole derivatives, including OBZ, suppressed KRAS-mutant lung cancer cells, but were not as effective as MTZ and FBZ [35]. In two types of pancreatic cancer cell lines (AsPC-1 and Capan-2), OBZ inhibited cell viability following PBZ, among the four benzimidazoles tested [25]. In a study conducted by Nygren et al., a benzimidazole group including OBZ was identified as one of several distinct clusters that were effective in suppressing tumor cell survival in HCT 116 and RKO colorectal cancer cell lines, upon screening of 1,600 molecules [48]. In addition, OBZ was found to be one of the 10 compounds that identified tumor-inhibitory effects upon screening of 2,000 compounds against M-14 and SK-Mel-19, two melanoma cell lines [71]. Research on prostate cancer cells (22Rv1 and PC-3) showed that the anti-tumor mechanisms of OBZ increased the expression of two well-known tumor suppressors, microRNA (miRNA)-204 and p53 [30].

RBZ is a metabolite (albendazole sulfoxide) of ABZ that shows anti-proliferative effects against a TNBC cell line (4T1) [34], as well as breast (MCF-7) [87], lung (NCI-H460) [87], and skin (A375-C5) [87] cancers; however, its effects were found to be milder than those of ABZ [34, 87]. In addition, RBZ effectively suppressed colorectal cancer cells (HT-29) [88], but was not effective at any concentration when tested in four colon cancer cell lines

(SW480, SW620, Caco2, and HCT8) [37]. Few studies have investigated the anti-tumor mechanisms of RBZ.

PBZ has shown anti-tumor effects in colorectal [85], lung [35], and pancreatic [25] cancer. In a study on colorectal cancer cell lines (RKO and HCT-116), PBZ was shown to be one of the two compounds with the lowest IC_{50} values, among a total of seven benzimidazoles tested [85]. Remarkably, it exerted the most potent cytotoxicity among the four benzimidazoles tested against pancreatic cancer [25]. In contrast, the anti-tumor effect of PBZ was not stronger than that of six other benzimidazoles tested, in the Z-score analysis for growth inhibition of KRAS-mutant and wild-type lung cancer cell lines, upon screening of 1271 compounds, where it was identified as one of 50 top-ranking compounds [35]. Similar to NCZ treatment, PBZ showed enhanced cytotoxicity and reduced chemotherapeutic resistance through ERK1/2-dependent HSF1 in colorectal cancer cells [85]. In addition, the inhibitory effect of PBZ was synergized when combined with gemcitabine, against pancreatic cancer cells (AsPC-1 and Capan-2) [25].

II-5-2. *In Vivo* Anti-cancer Effects

Only one study has reported the *in vivo* anti-tumor effects of the seven

benzimidazoles, in which OBZ (25 mg/kg p.o., in mice) was shown to increase the expression levels of miRNA-204 and p53, in addition to exerting repressing effects on androgen receptors and prostate-specific androgens in prostate 22Rv1 tumors [30].

III. Clinical Properties of Benzimidazole Anthelmintics

III-1. Clinical Evidences

Limited clinical evidence has been documented for benzimidazole anthelmintics, with most of it restricted to only three types of benzimidazoles: ABZ, CBZ, and MBZ (Table 1). For ABZ, one phase 1 clinical trial [89] and one pilot study [90] have been conducted. In both the studies, it was demonstrated that ABZ has modest anti-tumor effects, including reduction of tumor markers, and a well-tolerated safety profile; however, dramatic effects such as complete recovery or survival prolongation have not been reported. For CBZ, one phase 1 trial (NCT00003709) has been completed, but the results of the same cannot be found at ClinicalTrials.gov (https://clinicaltrials.gov/) or in any research article. Lastly, there are two case reports that present the anti-cancer activities of MBZ. In these case reports, which aimed to treat adrenal cancer [91] and metastatic colon cancer [92], metastases regressed without any significant adverse effects, upon treatment with MBZ. In particular, a man with adrenocortical carcinoma showed stable disease status for 19 months, during the application of MBZ. Meanwhile, although there are relatively many clinical trials being conducted on MBZ, most of them are scheduled to be completed after June 2022. Possible

reasons for many trials being conducted on MBZ might be its preclinical study history and dose convenience, since it had been approved for human use by the FDA and can be applied orally because of its relatively higher bioavailability. Consistent with the preclinical study of its anti-tumor efficacy, owing to its BBB-penetrating ability [19, 47, 60-63], three of the eight trials dealt with brain tumors (NCT01729260, NCT02644291, and NCT01837862). Of note, one phase 2a trial (NCT03628079) conducted on 11 patients with advanced cancer of the gastrointestinal or unknown origin was terminated earlier than planned because of a lack of effect. Moreover, the six recent trials that are currently ongoing (NCT04443049, NCT01729260, NCT01837862, NCT02366884, NCT03925662, and NCT02201381) have tested its anti-cancer effects in combination with other drugs, which might suggest weak anti-cancer effects of MBZ as a monotherapy and potential uses for synergizing effects with other drugs, as evidenced in the preclinical data.

Clinical evidence reveals that ABZ was administered at a dose of 10 mg/kg/day p.o., with two or three divided doses, in a pilot study [90]. In a phase 1 trial conducted in 36 patients, the maximum tolerated dose was 1,200 mg twice daily (b.i.d.) p.o. (2,400 mg/day) [89]. MBZ was administered at a dose of 100 mg b.i.d. p.o. in two case reports [91, 92], while no exact maximal tolerated

dose can be found in clinical trials, owing to the lack of reporting of results from these trials. All clinical evidences of benzimidazole drugs indicate that they were administered orally. ABZ has also been reported to be well tolerated in two studies [89, 90]. Mainly, fatigue and mild gastrointestinal upset were reported after ABZ treatment [89]; however, in some patients, hematologic adverse events such as myelosuppression [89] or neutropenia [90] were also observed. In two case reports related to MBZ, each of which described one person, no significant adverse effects were described [91, 92], but up to five-fold increases in levels of liver enzymes (aspartate aminotransferase and alanine aminotransferase) were detected in one patient [92]. Six clinical studies for the evaluation of benzimidazole anthelmintics as anti-tumor agents are currently ongoing, the results for which must be followed up on.

Table 1. Anti-cancer clinical evidences for benzimidazoles

Drug	Stage	Cancer type	Number of patients	Methods	Adverse effects	Results	Idendifier/ Ref.
Albendazole	Phase 1	Refractory solid tumors	36	Every day for 2 weeks, followed by 1 week of rest. Treatment was repeated in a 21-day cycle. 400–1,200 mg b.i.d. p.o.	ABZ was well tolerated. Fatigue and mild gastrointestinal upset (Major). Myelosuppression.	16% of patients showed a decrease in levels of tumor markers. Plasma VEGF level decreased in the first 8 h after ABZ administration.	[89]
Albendazole	Pilot Study	Colorectal cancer or hepatocellular carcinoma	7	10 mg/kg/day, with 2 or 3 divided doses p.o. (28 d). The maximum tolerated dose was 1,200 mg b.i.d.	ABZ was well tolerated. Severe neutropenia in three patients.	CEA decreased in two patients. CEA or α-feto protein stabilized in three patients.	[90]
Carbendazim	Phase 1	Unspecified adult solid tumor	25	P.o weekly for 3 consecutive weeks, followed by 1 week of rest. Treatment repeated in a 28-day cycle. Determining dose.	No results posted.	No results posted. Actual study completion date: November 2000	NCT0000 3709
Mebendazole	Case report	Adrenal cancer	1	100 mg b.i.d. p.o. for 19 months.	No significant adverse effects.	Metastases regressed. The patient's disease remained stable for 19 months, but showed progression after 24 months.	[91]
Mebendazole	Case report	Refractory metastatic colon cancer	1	100 mg b.i.d. p.o. for six weeks.	AST and ALT were increased up to >five times above the normal limit.	The metastases in the lungs and lymph nodes were near completely remissioned. A good portion of those in the liver were remissioned.	[92]

Mebendazole	Not applicable	Advanced hepatocellular carcinoma	170 (recruiting)	100 mg b.i.d. p.o. in combination with lenvatinib.	No results posted.	No results posted. Estimated study completion date: June 19, 2022	NCT04443049
Mebendazole	Phase 1	High-grade glioma	24	T.i.d. p.o. in a 28-day cycle, in combination with temozolomide. Determining dose.	No results posted.	No results posted. Actual study completion date: April 16, 2021	NCT01729260
Mebendazole	Phase 1	Recurrent pediatric brain cancers	21 (recruiting)	T.i.d. p.o. Determining dose.	No results posted.	No results posted. Estimated study completion date: June 2022	NCT02644291
Mebendazole	Phase 1/2	Pediatric gliomas	36 (recruiting)	50–200 mg/kg/day divided twice p.o., in combination with standard anti-tumor drugs	No results posted.	No results posted. Estimated study completion date: April 2023	NCT01837862
Mebendazole	Phase 2a	Advanced gastrointestinal cancer or cancer of unknown origin	11 (Terminated due to lack of effect)	50–4,000 mg b.i.d. p.o. for 16 weeks. Determining dose.	No results posted.	No results posted. Actual study completion date: January 16, 2019	NCT03628079
Mebendazole	Phase 2	Incurable and lethal Cancers	250 (recruiting)	Tolerable and safe doses for 10 to 12 months. Combination of two anti-protozoal drugs.	No results posted.	No results posted. Estimated study completion date: December 31, 2023	NCT02366884
Mebendazole	Phase 3	Colorectal cancer	40 (recruiting)	Folfox with avastin and MBZ.	No results posted.	No results posted. Estimated study completion date: December 2028	NCT03925662
Mebendazole	Phase 3	Cancer	207 (Not yet recruiting)	100 mg q.d. in combination with atorvastatin, metformin, and doxycycline.	No results posted.	No results posted. Estimated study completion date: September 22, 2026	NCT02201381

Abbreviations: b.i.d: twice daily; p.o.: oral administration; ABZ: albendazole; VEGF: vascular endothelial growth factor; CEA: carcinoembryonic antigen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; t.i.d.: three times a day; q.d.: once daily.

III-2. Pharmacokinetic Properties

There is limited pharmacokinetic data on the use of benzimidazole anthelmintics for humans, even with respect to ABZ, FBZ, FLB, MBZ, and OFZ (Table 2). The known common fact regarding the pharmacokinetic properties of benzimidazoles is that they are poorly soluble in water, which is the main reason for their low absorption and bioavailability [82, 93-95]. In addition, dietary fat can substantially increase the absorption of benzimidazoles [82, 95-98]. These five benzimidazoles are metabolized by first-pass metabolism [93, 95, 97, 99].

However, details of the pharmacokinetic aspects differ depending on the benzimidazole. First, the metabolisms of ABZ and FBZ shared similar patterns, but that of MBZ is somewhat different. The main metabolic products, fenbendazole sulfoxide (FBZSO) and its sulfone derivative ($FBZSO_2$), are produced upon first-pass metabolism of FBZ, while albendazole sulfoxide (ABZSO) and its sulfone derivative ($ABZSO_2$) are produced through sequential oxidation upon first-pass metabolism of ABZ [99]. The metabolism of both ABZ and FBZ is carried out by cytochrome P450 and flavin-monooxygenase [82], and the first metabolite of each, ABZSO and FBZSO, respectively, has two enantiomers in human plasma [99]. In contrast, MBZ is metabolized by extensive first-pass metabolism into many unidentified metabolites, and it is

unclear which enzyme(s) carries this out [82]. In addition to metabolic pathways, metabolic rates also vary depending on each benzimidazole. ABZ is known to be metabolized by very rapid first-pass metabolism ($T_{1/2}$<1.5 h) [100], compared to those of other benzimidazoles ($T_{1/2}$ of FLZ in tissue, 1–2 d [95]; $T_{1/2}$ of MBZ, 3–6 h [13]; and $T_{1/2}$ of OFZ, 8.5–11 h [93]). In terms of excretion, ABZ, FBZ, MBZ, and their metabolites are eliminated in the feces and urine [82, 96, 99]. FLZ [95] and MBZ [96] have been reported to be mostly excreted in the feces. In addition, the excretion route of OFZ has not been clearly described [100].

The most important point that should be considered when judging whether benzimidazoles can actually exhibit anti-cancer activities, as has been shown in a number of preclinical tests, is whether they can maintain the effective concentrations consistently in the bloodstream of human bodies. Based on several pharmacokinetic data, the maximum concentration (C_{max}) of each benzimidazole was as follows: ABZ; 0.047–0.1 μM at a dose of 400 mg [100]; FLZ, 0.016 μM at a dose of 2 g [95]; MBZ, 0.47 μM at a dose of 10 mg/kg [97]; and OFZ, 21.5 μM at a dose of 60 mg/kg [93]. When the C_{max} of each benzimidazole was compared to the IC_{50} values obtained in a number of *in vitro* tests [13], the C_{max} values of ABZ and FLZ at those doses were regarded as lower, to exert effective anti-cancer effects on a variety of cancer cells, except for a small number of cells. Furthermore, since ABZ is rapidly metabolized into

its main metabolites [94, 96, 101], the $T_{1/2}$ of ABZ was observed to be less than 1.5 h [100]. This rapid metabolism may also be an obstacle to the use of this drug as an effective option for cancer treatment. To repurpose benzimidazole anthelmintics as anti-cancer medicines, the major challenge would be improving their bioavailability, by developing new formulations for better solubility, absorption, and longer half-life, to achieve effective concentrations for enough time.

Table 2. Pharmacokinetic properties of benzimidazoles

Drug	Absorption	Distribution	Metabolism	Excretion	Ref.
Albendazole	· <5% · Poor solubility, as well as low absorption and bioavailability. · High inter-variabilities of peak levels. · A dose of 400 mg p.o. led to a C_{max} of 0.16–1.58 mg/L for ABZSO. · T_{max} of ABZ was <2–3 h. · Fat in the diet increased the absorption up to 6.5-fold. · T_{max} of ABZSO was 4.75 h. · C_{max} of ABZSO was 1.20 ± 0.44 μg/mL. · C_{max} of ABZ was 12.5 [0.047 μM] to 26.5 ng/mL [0.1 μM].	· ABZSO was widely distributed. About 70% of ABZSO was bound to plasma proteins, whereas about 90% of ABZ was bound to them. · ABZSO crossed the BBB. · ABZSO enantiomers were distributed about two-fold higher in the plasma than in the cerebrospinal fluid, in humans. · When treated with 400 mg ABZ, a small amount of ABZ was detected in the serum from 2–8 h after administration. · ABZSO was detected until 72 h in the blood.	· ABZ is metabolized to ABZSO by very rapid first-pass metabolism, and finally to ABZ sulfone through further conversion. · Metabolism is carried out by cytochrome P450 and other oxidases, including flavin-monooxygenase. · ABZSO has two enantiomers in the human plasma. (+)-ABZSO is the predominant enantiomeric form in the human plasma. · Increased CYP1A expression can cause auto-inductive effect of ABZ, upon repeated administration of ABZ.	· $T_{1/2}$ of ABZSO is 8–14 h. · $T_{1/2}$ of ABZ is <1.5 h. · ABZ and its metabolites are excreted in the urine and feces. · ABZSO is excreted in the urine quickly, from 4–72 h after administration. · ABZ concentrations are too low to measure in the urine.	[82, 94, 96, 99-101]
Fenbendazole	-	-	· FBZ is metabolized to FBZSO by first-pass metabolism, and finally to FBZ sulfone by means of further conversion. · Metabolism is carried out by cytochrome P450 and flavin-monooxygenase. · FBZSO has two enantiomers in the human plasma.	· FBZ and its metabolites are excreted in the urine and feces.	[99]

Flubendazole	· Poor solubility as well as low absorption and bioavailability. · A dose of 2 g p.o. led to a C_{max} that was lower than 5 ng/mL [0.016 µM] for FLZ. · Administration after a meal increases absorption.	-	· Initial biotransformation takes place through first-pass metabolism.	· FLZ is excreted in the feces (more than 80%) and urine. · $T_{1/2}$ in tissues is 1–2 d.	[95]
Mebendazole	· 5%–10% and 17%–22% · poor solubility. · Fat in the diet increased the absorption more than 5-fold. · C_{max} of MBZ was 137.4 ng/mL [0.47 µM], at a dose of 10 mg/kg. · T_{max} of MBZ was 2–4 h. · High inter-variabilities of peak levels.	· 90%–95% of it existed as bound to plasma proteins.	· MBZ is metabolized by extensive first-pass metabolism to many unidentified metabolites. · It is unclear which enzymes carry out this metabolism.	· MBZ and its metabolites are excreted in the feces and urine. · $T_{1/2}$ is 3–6 h.	[13, 82, 96, 97]
Oxfendazole	· Poor solubility, but higher than that of ABZ or FBZ. · C_{max} of OFZ was 6770 ng/mL [21.5 µM], at a dose of 60 mg/kg. · T_{max} of OFZ was 2–3 h. · Fat in the diet increased the C_{max} by 49%, and AUC by 86%.	-	· OFZ is metabolized to OFZ sulfone, FBZ, OFZ sulfate conjugates, and OFZ glucuronide conjugates.	· Minimal amount (<1% of dose) of OFZ is excreted in the urine. · $T_{1/2}$ is 8.5–11 h.	[93, 98]

Abbreviations: ABZ: albendazole; ABZSO: ABZ sulfoxide; T_{max}: time to peak drug concentration; C_{max}: maximum concentration; BBB: blood-brain barrier; FBZ: fenbendazole; FBZSO: FBZ sulfoxide; p.o.: oral administration; FLZ: flubendazole; $T_{1/2}$: half-life time; MBZ: mebendazole; OFZ: oxfendazole; AUC: area under the concentration-time curve.

IV. Discussion

Microtubule disruption has been regarded as one of the targets for cancer treatment since a long time [17]. As benzimidazole anthelmintics exert their anti-parasitic effect by disrupting microtubule polymerization, through binding with β-tubulin [18, 19], it might stand to reason that benzimidazoles display anti-proliferative effects in cancer cells. In fact, anti-cancer and anti-growth effects of benzimidazoles have been observed serendipitously upon their use as an anti-parasitic during tests in animals [19, 47, 76], with the anti-cancer efficacy of the benzimidazole group demonstrated in a number of *in vitro* and *in vivo* studies. Furthermore, its predominant cancer-suppressing activities compared to those of other compounds have also been demonstrated in screening tests [35, 48, 52, 65, 69, 71, 72]. The anti-cancer mechanisms of benzimidazoles have not been clearly elucidated, but multiple mechanisms have been identified for the same, which could possibly contribute to their cancer-suppressing effects. As shown in Figure 1, the effects are mainly mediated through disruption of microtubule polymerization [20-25], induction of apoptosis [18, 25-30], or inhibition of angiogenesis [31, 32] and metastasis [18, 25, 32, 33], and as recently reported, autophagy induction [18, 54, 55, 57, 102], glycolysis suppression [31, 49], immune system modulation [103], and cancer stemness inhibition [104]. In addition, it has also been revealed that this

chemical group displays advantages in suppressing not only tricky cancers such as TNBC [18, 27, 34, 53, 55, 64], brain cancer [19, 41, 47, 52, 60-63, 76, 77], and KRAS-mutant lung cancer [35], but also chemo-resistant cancer cells [56, 58, 67, 68] in preclinical studies, with the possibility of synergizing with established conventional therapies, including radiation [25, 35, 37, 39, 40, 57, 58, 72]. Therefore, expectations of repurposing this group as a cancer treatment have increased in recent years.

However, despite all these positive results in terms of anti-cancer effects, there is limited anti-cancer data in clinical evidence. One phase 1 clinical trial [89] and one pilot study [90] reported modest anti-tumor effects of ABZ, such as a reduction in the levels of tumor markers. In two case reports, metastases regressed after treatment with MBZ, in adrenal cancer [91] and metastatic colon cancer [92], and adrenocortical carcinoma did not progress for 19 months during the application of MBZ [91]. There is no study that reports an outstanding anti-tumor effect of these compounds in a massive population.

The current study attempted to understand why the anti-cancer effects were not remarkable in actual clinical settings. First, after reviewing a variety of pre-clinical studies, it was determined that even though many factors and pathways related to the anti-cancer effects of benzimidazoles have been identified, the comprehensive mechanisms or the exact main target(s) resulting in these anti-

cancer activities have not been completely clarified. Therefore, drug development for repurposing benzimidazoles as a cancer treatment inevitably faces certain uncertainties at this point of time, such as difficulties in patient selection during a clinical trial. Clinical trials related to application of benzimidazole for cancer treatment that have been conducted until now have recruited participants with mostly brain cancer (NCT01729260, NCT02644291, and NCT01837862), colon cancer (NCT03925662), or solid tumors without detailed classifications ([89], NCT00003709, NCT02366884, and NCT02201381). Clarification of the precise anti-cancer mechanism and the main targets would help narrow down the subject participants.

Second, the current study tried to understand the properties of the anti-cancer effects of benzimidazoles, to gain an answer to the low efficacies observed in clinical evidence, and identified several factors. Based on the preclinical results, the efficacies of benzimidazoles were revealed to be very different, depending on the different cancer cell lines and benzimidazole types. More specifically, in three screening studies [35, 48, 71] that tested against melanoma (M-14 and SK-Mel-19), K-RAS-mutant lung cancer (A-549, H-23, and H-1573), and colon cancer (HCT116 and RKO) cell lines, several benzimidazoles showed inhibitory effects on cancer cells, but the levels of these effects differed depending on the benzimidazole type. The benzimidazoles that exerted the most effective

suppression were MBZ against melanoma, MTZ and FBZ against K-RAS-mutant lung cancer, and OBZ and MBZ against colon cancer. In a study conducted by Králová et al., similar results were observed. ABZ and FLZ exhibited very high inhibition of colon cancer cell lines (SW480, SW620, Caco2, and HCT8), while RBZ was ineffective [37]. Thus, a specific benzimidazole does not seem to have a predominance of anti-cancer effects. Moreover, it was found evidence of the anti-cancer effects of benzimidazoles in an extensive study conducted by Michaelis et al. [52]. In this study, researchers tested the inhibitory effects of FLZ on 321 cancer cell lines with 26 cancer entities. They found that FLZ displayed an IC_{90} of less than 5 μM for all the 26 cancer entities. Above all, three entities, myeloma, neuroblastoma, and leukemia, showed high sensitivity to FLZ, with on an average IC_{90} of less than 1 μM, which was demonstrated to be achievable in mice, while only 117 (36%) of the total 321 cell lines displayed an IC_{90} of less than 1 μM. Based on this, it was determined that the anti-cancer effects of FLZ also depended on cancer entities. Cell line dependency has also been reported in the anti-cancer effects of paclitaxel, in which the mechanism of cytotoxicity was revealed to be by means of upregulating death receptor 5, thereby activating the extrinsic pathway of apoptosis in prostate cancer cell lines, but not in NSCLC or breast cancer cell lines [38]. These results indicate that differences in intracellular signal

transduction pathways between cell lines may cause cell line dependency. To confirm these observations and extend this idea to the anti-cancer effects of other benzimidazoles, extensive screening of other benzimidazoles should be performed in the near future.

This characteristic of variation in the anti-cancer effects on the basis of the type of cancer entities, cell lines, or benzimidazoles use, affects the results of the clinical trials as well. Because of this, benzimidazoles might have limitations in exerting effective suppression on extensive cancer entities or even a cancer entity with various cell lines, in clinical trials, unless their concentrations in the bloodstream are increased high enough to inhibit a wide range of cancer cells. Although various benzimidazoles have shown anti-tumor activities in many preclinical studies, a sufficient level of efficacy should be demonstrated in a large number of participants, through trials, in order for them to be developed as an anti-cancer therapy. Therefore, when planning a clinical trial, the experimental group could be specifically restricted to participants with a cancer type that has been demonstrated to be susceptible to the subject benzimidazole, or alternatively, a benzimidazole type that has already revealed its anti-cancer effects at relatively low concentrations could be selected; in either case, conducting trials on a large scale can be a good development strategy to help increase the effectiveness of the approach. In order to acquire information for

the determination of the abovementioned matters, more extensive preclinical data should be collected for benzimidazoles.

Finally, the last obstacle in obtaining prominent anti-cancer effects of benzimidazoles is their low bioavailability. As explained in terms of the pharmacokinetic properties, the C_{max} values for ABZ (0.047–0.1 μM at a dose of 400 mg one-time [100]) and FLZ (0.016 μM at a dose of 2 g one-time [95]) were regarded lower, as compared to the IC_{50} values observed in various *in vitro* tests [13]. In a pilot study and a phase 1 trial that reported the anti-cancer effects of ABZ, ABZ was administered at a dose of more than 400 mg, that is, 10 mg/kg/day with two or three divided doses p.o. [90], and 400–1,200 mg b.i.d. p.o. [89], respectively. As such, because of the poor solubility and absorption of benzimidazoles [82, 93-95], or rapid metabolism of ABZ [94, 96, 101] can also be attributed to their low anti-cancer efficacy in clinical settings, there is a need for various attempts using different excipients or novel formulation technologies, to increase the solubility and absorption of these compounds. In addition, in all the clinical evidence in Table 1, the benzimidazoles were administered orally, but administration by means of injection could also be considered for better efficacy, considering that most of *in vivo* tests for benzimidazoles, other than those for MBZ, did not use oral applications.

Part II. Experience with and Perceptions of Anthelmintics for Cancer Treatments among Cancer Patients in South Korea: A Cross-sectional Survey

I. Introduction

The term “drug repurposing” or “drug repositioning” refers to the idea that confers a novel and potential use of a drug that has been previously developed or approved for a specific clinical purpose [105]. In general, drug repurposing occurs when a particular disease has few remedies, and their limited treatment availability is coupled with their exceedingly high demand. This phenomenon is very common in the field of medicine, and can be attributed to the long period and high cost requirement of drug development [12, 106]. If previously existing and approved drugs can be made available in a shorter time period through reduced clinical trials, no process validations, and stability tests, patients can rapidly access an additional treatment option at a reasonable price.

Currently, drug repurposing is being studied as a novel strategy in various areas of drug development [105] including COVID-19, cardiovascular diseases, pulmonary arterial hypertension, and cancer. In recent years, drug repurposing

for COVID-19 therapies has increased rapidly, owing to publishing of more than hundreds of studies every year. Four major groups of drugs are being developed through drug repurposing for COVID-19 treatment: antivirals (lopinavir/ritonavir, oseltamivir, and remdesivir), immunosuppressors (eculizumab, dexamethasone, and budesonide), immunomodulators (camostat, interferons, and sargramostim) and other well-known drugs (azithromycin, doxycycline, and nitazoxanide) [107]. One example of drug repurposing is the use of antidiabetic drugs, such as sodium-glucose cotransporter 2 inhibitor (dapagliflozin), and glucagon-like peptide-1 receptor agonists (liraglutide and semaglutide) as potential cardiovascular drugs [108]. These medications were found to reduce the risk associated with cardiovascular disorders in people with or without diabetes. Another successful example of repurposing include the use of bosentan, iloprost, and sildenafil for the treatment of pulmonary arterial hypertension, which often causes serious outcomes [109].

Since there is no non-toxic, and effective standardized medications for cancer, numerous studies on drug repurposing are progressing in the field of cancer therapeutics as well. Zhang et al. grouped cancer therapeutic repurposing drugs into 10 groups based on their anti-cancer potential to inhibit the following cancer hallmarks: sustaining proliferative signaling (e.g., rapamycin and prazosin), evading growth suppressors (ritonavir, etc.), withstanding cell death

(artemisinin, etc.), inducing replicative immortality (curcumin, etc.), genome instability and mutation (mebendazole, etc.), reprogramming energy metabolism (metformin, etc.), inducing angiogenesis (itraconazole, etc.), activating invasion and metastasis (niclosamide, etc.), tumor-promoting inflammation (aspirin, etc.), and evading immune destruction (infectious disease vaccines) [12].

Recently in South Korea, there was huge controversy regarding the anti-cancer potential of anthelmintics when a man named Joe Tippens claimed to have completely cured his lung cancer by taking a dog deworming drug (communicated through YouTube in 2019), the active pharmaceutical ingredient of this drug being fenbendazole. [14] The treatment regimen of Joe Tippens was as follows [110]: ① curcumin 600 mg per day, ② cannabidiol oil 25 mg per day, ③ fenbendazole 222 mg per day for 3 consecutive days at an interval of 4 days. Following this incident, for several months pharmacies experienced shortages of anthelmintics, including fenbendazole, due to the sudden increase in demand for these drugs. The use of anthelmintics in cancer patients without the consent or prescription from medical institutions continued. Although many cancer patients continue to take anthelmintics, no study has been conducted on their perceptions, actual experiences of medication, and the visible results of the treatment.

This study thus aimed to understand medication methods, perceptions of anti-

cancer efficacy, and adverse effects of anthelmintics among cancer patients in South Korea. To this end, a structural survey was conducted to collect data from cancer patients who had been practicing anthelmintics therapy to treat cancer.

II. Methods

II-1. Sample and Settings

Cancer patients were recruited from two mega online communities that were established to exchange beneficial information about the use of anthelmintics. The recruitment notice was advertised on the respective community webpage, volunteers willing to participate in the survey contacted the representative research team. A survey link was sent to the volunteers through online chat boxes to prevent any unauthorized member from accessing the link. The sample included cancer patients of age 19 years and above, who had taken anthelmintics for cancer treatment. From a total of the 168 participants, 86 patients completed the survey. This survey was conducted using a structured questionnaire containing 28 questions (6 on general characteristics, 21 on the survey topics, and 1 on free description for their opinions) on an online platform (DOOIT^{TM}) from April 2021 to July 2021. It took approximately 30 min for each participant to complete the survey.

II-2. Survey Structure

This survey was largely divided into six parts: i) the characteristics of patients, ii)

the methods of anthelmintics administration, iii) effectiveness of anthelmintics, iv) adverse effects of anthelmintics, v) communications with clinicians, and vi) free description on their experiences. For the characteristics of patients, gender, age, education, and cancer diagnosis details were collected. In the second part, the duration and mode of anthelmintics administration, the distributor of the drugs, name of the drugs, and dosage of the drugs were investigated. In the third part, the patients' perceptions regarding the anti-tumor efficacy of anthelmintics and the reasons for their evaluation were enquired. The fourth part explored about the occurrence of adverse effects and their detailed explanations, such as type, frequency, duration, and severity. In the fifth part, information regarding clinicians support, comments and consent regarding the usage of anthelmintics were evaluated. Finally, in the sixth part, all participants were given a chance to share their experiences of taking anthelmintics.

II-3. Statistics

Survey responses were downloaded from DOOIT in Excel file format. The analysis was performed in Microsoft Excel 2010 (Microsoft Co., Redmond, WA, USA). Frequencies and percentages are used for categorical variables, and for continuous variables such as age or duration, mean, standard deviation, and

median are used.

II-4. Ethics Approval

Approval was obtained on April 20, 2021, from the Institutional Review Board at Chosun University (Institutional Review Board No.: 2-1041055-AB-N-01-2021-7), South Korea. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

II-5. Consent to Participate

Informed consent was obtained from all individual participants included in the study through agreement of them with the instructions presented before the survey.

III. Results

III-1. General Characteristics of Patients

From a total of the 168 participants, 86 patients completed the survey, and the general characteristics of the patients are represented in Table 1. The patients included 40 men and 46 women with a mean age of 55.2±13.0 years. Among the participants, 55 (64.0%) were college graduates. The cancers diagnosed in the participants included breast (20.9%, 18/86), lung (10.5%, 9/86), intestinal (10.5%, 9/86), liver (8.1%, 7/86), and gastric cancer (5.8%, 5/86). Most of the patients (74.4%, 64/86) were diagnosed with cancer between the years 2016–2020, of whom 52.3% (45/86) were at stage 4 at the time of diagnosis.

Table 1. The general characteristics of patients

Characteristic		No. of subjects	%
Age (years)	Average±SD	55.2±13.0	
	Min-Max (median)	19-90 (55)	
	19	1	1.2
	20–29	0	0
	30–39	8	9.3
	40–49	22	25.6
	50–59	20	23.3
	60–69	22	25.6
	70–79	11	12.8
	80–89	1	1.2
	90–99	1	1.2
Gender	Male	40	46.5
	Female	46	53.5
Education status	No education	2	2.3
	Elementary school graduate	2	2.3
	Middle school graduate	4	4.7

	High School graduate	23	26.7
	College graduate	55	64.0
Diagnosis	Lung	9	10.5
	Gastric	5	5.8
	Liver	7	8.1
	Intestinal	9	10.5
	Breast	18	20.9
	Others	38	44.2
Stage	1	9	10.5
	2	14	16.3
	3	18	20.9
	4	45	52.3
Time of diagnosis (year)	1996–2000	2	2.3
	2001–2005	1	1.2
	2006–2010	3	3.5
	2011–2015	13	15.1
	2016–2020	64	74.4
	2021–	3	3.5

III-2. Taking Anthelmintics as a Cancer Treatment

The details of taking anthelmintics as a cancer treatment are presented in Table 2. The majority of patients (96.5%, 83/86) started taking anthelmintics from 2019, the year Joe Tippens opened the video to the public. The mean duration from the time of diagnosis of cancer until the onset of anthelmintics treatment for each participant was calculated to be 12.5 months. The mean duration of anthelmintic usage was 10.5±7.8 months. To a question that allowed patients to choose multiple answers about the time period of anthelmintic use, the majority of responses (42.9%, 42/98) declared that the drugs were taken during their chemotherapy. Most of participants (64.0%, 55/86) revealed that they were continuing anthelmintics therapy.

For the question regarding the reason behind the decision of selecting anthelmintics as a therapeutic option, the participants responded as follows: based on information available on social media, such as YouTube (41.9%, 36/86), online communities (25.6%, 22/86), or online news (11.6%, 10/86). It was also revealed that they preferred shopping online (55.1% of responses, 65/118) for anthelmintics rather than purchasing them from community pharmacies (33.1%, 39/118). In response to the question about the types of anthelmintics preferred, the most preferred anthelmintics was ivermectin (21.0%

of responses, 54/257) belonged to the non-benzimidazole group, followed by albendazole (20.2%, 52/257) and fenbendazole (17.5%, 45/257). The patients used an average of three kinds of anthelmintics, each having different active pharmaceutical ingredients, either alone or in combination. The maximum number of anthelmintics used by one person was eight. More than half of the responses (53.5%, 54/101) declared that they took the medicines in routine schedules composed of consecutive daily intake and several days of a break. The most frequently selected option for frequency of medicine intake was twice a day (51.5% of responses, 50/97).

Table 2. Details of taking anthelmintics as a cancer treatment

Survey question	Response	No. of responses	%
1. When did you begin to take anthelmintics for cancer treatment?	1996–2000	1	1.2
	2001–2005	1	1.2
	2006–2010	0	0
	2011–2015	0	0
	2016–2020	78	90.7
	2021–	6	7.0
2. Please state how many months you have taken anthelmintics for cancer treatment.	Average±SD	10.5±7.8	
	Min-Max (median)	1-44 (10)	
3. When did you take anthelmintics?	After diagnosis, before chemotherapy	25	25.5
	During chemotherapy	42	42.9
	Resting chemotherapy	20	20.4
	Discontinuing chemotherapy	11	11.2
4. Are you still	Yes	55	64.0

taking anthelmintics?	No	31	36.0
5. What made you take anthelmintics?	Information from TV news	5	5.8
	Information from YouTube	36	41.9
	Information from online news	10	11.6
	Information from online communities	22	25.6
	Information from acquaintances	5	5.8
	Recommendation of a clinician	3	3.5
	Others	5	5.8
6. Where did you purchase anthelmintics?	Local pharmacy	39	33.1
	Internet shopping	65	55.1
	Others	14	11.9
7. Please state the name of the anthelmintic you	Albendazole	52	20.2
	Fenbendazole	45	17.5
	Flubendazole	5	1.9

have taken for cancer treatment.	Mebendazole	42	16.3
	Trichlabendazole	1	0.4
	Niclosamide	30	11.7
	Nitazosanide	14	5.4
	Praziquantel	5	1.9
	Ivermectin	54	21.0
	Pyrvinium	8	3.1
	Do not know	1	0.4
8. How did you take anthelmintics?	Daily without resting	31	30.7
	In a routine schedule with resting	54	53.5
	Intermittently	7	6.9
	Others	9	8.9
9. How many times a day did you take anthelmintics?	Once	26	26.8
	Twice	50	51.5
	More than three times	21	21.6
	Do not know	0	0

III-3. Perceptions for Anti-cancer Efficacies of Anthelmintics

The perceptions of anti-cancer efficacy of anthelmintics are described in Table 3. The majority of the patients (79.1%, 68/86) chose “yes” as an option when they were questioned whether the anthelmintics administration was effective for their cancer treatment. When the participants were asked why they thought the treatment was effective, 42.9% (45/78) of responses indicated that the treatments improved their physical conditions. Moreover, 28.6% (30/78) and 2.9% (3/78) pointed out that the spread of their cancer-affected area reduced and the number of cancerous masses decreased, respectively. In contrast, 20.9% (18/86) of participants chose “no” for the same question, stating that the use of anthelmintics worsened their cancer status (44.4% of responses, 8/18), or had no effect (33.3%, 6/18).

Table 3. Perceptions of the anti-tumor efficacies of anthelmintics

Survey question	Response	No. of responses	%
10. Do you think anthelmintics were effective in your cancer treatment?	Yes	68	79.1
	No	18	20.9
11. What made you think anthelmintics were effective in your cancer treatment?	The decline in cancer size	30	28.6
	Decrease in the number of cancer masses	3	2.9
	Improving of physical condition	45	42.9
	Others	27	25.7
12. What made you think anthelmintics were non-effective in your cancer treatment?	No change in cancer status	6	33.3
	Worsening cancer status	8	44.4
	Others	4	22.2

III-4. Perceptions of Adverse Effects of Anthelmintics

The perceptions of adverse effects of anthelmintics are shown in Table 4. The majority of the participants (73.3%, 63/86) chose the option “no” when questioned whether they had experienced any adverse effects upon the administration of anthelmintics for their cancer treatment. For the participants who responded “yes” (26.7%, 23/86), further questions regarding the nature of the side effects were provided. In this regard, the majority of the patients (21.4% of responses, 6/28) responded positively for gastrointestinal side effects, followed by liver abnormality and hematological effects at 10.7% (3/28) each, respectively. Regarding the frequencies of occurrence of adverse effects, most of the participants (47.8%, 11/23) selected the answer “more than three times.” With regards to the onset of adverse effects, “after a month” was mostly chosen (41.7% of responses, 10/24), and “within a month” followed (25.0%, 6/24). The severity of adverse effects was mostly voted for “somewhat uncomfortable” (34.6% of responses, 9/26) and “uncomfortable, but endurable” (30.8%, 8/26). Concerning the measures taken to relieve adverse effects, “discontinuing anthelmintics course” was mostly chosen (37.9% of responses, 11/29), and “continuing to take the same anthelmintics despite the adverse effects” followed (27.6%, 8/29).

Table 4. Perceptions of adverse effects of anthelmintics

Survey question	Response	No. of responses	%
13. Have you experienced any adverse effects after taking anthelmintics?	Yes	23	26.7
	No	63	73.3
14. What kind of adverse effects have you experienced that you think were caused by taking anthelmintics?	Gastrointestinal effects	6	21.4
	Liver abnormality	3	10.7
	Hematological effects	3	10.7
	Others	16	57.1
15. How many times have you experienced adverse effects by taking anthelmintics?	Once	9	39.1
	Twice	3	13.0
	More than three times	11	47.8
16. When did the adverse effects occur since the first use of anthelmintics?	Within a day	3	12.5
	Within a week	5	20.8
	Within a month	6	25.0
	After a month	10	41.7
17. How long did the adverse effects last?	A day	8	33.3
	A week	10	41.7
	A month	4	16.7

	More than a month	2	8.3
18. How severe were the adverse effects?	Not uncomfortable, but worsening of hematological parameters	7	26.9
	A bit uncomfortable	9	34.6
	Uncomfortable, but endurable	8	30.8
	Very severe, interfering daily life	2	7.7
19. What action did you take to relieve the adverse effects?	Discontinuing of anthelmintics	11	37.9
	Change of types of anthelmintics	4	13.8
	Continuing using the same anthelmintics	8	27.6
	Additional use of different medicines	1	3.4
	Others	5	17.2

III-5. Communications with Clinicians

The details of communications with clinicians are listed in Table 5. For the question regarding consent of the respective clinicians about taking anthelmintics for cancer treatment, the majority of patients (96.5%, 83/86) answered “no.” For the three patients who replied “yes,” follow-up question about the clinician’s support was provided. Two of them (66.7%) stated that the clinicians did not help anything, and the remaining one (33%) replied that the clinician recommended the patient to not take the anthelmintics due to the possibility of liver toxicity.

Table 5. Details of communications with clinicians

Survey question	Response	No. of subjects	%
20. Have you informed your clinician about taking anthelmintics to treat your cancer?	Yes	3	3.5
	No	83	96.5
21. What kind of support have you received from a clinician regarding taking anthelmintics?	Advice for helpful anthelmintic types	0	0
	Guidance for anthelmintic medication	0	0
	Taking actions for adverse effects from anthelmintic medication	0	0
	No support	2	66.7
	Others	1	33.3

III-6. Free Description on Their Experiences

Finally, all patients were required to make notes on their experiences with anthelmintic therapy for cancer treatment. Most of them gave a positive feedback regarding the use of anthelmintics for cancer therapy. Some patients suggested that anthelmintics should be made available in combination with the clinically approved chemotherapy medications in the future, through clinical trials or drug development processes, whereas a few others expressed concerns about the underlying adverse effects.

IV. Discussion

Although the application of anthelmintic in cancer treatment often takes place among cancer patients, there is a lack of information regarding their behavior or perceptions. This study is the first to investigate the factors governing the use of anthelmintics as a treatment for cancer, such as motivation, mode of intake, types of anthelmintics, etc., and the perceptions of their effectiveness in cancer treatment and its adverse effects, among cancer patients of South Korea. The participants of the present survey were mainly college graduates (64.0%, 55/86), and most of them (73.3%, 63/86) had advanced cancers. The results showed that large portions of participants depended information from social media or online platforms when they decided to start anthelmintics therapies or attempted to buy those medicines. The majority of patients (96.5%, 83/86) revealed that they started taking anthelmintics in 2019, the year Joe Tippens had released the video; overall 42% of patients (36/86) indicated that the information from YouTube motivated them to try anthelmintics. Based on these two results, it can be speculated that the onset of anthelmintics in South Korea was mostly triggered by the YouTube video that was mentioned in the introduction.

Even though the video largely affected the patient's decision on anthelmintics treatment, the survey results revealed that almost all cancer patients taking

anthelmintics tried to modify their methods based on the findings of others and through the communication of available information. First, it was observed that fenbendazole, initially mentioned by Joe Tippens as an effective drug, expanded to other types of anthelmintics, including ivermectin (of the non-benzimidazole group) and other benzimidazoles, such as albendazole and flubendazole. Second, inquiry about the type of anthelmintics used indicated that almost all the patients were aware of the generic names of these medicines, and it means they made an effort to extend their knowledge about anthelmintics therapies. Third, most of the patients (53.5% of responses, 54/101) followed Joe Tippens's method, composed of several consecutive days of medicine intake and a break of a few days per week. However, the majority of the patients (51.5% of the responses, 50/97) took anthelmintics twice a day, which was different from Joe Tippen's regimen, who took 1 g of canine anthelmintics (222 mg fenbendazole) per day in the form of granules, which did not contain any detailed descriptions about intake frequencies per day. These results imply that the patients actively improve the regimen of taking anthelmintics for the cancer treatment, but not dependent on the Tippens' method.

Concerning the efficacies of anthelmintics, more than two-thirds of the patients considered anthelmintic therapy as an effective method of cancer treatment. The positive perceptions on the efficacies were related to the

improvement of their physical conditions (42.9% of responses, 45/105), the decline of cancer size (28.6%, 30/78), or the number of cancer masses (2.9%, 3/78). Accordingly, it needs to assess the effectiveness of anthelmintics in cancer treatment more, and further evaluations are required to reveal which factors affected the efficacy gap, such as individual differences or cancer types.

The most frequent type of adverse effect observed was gastrointestinal effects (21.4% of responses, 6/28); out of total cases, only 7.7% (2/26) indicated that these adverse effects were severe and affected their daily lives. More than two-thirds (73.3%, 63/86) of the participants declared that they did not experience any adverse effects. This is in line with the explanations in the previous studies that anthelmintics including benzimidazole derivatives, ivermectin, and praziquantel are in general known to be safe as demonstrated by use over a long period. [13, 96, 111] In this research, there were several concerns regarding adverse effects, although serious adverse cases are rare. First, since there was no medical guidance about anthelmintic therapies for cancer treatment, the patients could use higher doses than the optimal doses. Considering that some severe cases were caused by the administration of high doses of albendazole or praziquantel for a long duration in patients with poor liver function, [96] these arbitrary decisions of dosage might lead some patients to adverse effects. Second, participants tended to keep anthelmintics treatment long term; the average

duration of anthelmintics administration in all patients was 10.5 months. These raise concerns about possibilities of adverse effects from long-term intake. Third, the majority of patients (42.9% of responses, 42/98) replied that they took anthelmintics during the chemotherapy period. This result suggests that drug-drug interactions following combination with chemotherapy can be triggered. Fourth, combination therapies of anthelmintics that might be used by the participants also have possibilities to cause drug-drug interactions. For instance, there was a case report that described a case of a patient who developed psychosis due to the combined use of albendazole and ivermectin. [112] In that albendazole and ivermectin were revealed to be highly chosen as anthelmintics therapies by the patients, the combinations had chances of albendazole-ivermectin interactions. Fifth, when the adverse effects occurred, some patients (27.6%, 8/29) continued to take the same anthelmintics. These results suggest the necessity of guidance by clinicians regarding the safety of anthelmintic use to prevent any harmful effects.

It was observed that the majority of the patients (96.5%, 83/86) failed to inform their clinicians about the use of anthelmintics for cancer treatment. This result was consistent with previous study results, [2, 3] which showed that a large proportion of patients refused to consult their clinicians regarding alternative medicines. Moreover, those patients (3.5%, 3/86) who informed their

clinicians about the therapy described that they did not receive any support from the clinicians. This information reflects the insufficiency of communication between cancer patients and their clinicians regarding the use of anthelmintics; thus, there needs more interest of the clinicians in the safety as well as the effectiveness of anthelmintics in cancer therapies.

This study has several limitations that need to be addressed. First, since the recruitment and organization of the survey were performed online, the findings of this study may not be generalizable to the generic population of cancer patient, which includes patients who are not familiar with internet platforms. Second, since many patients took anthelmintics during chemotherapy, their combined effect with anthelmintics could affect results. Third, information about exact various dosages that the patients taken remains unknown by taking a tool using multiple-choice question. Finally, the survey was an investigation of the patients' perceptions alone, and there was not enough evidence or medical data available to evaluate the results.

Conclusion

PART I. Review on Preclinical and Clinical Evidences of Anti-cancer Effects of Benzimidazole Anthelmintics

Although the anthelmintics of the benzimidazole group have shown anti-cancer effects in many *in vitro* and *in vivo* studies, there is still limited clinical evidence regarding the same. Moreover, only a few modest efficacies have been observed with ABZ and MBZ. It is presumed that these modest efficacies are owing to the facts that the main targets of these drugs and their multiple anti-cancer properties are not elucidated yet, in addition to which these compounds suffer from the limitation of low bioavailability. Therefore, upon additional efforts in terms of novel formulation and development strategies, the anti-cancer effects of benzimidazoles could be significantly enhanced, even for clinical applications.

PART II. Experience with and Perceptions of Anthelmintics for Cancer Treatments among Cancer Patients in South Korea: A Cross-sectional Survey

Based on the current study, it might be worth evaluating the benefit and risk of

anthelmintics in cancer treatment through further clinical trials. And communication between the clinicians and cancer patients also needs to be enhanced affirmatively regarding the use of anthelmintics to prevent adverse effects. Furthermore, it is required the more active involvement of government in blind spots of health security.

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