Benzimidazole Adjuvant Therapy: A Review of Efficacy and Safety in Patients with Cancer

by

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Abstract

This literature review aims to compile previous studies' data and explore the effects of benzimidazoles as an adjuvant therapy and its relationship to a patient's cancer biomarkers, tumor progression, and quality of life. In addition to the aforementioned, we will attempt to review data to identify the safety and toxicity profile of benzimidazoles within a population of patients currently diagnosed with cancer to determine if this class of medications could be implemented along with current cancer treatment regimens to increase efficacy and tolerance. To determine this, the databases of CINAHL, ClinicalKey, and PubMed were searched with the keywords mentioned below. Studies include those after the year 2000, and those focusing on the use of benzimidazoles on human cancer cells and tissue, case studies, and all phases of clinical trials. Excluded were those using non-human study subjects, those before the year 2000, and those not applicable based on a population not being those with cancer. These parameters resulted in 11 applicable studies available for this review. The review resulted in the realization that benzimidazole use in those with cancer is in its early stages of research. This being said, many of these early-stage trials did show promise that benzimidazoles may show antineoplastic characteristics with less severe side effects than conventional cancer treatments. Additionally, safety and toxicity properties among participants appeared to be similar to their use in those with helminth infections. Larger-scale clinical trials will be necessary to further understand the role benzimidazoles may have as antineoplastic agents.

Keywords: benzimidazoles, albendazole, mebendazole, flubendazole, adjuvant therapy, anticancer, antitumor, and drug repositioning.

Introduction

According to the American Cancer Society, over 1.9 million new cancer diagnoses are expected across the United States in 2022. This is an increase from previous years, contributing to approximately 610,000 expected cancer deaths in the United States alone (American Cancer Society, 2022). While chemotherapy and radiation therapies have been widely studied and considered first-line therapy, the question remains if more can be done to improve the efficacy of current conventional treatment and quality-of-life measures for those with cancer. Although new therapies and drug classes are being researched, others have theorized that the answer may lie within our current formularies using a drug with a completely different current indication. The idea is that research should be focused on repurposing drugs that already have a known safety and toxicity profile, fast-tracking the process.

In their study looking at repurposing drugs as cancer therapy, Rodrigues, Duarte, & Vale (2022) state that:

> Drug repurposing, also known as drug repositioning, is a strategy that explores alternative uses of an already approved drug, for other diseases besides its original indication. It has been proposed by several authors as an alternative way to increase the number of therapeutic weapons available for cancer treatment and presents many advantages compared to de novo cancer drug development. There is no need for extensive studies because the drug's pharmacokinetic and pharmacodynamic profiles are already fully characterized, which shortens the translational process and consequently lowers the associated costs, contributing to a major success of the process (para.4).

Recently, benzimidazoles have been investigated as a practical adjuvant therapy for those with cancer who are receiving conventional therapies such as chemo, radiation, and surgical intervention. The findings will be discussed below, however, an overview of the history of the drug class is important to fully understand the possible repurposing. According to the National Center for Biotechnology Information (2022), Benzimidazoles are classified as Anthelmintics and their purpose is defined as an agent working to kill a parasitic worm, primarily used in animals and humans as a treatment for helminthiasis. Essentially, the drug class can be considered an antiparasitic, or "de-wormer". As will be further discussed as it pertains to its ability to fight cancer, the MOA is defined as the prevention of tubulin polymerization or spindle movement, with administration resulting in aneuploidy (National Center for Biotechnology Information, 2022). Due to the nature of how this works against parasites, those looking to repurpose this drug class recognized this MOA as having similar characteristics to other antineoplastic drugs, leading to the presumption of similar outcomes.

It is well known that conventional cancer treatments are not a pleasant experience, with many patients reporting frequent occurrences of neutropenia, lymphedema, alopecia, nausea and vomiting, cognitive issues, pain, and DVTs, among others as reported by the Centers for Disease Control and Prevention (Side Effects of Cancer Treatment, 2022). The American Cancer Society adds to this stating that 1 in 4 cancer survivors report a decreased quality of life with a more significant decrease associated with invasive and aggressive treatments (American Cancer Society, 2019). With these known issues tied to conventional cancer therapy, finding a drug to repurpose as an adjuvant therapy may allow for a shorter duration or intensity of these conventional therapies with the hope of retaining quality of life after treatment while retaining efficacy of therapy.

This scholarly project aims to compile previous studies' data and explore the effects of benzimidazoles as adjuvant therapy and its relationship to a patient's cancer biomarkers, tumor progression, and quality of life. Additionally, we will attempt to identify the safety and toxicity profile of benzimidazoles in patients with cancer as a target population. The end goal of this project is to provide the reader with the most up-to-date information to make an educated decision on whether the relationship between benzimidazole therapy and the previously mentioned cancer measures is positive, neutral, or negative.

Statement of Problem

According to the CDC, Cancers are the 2nd leading cause of death in the United States now accounting for over 600,000 deaths each year as well as nearly 1.8 million people being diagnosed annually. While chemotherapy and radiation therapies have been widely studied and shown effective, many wonder what more can be done to improve the efficacy of current treatment and quality of life measures as it is well known that traditional cancer treatments come with a significant amount of undesired and life-altering side effects that persist even after remission of cancer. Recently, benzimidazoles have been investigated as a viable adjuvant therapy for those with cancer and receiving conventional therapies such as chemo, radiation, and surgical intervention with hopes of reducing side effects and increasing quality of life during treatment (American Cancer Society, 2022).

Scholarly Project PICO Question

In patients with cancer who have received conventional therapy, does the addition of adjuvant benzimidazoles versus those receiving conventional therapy or observation alone show improved safety and efficacy with increased antitumor tissue response, survival measures, and reduction in cancer biomarkers?

Methodology

To identify applicable studies for this literature review, the electronic databases of PubMed, ClinicalKey, and CINAHL were systematically searched with initial keywords and MeSH indexing terms to include: “benzimidazoles, albendazole, mebendazole, flubendazole, adjuvant therapy, anticancer, antitumor, and drug repositioning”. PubMed resulted in 3,061 articles with the initial search under these terms with English as the primary language. ClinicalKey and CINAHL databases were searched in the same systematic way as PubMed with the same keywords with no additional studies identified, thus PubMed findings are reported and used in this literature review.

Further filters were applied to limit studies to the human species with all animal models excluded. All meta-analyses and systematic reviews were then excluded to include primary source data alone. Human cell and tissue studies, case studies, and all phases of clinical trials were included in the search after searching clinical trials alone which yielded limited results. Studies were limited to being published after the year 2000 and resulted in 275 articles. Articles were then analyzed and excluded unless found to be focused on studying at least one of the drugs under the classification of benzimidazoles. Furthermore, articles were excluded unless the endpoints of studies investigated the mechanism of action, safety, or efficacy of benzimidazoles within a population of patients with active cancer and treatment or in human cancer cell lines. Eleven total articles were found applicable for this literature review based on these inclusion and exclusion criteria.

Criteria Defined

Common Terminology Criteria for Adverse Effects

The following studies incorporate grading based on the Common Terminology Criteria for Adverse Effects (CTCAE) v5.0 created by the Division of Cancer Treatment and Diagnosis which resides within the National Cancer Institute (NCI). According to the NCI (2017), an adverse effect is defined within CTCAE as:

> any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. (p.2)

The grades are a representation of the severity of the event and are explained by the NCI (2017) as:

> **Grade 1:** Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
>
> **Grade 2:** Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
>
> **Grade 3:** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
>
> **Grade 4:** Life-threatening consequences; urgent intervention indicated.
>
> **Grade 5:** Death related to AE. (p.2)

CTCAE is intended to define and assign severity to symptoms for better comparison and is not all-encompassing. Some adverse effects do not fall within the grades and may need to be

defined independently within a study (National Cancer Institute - Division of Cancer Treatment and Diagnosis, 2017).

Response Evaluation Criteria in Solid Tumors

According to Eisenhauer et al. (2009), the evaluation of tumor response to medications is also commonly measured with the Response Evaluation Criteria in Solid Tumors (RECIST). In a revision publication, Eisenhauer et al. define the RECIST criteria and its most recent application of standardizing the measurement of therapeutic tumor response of solid tumors. The following will summarize RECIST for application within this literature review, but a full explanation and description of these parameters can be found within the reference list. For RECIST to be applied, measurable disease must first be present, defined as having at least one measurable lesion measuring 10mm by CT or calipers or 20mm by chest X-ray. Target lesions are then identified by their specific criteria and monitored over time preferably by CT or MRI. Eisenhauer et al. (2009), state the findings are reported in the following means:

> **Complete Response (CR):** Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have a reduction in the short axis to <10 mm.
>
> **Partial Response (PR):** At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
>
> **Progressive Disease (PD):** At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

> **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study. (pp. 232-233)

These parameters will be referred to throughout the remainder of this literature review and can be referenced for their definition here.

Literature Review

Safety Profile of Benzimidazoles

To determine the safety and dosing of mebendazole and ultimately the maximum tolerated dose (MTD), Gallia et al. (2020) conducted a single-institution, non-randomized, phase 1 trial in patients with newly diagnosed malignant glioma brain tumors. The study design included typical treatment of patients with gliomas including surgery, temozolomide (standard adjuvant therapy), radiation therapy, followed by concurrent temozolomide and oral mebendazole (MBZ), and finally MBZ monotherapy until progression or withdrawal from the study occurred. The dosing of MBZ was administered in a dose-escalation fashion with doses starting at 25 mg/kg/day increasing to 50, 100, and 200 mg/kg/day. Patients were selected with specific requirements including being 18 years of age, having histology-confirmed and newly diagnosed malignant glioma, adequate organ and bone marrow function, and completion of the previously mentioned treatment regimen without significant toxicity. Twenty-four patients met these criteria and were enrolled in the study with a median age of 49.8, 15 of those being male and nine being female with all participants being Caucasians. All cancers were types of gliomas including 18 glioblastomas, five anaplastic astrocytomas, and one anaplastic infiltrating glioma. There was no control group, as the main goal of this study was to determine the safety and MTD of MBZ in

this patient population, but the findings of anticancer effects were documented and discussed later in this article to provide a suggestion of continuation or discontinuation of further trials.

To ensure serum levels were consistent, mean MBZ serum levels and their relationship to metabolites were monitored by Gallia et al. (2020). Additionally, the adverse effects (AE) of medication escalation as reported by participants were monitored. These were then reported with CTCAE grades assigned as appropriate along with descriptive statistics used for toxicity findings. Dose-limiting toxicities (DLT), defined as grade 3 or above hematological events and grade 4 for AE, were monitored as an exit point for patient safety. (See CTCAE above). Gallia et al. found that during the first cycle of the dose-escalation period of MBZ administration, no DLTs were noted among all dose ranges up to the max dose of 200mg/kg/day. With the study's multiple cycle administration design, repeat cycles of MBZ only resulted in DLT in 4 out of 24 patients and this occurred at the max dose of 200mg/kg/day. These DLTs were reported as elevation of alanine aminotransferase (ALT) and aspartate transaminase (AST) with six total DLTs reported within these same four patients. These toxicities were reported to be reversible with either reduction in dose to 100 mg/kg/day (3 patients) or complete discontinuation of MBZ (1 case). One of these patients was found to have four additional AEs requiring removal from the trial. There were no AEs for the duration of the trial that required hospitalization or death attributable to MBZ (Gallia et al., 2020).

While this study does include the pertinent cancer population and appropriate drug therapy, the study lacks a control group and has a low n value. Also lacking is the specific data on what type of AEs were encountered along with the statistical significance of these findings. The main value of this study, and its purpose, was to assess the ability of mebendazole to be tolerated at doses higher than previously administered and monitored via objective measurement

of liver and renal parameters, as discussed. MBZ was able to do this with a low percentage of participants reaching a DLT, resulting in the possibility of further research into specific AEs and efficacies. The study reported on overall survival (OS) and progression-free survival PFS (PFS) which are valuable measures but had no comparison to a control group which is discussed in a later theme.

Using a prospective, randomized double-blind placebo-controlled study, Hegazy et al. (2022) researched the efficacy and safety of MBZ as an adjuvant therapy after treatment with bevacizumab and FOLFOX4 in patients with metastatic colorectal cancer. They determined the eligibility of 40 individuals aged 18-65 years and placed them into a placebo-control group and a Mebendazole group via a randomized sealed envelope method. The control group of 20 participants reported a female-to-male ratio of 9:11, a median age of 55.5, and an avg BMI of 28.23. Metastasis in patients included liver (12), lung (5), and other (12) giving a wide variety of metastasis differentiation. The MBZ group participants reported a female-to-male ratio of 8:12, a median age of 47, and an avg BMI of 28.05 creating a similar group to the control. Metastasis includes liver (14), lung (5), and other (8). These groups were followed over a mean duration of 12 months to identify the efficacy and safety profile of MBZ (Hegazy et al., 2022).

Analysis of the safety and drug tolerability were desired outcome measures due to the necessity of a drug to be safe and tolerable to provide any benefit of its efficacy to then be warranted as a viable alternative treatment to conventional therapy or observation alone. To define and grade safety and tolerance, Hegazy et al. (2022) utilized the NCI-CTCAE which can be referenced above. Following analysis of data, the most encountered significant adverse effects (AE) were gastrointestinal-related. Abdominal pain was the most reported AE with 13 patients (9 CTCAE grade 1 and 3 CTCAE grade 2) compared to the control group with six reporting

abdominal pain (6 CTCAE grade 1) showing a significant increase over the control group ($p=0.029$). Diarrhea was the second most prevalent AE with 10 instances (7 CTCAE grade 1 and 2 CTCAE grade 2) reported in contrast to the control group reporting three instances of diarrhea (2 CTCAE grade 1 and 1 CTCAE grade 2) also increased over the control group, respectively ($p=0.02$). Other reported symptoms, but not within statistical significance, include elevated liver and renal parameters. Liver parameters were reported to be increased in 17.5% of the control group compared to 22.5% in the MBZ group (ALT $p=0.741$ and AST $p=1$). Renal measure parameter increases were reported by 45% of the control group and 30% of the MBZ group, respectively (SCr $p=0.262$ and eCrCl $p=0.348$). Due to similar findings within the control group and p values outside of the limitations set of <0.05 to be statistically significant, Hegazy et al. theorized these findings to be associated with chemotherapy-induced cytotoxicity rather than MBZ administration due to both groups being post-chemotherapy treatment per the study design. It is of note that this study included patients with colorectal cancer who have high rates of metastasis to the liver, contributing to the likelihood of these findings being associated with already compromised liver health (Hegazy et al., 2022).

In review, the n value of 40 does limit the validity of the study, but the remainder of the study including the group variation, random assignment to a control group, objective measures of antitumor and tolerability/adverse effects, and statistical analysis appears to strengthen the data of this study. There is no apparent bias as the funding was not received from the public, commercial, or not-for-profit sectors.

In a prospective, open-label, phase 2a study, Mansoori et al. (2021) conducted a study questioning the safety and efficacy of MBZ in patients with advanced cancer who are no longer able to or have completed standard drug treatment for their cancer, fitting the primary patient

population of this review. This study was broken down into two segments, the first being week one analyzing the pharmacokinetics of MBZ followed by the treatment phase for 16 weeks to follow the full effect of MBZ on these participants. Participants were required to be 18 years of age or older with diagnosed and measurable cancer according to the RECIST criteria. These participants were also required to have had previous standard or experimental therapy to which this advanced cancer was refractory. Exclusion of participants included therapy within the previous three weeks, ongoing or recent infection, hepatic dysfunction or hepatocellular carcinoma, or any other major organ dysfunction findings. Thirty patients were initially identified as qualified participants for the study. Due to difficulties in maintaining desired serum levels of MBZ with the dose of up to 4g per day, only 11 patients met the criteria to be followed and considered for evaluation within the study. The median age of these patients was 55.8 years, gender consisting of five being female and six being male with a median weight of 71.7kg. All patients were diagnosed with metastatic GI, hepatic, or pancreatic cancer refractory to previous chemotherapy. Previous intended curative surgery was also reported in seven of these patients (Mansoori et al. 2021).

Ten patients ultimately reached the treatment phase of this study. Mansoori et al. (2021) subsequently ended the study after clinical deterioration or radiologic progression of disease (PD) with a median treatment phase of 52 days. While the study ended prematurely, the data over the treatment period was analyzed. Regarding the safety profile of MBZ observed within this study, overall MBZ was reported to be well tolerated up to doses of 4g/day. Abdominal pain, decreased appetite, nausea, and vomiting were the most reported adverse effects. Also reported by the study were nine serious adverse events, three of which were fatal. The study reports these findings as being not related to the MBZ therapy and attributed these to predisposed patient

factors. With these findings, the study also reports no dose-limiting toxicities with no significant changes in vital signs, physical examinations, ECGs, and hematological/biochemistry results. With proposed anticancer levels requiring high serum concentration, the pharmacokinetics of MBZ were also analyzed. Out of the reported number of patients participating in the pharmacokinetic phase of the trial, only two patients briefly reached the target concentration with four total reaching this target concentration throughout the trial, limiting the ability of MBZ to maintain steady-state serum concentrations in this study (Mansoori et al. 2021). The relevance and strengths and weaknesses of this study will be discussed below along with the results of efficacy noted within the study in a theme below.

In an open-label, non-controlled, phase I dose-escalation study Morris et al. (2001) set out to investigate the safety and tolerability of albendazole (ABZ). ABZ, a benzimidazole, developed in 1975 for veterinarian anthelmintic therapy has also successfully been used for the treatment of helminth infections in humans. It is theorized that, at higher doses, this medication could be repurposed as adjuvant therapy in cancer leading to this study aiming to determine a max tolerable and safe dose. To be eligible for participation, patients required a biopsy showing advanced or metastatic cancer refractory to conventional treatment. Patients were required to be over the age of 18, > 3 weeks past any prior chemotherapy, have recovered from reversible side effects, and > 2 weeks from any radiotherapy. These recovery measures were determined by participants having adequate hematological function with an absolute neutrophil count (ANC) >1,500/mm^3 and platelets > 100,000 mm^3, adequate liver function with bilirubin <2 times the normal upper limit, AST and ALT <10 times the upper limit of normal, and adequate renal function with calculated creatinine clearance > 60ml/min. All measures were required to assure the patient is metabolically capable of clearing medications to show true toxicity and dose

relationship with a normal baseline of values which were monitored by Morris et al. to determine the safety and tolerance profile of ABZ.

Morris et al. (2001) identified 36 patients per the criteria which included 14 females and 22 males. The median age was 65 years with a mean weight of 69.2kg. The majority of tumor types involved the GI, respiratory, or reproductive system. Patients received an oral dose of 400mg of ABZ twice daily up to the escalation endpoint of 1,200mg twice daily. With this escalation dosing, patients were monitored for endpoint measures of dose-limiting toxicity (DLT) and maximum tolerated dose (MTD). DLT is defined as grades of neutropenia, fever, and non-hematological toxicity, and MTD is defined as the dose at which 2/6 participants reached a measure of DLT during the first 8 weeks of treatment requiring adjustment or cessation of medication (Morris et al., 2001).

Out of the 36 patients enrolled, Morris et al. (2001) reported one patient that reached DLT at 1,600mg daily intake and two patients reached DLT at 2400mg daily intake. The most regularly noted adverse effects were fatigue, myelosuppression, and minor gastrointestinal side effects. Less frequently occurring were cough, neuropathy, alopecia, rash, and peripheral edema, all of which were rated as non-severe at CTCAE grade 2 or below. Fatigue, infection/fever, and confusion were all reported as the most severe at CTCAE grade 4, although infrequent. One patient, with severe end-stage metastatic colorectal cancer and severe liver involvement, died from neutropenic sepsis.

Morris et al. (2001) determined, based on these previously mentioned measures, the MTD of ABZ is equal to or greater than 2,400mg with more trials required to determine the pharmacokinetic differences of patients who did reach a DLT. While the common side effects of bone marrow suppression and elevated liver enzymes that are known with ABZ used as an

anthelmintic were encountered, the study found in the population of patients with metastatic cancer, ABZ is tolerated and relatively safe warranting further testing for its efficacy as an antitumor agent.

Limiting factors of the study include a low n value with no control or blinded group to differentiate whether these findings were truly caused by albendazole versus natural progression or long-term effects of previous therapy. Due to the design of the study, the goal of determining MTD and safety was performed using appropriate measures and dose escalation provided valuable information for repurposing the drug in patients with cancer. Also strengthening the study was the use of patients with metastasis versus healthy patients to determine the safety and tolerability, as this is the target population in which repurposing would take place. There was also no mention of funding or statistical measures to identify the significance of these findings. The age of the study is also a limiting factor, but the study was included in the review due to the specificity of the population studied versus other studies using populations targeted for anthelmintic therapy.

Effects of Benzimidazoles on Human Cancer Cells

In a study researching Mebendazole (MBZ) and its ability to overcome cisplatin-resistant ovarian cancer, Huang et al. (2021) isolated resistant cancerous cell lines and tested the ability of MBZ to sensitize the cells to cisplatin via concurrent MBZ administration, with the goal of causing cell death of the resistant cell lines. These experiments were quantitative in nature, and each was performed in a triplicate fashion to ensure accuracy and identify outliers. To ensure cells were truly cisplatin-resistant, two ovarian cell lines (OVCAR8CR and SKOV3CR) were isolated and exposed to two treatments of cisplatin with selection when, even after treatment, stable and robust cell lines remained. These cell lines were then selected as either a control or

experimental cell line being treated with various concentrations of either cisplatin, mebendazole, or both. Through systematic procedural lab methods, the IC50, migration, and quantitative apoptotic values were determined and reported per treatment group (Huang et al., 2021).

The study first analyzed the ability of MBZ to inhibit the viability of ovarian cancer cells. The quantitative results indicated that MBZ alone significantly decreased the cell viability of the cells with just 0.25 μM of MBZ and completely irradicated the cell lines at 4.0 μM of MBZ in the OVCAR8CR cells. Huang et al. found similar results, although less, in the SKOV3CR lines. Cell proliferation inhibition was reported at IC50 of 0.28 μM for OVCAR8CR and 0.61 μM SKOV3CR, respectively, indicating the ability of MBZ to overcome cisplatin-resistant cell lines and effectively inhibit cell viability. These were compared to the control group which did not indicate any of these values and showed continued cell viability ($p<0.01$).

Investigating another important factor in patients with cancer, Huang et al. (2021) reported MBZ efficiently inhibited the wound healing/migration of these cell lines. This was determined by creating a scar line within the cell cultures and monitoring the ability to prevent wound closure versus a control group with the treatment of MBZ. They found that MBZ was able to retain an 80% wound gap compared to the control at 40 hours after treatment. Also analyzed was the ability of MBZ to initiate apoptosis with a significant increase of apoptotic cells noted in the MBZ-treated cells versus that of the control group ($p<0.01 – 0.05$ dose-dependent and cell-line dependent).

While MBZ was analyzed alone, Huang et al. (2021) also set out to determine the ability of MBZ to work synergistically with cisplatin to reduce cell growth markers and tumor measures. The study monitored colony growth of cell lines treated with MBZ to sensitize them to cisplatin treatment and found there was a significant increase in cisplatin-based cellular toxicity

compared to that of MBZ or cisplatin alone ($p<0.01 – 0.05$ dose-dependent and cell-line dependent). These findings resulted in the discussion of further progression of trials to determine the efficacy in situ (Huang et al., 2021).

The nature of this laboratory study was performed with proper triplicate technique and a control group. Statistical analyses were well described, and p values were within the limits of being statistically significant. The funding of this study was primarily via grants, government or educational institutions that were not involved in the study design or any other aspect of the study that could alter outcomes.

In a study to determine the efficacy of MBZ as a repurposed drug in the treatment of colon cancer, Nygren et al. (2013) isolated MBZ and ABZ from a group of 1,600 compounds with previous clinical use as having the potential to be repurposed as a treatment for those with colorectal cancer. The study isolated colon cancer cell lines of HCT 116 and RKO which were then cultured using a systematic and controlled protocol to ensure equal concentration and characteristics. These cell lines were then used for the primary screening with the treatment of all 1,600 compounds to identify possible compounds with efficacy against these cancerous cell lines. Second, colon cancer lines HT29 and SW626, along with non-malignant epithelial cell lines MCF 10A, renal RPTEC/TERT1, and hepatic cell line NeHepLxHT were all isolated and prepared via the same protocol on culture for further validation experiments. These cell lines were then exposed to different concentrations of each drug via an Echo dispenser which dispenses equal concentrations to each culture plate to ensure consistency. Cell lines were then monitored for cytotoxic effects via the hydrolysis of fluorescein diacetate, which is produced by plasma membranes indicating an intact and surviving cell. Also included were two columns without drugs containing only previously mentioned cell lines serving as a control. One column

with medium only was included serving as a blank to ensure the quality of the procedure (Nygren et al., 2013).

To determine the efficacy following the drug administration, the survival index (SI) was determined to display the efficacy. Nygren et al. (2013) define this as assessing the fluorescence-stained cells which represents the percentage of survival of a well minus that of the control cells overall indicating the survival of cells following treatment in relation to untreated cells. To be considered for further testing, a medication must have an SI of < 40% in both reported colon cancer lines. To determine statistical significance, Nygren et al. (2013) state,

> Z scores are determined for each experiment/cell line pair by the subtraction from its intensity by the experiment mean (across the 60 cell lines) and division by the standard deviation of the experiment (across the 60 cell lines). The z-score average was then calculated as the mean across all experiments that passed quality control criteria. The z score will have a mean of zero and a standard deviation of one. (p. 5)

A total of 68 drugs were determined by Nygren et al. (2013) to fall within the SI for significant efficacy against these cell lines. Antineoplastic drugs showed the highest efficacy followed by antiparasitic, antiseptic/antibacterial, cardiovascular, antifungal, and central nervous system, respectively. As with known antineoplastics, such as anthracyclines and vinca alkaloids, benzimidazoles showed statistically significant effects on the cancer cell lines (correlation coefficient of 0.64 and considered statistically significant). Due to MBZ and ABZ having a significant pharmaceutical and clinical use history as anthelmintics and falling within the ideal SI, they were prioritized for further experimentation. Nygren et al. further exposed cancerous cell lines and MBZ showed significant inhibitory findings with 80% of colon cancer cell lines

showing sensitivity, whereas lung and renal cancer cell lines indicated 25 and 28% sensitivity. (z-score < 2 SD). Furthermore, MBZ showed a concentration of a medication resulting in inhibiting growth to 50% (IC_{50}) of <5 μM in all 5-colon cancer cell lines but did not show significant activity within non-malignant cell lines indicating highly targeted and therapeutic properties which would be a desirable characteristic for drug repurposing (Nygren et al., 2013).

This study contains well-prepared and analyzed data with cancer cell lines obtained from a reputable source and handled appropriately, strengthening the study's data. The design of the study takes a broad approach with no initial medications determined sourcing of 1,600 compounds and letting their SI and Z scores determine what compounds would advance to further testing, indicating no bias on trying to promote a certain class of medications. After narrowing down compounds, a systematic and highly controlled process was implemented to further test these medications on other types of cancer cells, reporting their efficacy on the SI along with their correlation coefficient values. Study weaknesses include reporting statistics in relation to the percentage of change from control making it difficult to visualize the values of the untreated controls in comparison to the compounds themselves. Support of the study includes the Swedish Cancer Society, Swedish Foundation for Strategic Research, and the Lions Research Fund with no apparent bias or funding source concerns.

In a human tissue study investigating the efficacy of ABZ in combination with conventional radiation therapy on multiple myeloma (MM) and small cell lung cancer (SCLC), Patel et al. (2011) obtained isolated cell lines and exposed them to ABZ and radiation to identify if the drug has anticancer characteristics when used in combination with radiation. Cell lines A375 and A2058 MM cell lines and H153 and H446 SCLC cell lines were seeded and incubated with each assay being performed in triplicate with all values reported as mean values while

following standard procedures and protocols to ensure consistency and accuracy of results. These cell lines were then treated with ABZ at doses of 0nM (control),100nM, and 500nM and then irradiated with MV photons (equivalent to conventional irradiation) ranging from 1 to 8Gy, with others remaining free of radiation for control measures. Following the completion of treatment with ABZ, program calculations of the Combination Index (CI) determined whether the interaction had synergistic effects (CI<0.90), additive effects (CI >0.90 – <1.0), or antagonistic effects (CI >1.0). To determine statistical significance, p values were determined and a p value of < 0.05 was considered statistically significant (Patel et al., 2011).

The study found that as a single agent ABZ, at clinically achievable concentrations, was able to inhibit proliferation as a DNA damaging agent and microtubule destabilization in both previously mentioned MM and SCLC cancer cell lines with exposure to ABZ. MM cell lines treated with 500nM averaged 42.35% inhibition of growth and SCLC lines averaged 43.6% growth inhibition with higher doses not being found to significantly increase the further growth inhibition of these cell lines ($p < 0.05$). To further investigate the role of ABZ in adjuvant cancer therapy, Patel et al. (2011) determined the ability of ABZ to sensitize these cell lines to achieve better results of radiation therapy and limit the duration of therapy as side effects of ABZ have been reported as being time-dependent, thus a 12-hour incubation time frame was investigated. The study exposed these cell lines to increasing concentrations of ABZ for a shorter period along with radiation. Patel et al. found that, when compared to irradiated-only cell lines (61.6% cell survival), the time-limited ABZ treated group was found to have decreased by 33.5% (28.1% cell survival) compared to approximately 43% in the 72-hour group as shown above ($p < 0.05$).

As previously mentioned, Patel et al. (2011) set CI intervals to categorize responses of these combination studies. With pretreatment of 500nM ABZ and radiation at 2Gy, the

relationship was noted to be synergistic (CI < 0.90). Treatment with 100nM and 2Gy radiation was found to be additive with a CI of (0.90 – 1.0). To end the study, Patel et al. investigated the efficacy of the pretreatment of ABZ to determine any potentiation of radiation-induced apoptosis. Findings were consistent with cellular apoptosis pathway properties like that of treatment with 10Gy irradiation alone, indicating ABZ monotherapy has strong similarities to radiation therapy.

Overall, the study took measures to ensure proper procedures were in place to ensure accurate findings with appropriate lab procedures and triplicate methods. The authors of this study analyzed ABZ in multiple fashions testing its efficacy, or lack thereof, in multiple doses and in combination with other forms of conventional cancer therapy. While some p values were reported, other areas of this study lacked statistical analysis to adequately determine the significance of the data. There were no reports of conflicting interest by the authors and no concerns with funding or affiliations.

In a study to determine the effects of anthelmintics on Triple Negative BC (TNBC), Zhang et al. (2019), obtained multiple human cell lines of TNBC and prepared them per specific laboratory protocols to ensure consistency and accurate results. An initial screening was performed to include 20 anthelmintic drugs and their effects on sensitizing TNBC cells to irradiation. Seven of the 20 drugs screened were found to have sensitizing effects with the other drugs found to either have no effect or even promote IR-induced dedifferentiation (decreased sensitivity to irradiation). MBZ was selected due to having the highest toxicity to the majority of TNBC cells, anticancer and antitumor characteristics reported in previous studies, and a favorable low-toxicity profile in previous clinical use, according to Zhang et al. The study then

moved to test MBZ effects on the breast cancer-initiating cells (BCIC) with the use of monitoring ALDH1 reported as an accurate and reliable marker for the viability of BCICs.

These TNBC cell lines were treated by Zhang et al. (2019) with a single dose of MBZ resulting in a reduction in viable BCICs along with reducing the ability to self-renew in two of the TNBC cell lines, with an overall reduction in BCICs in the TNBC cells ($p < 0.01$). Also noted within the study was MBZ's ability to reduce the hedgehog signaling pathway that plays a role in the TNBCs cell's ability to maintain a cancer-initiating characteristic in normal and cancer cells by regulating a downstream effector of the hedgehog pathway ($p < 0.0001$). The ability of MBZ to improve efficacy by sensitizing cells to radiation was also evaluated by administering a single dose of MBZ followed by increasing doses of radiation. Zhang et al. then compared these results to control groups receiving radiation alone. Results included MBZ-treated cells showing significantly increased sensitivity to radiation in two of the TNBC cell lines with a lower surviving fraction, regardless of the radiation dose ($p < 0.0001$). The effects of MBZ on apoptosis and other cellular markers were performed on mice models and were excluded from this review due to adherence to our methods section.

Overall, this study followed appropriate protocols to produce data valuable to the assessment of MBZ efficacy in TNBC cells. The study provided appropriate statistical analysis to determine the statistical significance of the data reviewed. A weakness of the study includes using mice models for investigation of cell cycle inhibition, as these were excluded from this review in the methodology, but in vitro studies discussed provide valuable data for discussion.

In a human tissue study, Zhu et al. (2022) studied the effects of Albendazole (ABZ) on isolated melanoma cancerous cell lines A375 and SK-Mel-28 to determine if any anticancer properties were present. These cell lines were then exposed to ABZ in monotherapy and

synergistically with Palbociclib, a conventional CD4 inhibitor. The cultures were all procured following laboratory standards to ensure consistency between groups. The study reports first investigating the effects of ABZ on melanoma cells vs normal human keratinocyte HaCaT cells as a control.

Zhu et al. (2022) found results indicated that the melanoma cell lines previously mentioned showed decreased viability of 50%, even with low doses, while showing no obvious viability changes in the normal HaCaT keratinocyte cell lines indicating a low toxicity level to normal human skin cells ($p < 0.001$). Secondly, the study wanted to further investigate the MOA of ABZ and its effects on the cell cycle. Regulatory protein levels and flow cytometry analysis of each cell cycle were monitored for effects when exposed to ABZ. ABZ was found to induce cell arrest within the G2/M cycle when monitoring cell cycle-specific proteins and induce apoptosis determined by cell viability when compared to controls ($p < 0.001$). When analyzing the effects of synergy as previously mentioned, ABZ in combination with the conventional therapy of CD4 inhibitor Palbociclib increased cell G2/M cell cycle arrest and apoptosis more so than either of the treatments alone, indicating synergistic effects. In monotherapy, a slight increase in efficacy was noticed in ABZ monotherapy vs Palbociclib monotherapy. All data from this study was extrapolated from charts that Zhu et al. report all these findings to be within statistically significant ranges. ($p < 0.05$, $p < 0.01$, $p < 0.001$).

This study also included mouse models which were excluded due to being an exclusion criterion within the methodology of this review. This study shows the strengths of using multiple cell lines to ensure variability and efficacy against multiple cell lines of melanoma. The study also included a control on all study methods to improve the statistical significance of the data.

Graphics representing the findings along with actual photos of cell viability were available to provide a visual of efficacy along with all data analyzed being statistically significant.

Effects of Benzimidazoles on Survival Measures, Response Rates, and Biomarkers

In a single-patient case study, Dobrosotskaya et al. (2011) monitored the long-term effects of mebendazole in a patient with metastatic adrenocortical carcinoma (ACC) refractory to and intolerant of conventional therapy. The patient is a 48-year-old male with no significant medical history and an adequate functional status who was initially found to have an isolated adrenal mass. Surgical resection was performed with negative margins for ACC on pathology. Subsequent imaging and follow-up revealed metastatic disease, which was then treated with mitotane, 5-fluorouracil, bevacizumab, radiation, and other traditional therapies without a reduction or cessation of disease progression. Following these failed therapies, all therapies were discontinued and MBZ was initiated with 200mg given orally each day along with palliative radiation of the tumor site for analgesic effects. The dose of MBZ remained constant for the duration of this study. It was well tolerated, with some nausea reported with initial mediation administration that subsequently subsided after an unreported amount of time (Dobrosotskaya et al., 2011).

To determine the efficacy, MBZ was administered and Dobrosotskaya et al. (2011) monitored the primary metastasis lesions of the liver and graded them with RECIST criteria. Upon follow-up two months into treatment, hepatic metastasis was defined as “stable” per RECIST criteria with a 17% to 42% decrease in the diameter of liver metastatic lesions. With continued monitoring and MBZ therapy, the patient’s metastatic disease fluctuated without significant fluctuation to progression or regression for a total of 19 months. During these 19 months, the patient reported quality of life measures returning to that of pre-diagnosis levels.

During this time, comprehensive metabolic panels were obtained with no findings of toxicities from mebendazole therapy. They report these findings lasted until his 24-month follow-up where metastatic disease had shown progression and the addition of other therapies was initiated essentially ending this case study (Dobrosotskaya et al., 2011).

While being a single-patient study limits the significance of these findings, the documented CT scans monitoring these lesions do show the results of MBZ use in this patient. Also of note, continued palliative radiation may have affected these findings, although the study reports these were targeted at his right flank and did not report suspected involvement of the hepatic lesions. Being a single case study, the only objective findings reported are that of the CT scan and RECIST criteria, but there is no way to confirm if this is due to the therapy as this could have been the natural progression of this patient's disease, weakening the value of this information.

As previously discussed in the safety portion of this literature review, Hegazy et al. (2022) researched the efficacy and safety of MBZ as an adjuvant therapy after bevacizumab and FOLFOX4 for metastatic colorectal cancer. They determined the eligibility of 40 individuals and randomly placed them into either an MBZ treatment group or a control group. These groups were followed for a median duration of 12 months to determine one-year overall survival and progression-free survival. Overall response rate (ORR) was monitored via serial CT scans and classified as in complete remission, partial response, stable disease, or increase in target lesion. These measures (ORR and biomarkers) contribute to the primary endpoint of investigating the anti-tumor activity (of mebendazole). Secondarily, Hegazy et al. set the endpoints of PFS, OS, and safety and tolerability to the adjuvant MBZ.

Hegazy et al. (2022) report finding MBZ was well tolerated in conjunction with bevacizumab and FOLFOX4 therapy as previously reviewed. The ORR was analyzed at a 12-week checkpoint and at the median duration of 12 months. The 12-week control group ORR showed 0 at complete response, 2 patients with partial response, 3 patients with stable disease, and 15 patients with progressive disease, respectively ($p < 0.001$). The MBZ treatment group was found to have 2 patients with complete response, 11 patients with partial response, 4 patients with stable disease, and 3 patients with progressive disease ($p = 0.001$). At the 12-month median mark, Hegazy et al. report the control group has 1 patient with complete response, 9 patients with partial response, 4 patients with stable disease, and 6 patients with progressive disease. The MBZ group at the 12-month median mark has 2 patients with complete response, 9 patients with partial response, 2 patients with stable disease, and 7 patients with progressive disease, although not considered statistically significant ($p = 0.783$). PFS is reported with the control group showing a median PFS of 3 months while the MBZ group is found to have a PFS of 9.25 months, respectively ($p < 0.001$). One-year OS is also found to not hold statistical significance but is reported a median of 12 patients surviving to one year in the control and MBZ group ($p = 0.297$).

Hegazy et al. (2022) also monitored common cancer biomarkers within each group for the duration of the study. Biomarkers were obtained at baseline between both groups and were found to have a non-significant difference between the control and the MBZ treatment group. After three months, these biomarkers were analyzed again with a significant decrease noted within the MBZ group in both biomarkers vascular endothelial growth factor (VEGF) and carcinoembryonic antigen CEA ($P<0.05$). Within the control group, the data showed a significant increase of VEGF ($p < 0.01$) and CEA ($p < 0.05$) indicating MBZ does show an effect on cancer

biomarkers (Hegazy et al., 2022). The strengths and weaknesses of this study can be found above in the review of the safety of MBZ from this article as it applies to this theme as well.

Mansoori et al. (2021) also evaluated the efficacy of MBZ in the phase 2a trial while also assessing the safety of MBZ as previously discussed (see above). Overall tumor response was evaluated at 8 weeks and 16 weeks. Eight patients were evaluated in 8-week follow-up CT imaging and seven of these patients had findings consistent with progressive disease showing new lesions within the liver and/or lung. One patient showed necrosis of metastatic tissue and was continued on MBZ adjuvant therapy per the study design. Four other patients who had previously discontinued MBZ therapy prior to week eight, due to complications, also underwent CT imaging to evaluate the effects on their duration of treatment. Three of these patients continued to show progressive disease while one restarted chemotherapy at this time and had shown tumor regression. Four of these patients were further investigated for signs of hyperprogressive disease defined as the phenomenon of cancer drug-related treatment with continued tumor progression at similar or higher rates after cessation of drugs than prior to initial therapy (Mansoori et al, 2021). The study also found that the time to tumor progression (TTP) included a median of 162 days in the period prior to MBZ therapy and a median of 59 days while on the MBZ therapy, showing a quicker time to progression of tumor while receiving MBZ therapy. Overall, Mansoori et al. (2021) noted a gradual decline in patients' status between screening and the pre-termination phase of the trial.

This study lacks n value and a control group, relying on the same patients before treatment as a control, weakening the study statistically. Premature discontinuation of this trial raises concerns about the efficacy or even harm due to increased TTP. There was no statistical data or other factors to consider when determining the strengths and weaknesses of this study. The

funding of this study was open access and provided by Uppsala University. To note, three of the authors of this study are co-founders of Repos Pharma, two others are chairmen on the board, and two stated no conflict of interest in this study.

Following in vitro studies showing promising results as previously reviewed above (Nygren, 2013), Nygren and Larsson (2014) presented a case study of a 74-year-old man with metastatic colon cancer which was resected followed by palliative chemotherapy for metastatic lung and aortic lymph node involvement with partial remission noted. Progressive disease returned with conventional therapies initiated including platinum and monoclonal antibody therapies resulting in stable disease. Therapy was stopped due to intolerance of platinum-based therapy and further conventional therapies were continued with progression of disease to include newly found liver metastasis. With the patient failing most standard therapies and not having significant organ dysfunction from disease, he became a candidate for MBZ therapy with previous preclinical trials showing possible antitumor effects.

Nygren and Larsson (2014) began therapy with the patient who was given MBZ at a dose of 100mg BID for six weeks. Upon completion of the MBZ therapy, there was no report of adverse effects and near complete remission of lung and lymph node metastasis with partial remission of liver lesions, identified by follow-up and comparative CT scans. Of mention alluding to previous reviews of the safety and tolerability of MBZ, the patient did experience a spike in ASL and ALT levels which quickly subsided after temporary discontinuation of MBZ and stabilization of liver enzymes after halving the dose of MBZ. Follow-up CT confirmed previous findings and indicated stable disease at the time of their article publication (Nygren & Larsson, 2014).

While being a single case study, the data suggest MBZ may have antitumor capabilities in colon cancer, after standard therapies are found to be ineffective. The study itself uses the patient

as a control with traditional therapy followed by MBZ monotherapy, being able to infer a comparison between the two treatments. The data is limited to having a low n value and no ability to assign statistical significance to the case. There is no report of conflicts of interest by the authors who are solely involved in the writing of the article.

Discussion

When discussing the results of these studies, it is important to consider and understand the linear method of pharmaceutical implementation which starts with basic drug investigation and theories and eventually leads to large controlled, double-blinded, randomized clinical trials on a specific patient group. Between these two points on a timeline are many different phases of laboratory studies, case studies, and preclinical trials that are ultimately used to determine if the topic should be further studied and progressed to the clinical trial phase. After reviewing this literature, it appears that determining benzimidazoles' safety and efficacy in the population of those who have cancer as an additional therapy has just recently moved into the clinical trials phase, therefore, few large clinical studies are available to discuss. We will focus on the discussion of the literature that has been published thus far in hopes of presenting the factual data to determine if it suggests that there is, or is not, sufficient evidence for these studies to move forward into larger clinical trials.

Safety Profile

As noted in the introduction, Benzimidazoles have a well-documented and researched safety profile when used as an anthelmintic which was the initial purpose for the development of the drug class. While we can infer the safety profile from these studies and years of prescribing this medication class in practice, there is importance in looking at the safety profile of benzimidazoles within the specific population where repurposing would occur, such as in

patients being treated for cancer. To determine the safety of use within the population of those being treated for cancer, topics of adequate serum concentration, adverse effects, and dose-limiting toxicities will be discussed.

When investigating the ability of benzimidazoles to reach an effective serum concentration, it is also important to understand the ratio of increased serum concentrations to MTD and DLTs. Gailla et al. (2020) were able to determine that MBZ presented no DLTs up to a dose of 200mg/kg/day. With repeat doses, which would be common for use as an antineoplastic agent, they reported 4 out of 26 patients saw increases in the AST and ALT but stated these were not concerning as it was easily reversible by lowering the patient's dose or discontinuation of the drug (Gailla et al., 2020). Similarly, Hegazy et al. (2022) reported an increase in liver enzymes of 17.5% in the control group versus the MBZ group showing 22.5%, respectively. (ALT P = 0.741 and AST P = 1). Interestingly, although not statistically significant, the study attributes this to a post-chemotherapy state as all patients had received chemotherapy in the past. The study to determine MTD by Gailla et al. (2020) being preclinical had no control group but had similar findings in their similar patient population. These patients all had previously received chemotherapy as well, which could have been attributed to the increased AST/ALT noted by Gailla et al. (2020) with no reporting of statistical analysis for this finding.

Also of note is that while these studies report benzimidazoles do not appear to have significant toxicities associated with them, patients must be healthy enough at the start of the trial to receive these doses and maintain administration to a desired serum concentration level. The study provided by Mansoori et al. (2021) does raise some questions as to whether these meaningful serum concentrations are truly achievable by certain patient populations. They attempted to follow 30 patients with advanced metastatic cancer which was refractory to all other

conventional therapies. They reported that only 11 of these patients were able to achieve desired serum concentrations to a level that met the criteria for the study. Once the study was initiated, 10 patients reached the treatment phase and the study was ultimately stopped due to the clinical deterioration and progression of disease raising the question if this was due to the administration of MBZ versus the clinical state of these patients at initiation of the study as they reported no biochemical or hematological cause for the change in patient condition (Mansoori et al., 2021). In comparison, Morris et al. (2001) reported only 3 patients out of 36 having a DLT up to their dose of 2400mg/day versus Mansoori et al. but did report one death of a participant due to neutropenic sepsis who did bring patients up to a dose of 4,000mg/day and had the results previously discussed.

After comparing the findings of these studies and analyzing aspects of benzimidazoles in similar patient populations, there appear to be varied outcomes regarding the objective measures such as MTDs and DLTs versus the more subjective clinical outcome seen in these studies. Benzimidazoles do appear to cause increases in liver function enzymes, but these studies suggest that they may be reversible, and possibly related to previous chemotherapy. Of concern is the varied outcomes of the studies with certain studies ending early from patient deterioration, and others being able to continue without significant toxicities. In reference to the original question of benzimidazoles and their comparison to conventional therapies and their toxicities, the medication has only been studied in those patients who have already received chemotherapy and not in place of said treatment. Although, when analyzing the study patients were routinely required to be off chemotherapy, radiation, and other forms of therapy for an extended period due to their reported toxicities such as having a significant effect on liver enzymes as can be seen in the above literature review. Regarding the toxicity of benzimidazoles and their ability to be

used at desired serum concentrations, it appears more large-scale clinical trials are indicated to provide statistically significant and relevant data on this matter with comparison to a control group of those who received conventional therapies alone.

Along with the necessity for low toxicity of a drug, the target population must also tolerate the therapy without significant AEs that would limit the patient's ability to continue the adjuvant therapy. As listed above, drug classes are subject to the CTCAE criteria to determine their tolerance in a patient population and are graded based on the responses of AEs reported by patients while on drug therapy. As a comparison, the subjects themselves become the control to hypothesize that these reported AEs are in fact due to the initiation of therapy. As mentioned in the literature review, participants were screened prior due to reported similar AEs associated with chemotherapy, radiation, and other conventional methods of cancer treatment and were required to be symptom-free.

As reported by Hegazy et al. (2022), the most common AEs within the MBZ group were related to the gastrointestinal tract with abdominal pain and diarrhea being the most reported AEs. None of these adverse effects were graded higher than a CTCAE grade 2, indicating a very tolerable, although common, and non-severe AE profile. Of note, the MBZ group did experience 57% of the group having these two AEs versus the control group where 23% of the group reported these complaints, suggesting that MBZ does significantly increase the occurrence of abdominal pain and diarrhea, although not severe ($p = 0.029$, $p = 0.02$). Mansoori et al. (2021) reported similar findings of the AE profile of MBZ including abdominal pain, nausea, vomiting, and decreased appetite, but reported significantly different findings based on the severity of these reporting nine serious events, three of which were fatal. The study does go on to state they do not believe these are to be related to MBZ therapy, but patient-predisposed factors were the

causative factors for these AEs. To support Hegazy et al., although a different medication within the class of benzimidazoles, Morris et al. (2001) reports similar findings to include minor gastrointestinal AEs, fatigue, and myelosuppression all primarily graded less than CTCAE 2. Infrequently reported by Morris et al. were fatigue, infection/fever, and confusion up to grade 4 CTCAE.

These findings, while subjective based on patient reporting, do include a control group for comparison in the Hegazy study and are graded according to the CTCAE in all studies providing good comparison and continuity to compare the AEs across these studies. Once again looking at the participants prior to these studies, we can infer they were symptom-free to meet the criteria for the study creating a retrospective control to compare their AEs to once they began the trial mimicking that of a patient who has completed conventional therapy and would be on observational status. Overall, there appears to be evidence that suggests benzimidazoles as a class has moderate to high occurrence of AEs mostly related to the gastrointestinal tract, which does not appear to be severe across most studies. There remains the question as to whether some of these patients did have significant AEs that the studies attributed to predisposed disease or factors, leaving questions as to how often this would happen in a clinical application and what the true cause of these findings was. Initial evidence suggests that in comparison to observational care alone after conventional therapy, Benzimidazoles may have a safety profile and tolerance level that supports further large-scale clinical trials to determine their efficacy as an anticancer medication and confirm the initial safety and tolerability data.

Mechanism of Action

With a safety profile discussed, it brings into question the efficacy of benzimidazoles as anticancer agents and where exactly they would fit into the treatment of a patient population of

those with cancer. Due to very few clinical trials, there remains a large value placed on the physiological action of benzimidazoles within lab-obtained human tissues to theorize their *in-situ* effects based on *in-vitro* results. The following discussion will compare the *in -vitro* studies of human cancer cells in a fashion of analyzing the efficacy of this drug class in various applications including the MOA, ability to sensitize cancer cells, ability to act synergistically with conventional therapies, and monotherapy.

To better understand the actions of benzimidazole and to compare them to antineoplastic agents, many of the previously mentioned studies in the literature review found MOAs that were consistent with other known antineoplastic agents. ABZ was reported to inhibit proliferation as a DNA-damaging agent and was noted to cause microtubule destabilization in MM and SCLC cancer lines state Patel et al. (2022) ($p < 0.01$). Similarly, Zhang et al. (2019) reported MBZ was found to reduce breast cancer-initiating cells viability as well as reduce hedgehog signaling pathways which also affects the breast cancer cells' ability to initiate cancerous activity ($p < 0.0001$) as well as Zhu et al. (2022) reporting cell arrest within the G2/M phase of the cell cycle ($p < 0.001$). Interestingly, in comparison to many conventional cancer therapies that are well known to cause harm to healthy human tissue, Zhu et al. found that not only was ABZ effective as an antineoplastic agent, but it was highly specific to the cancer cell lines showing low toxicity to the human cell control groups ($p < 0.001$). This was also noted by Nygren et al. (2013) as well as Zhu et al. in their normal tissue controls ($p < 0.001$). An initial review of these studies shows promise based on their MOA being comparable to known effective antineoplastics and, if *in-vitro* studies are a good predictor of *in-situ* studies, this drug class has the potential to show high specificity to cancer cells resulting in a likely higher safety and tolerability profile as was noted within the safety profile of benzimidazoles.

Benzimidazoles as Monotherapy

When considering benzimidazoles as a monotherapy, multiple *in-vitro* studies showed efficacy in reducing cancer cell viability indicating probable antineoplastic effects when employed as a single drug. In ovarian cancer, Huang et al. (2021) reported that MBZ monotherapy significantly decreased cell viability with low doses ($p < 0.01$). Similarly, Nygren et al. (2013) reported exposing various cancerous cell lines to MBZ and also found significant inhibitory characteristics compared to that of controls. Interestingly, Nygren reported a much more varied response to certain strains and types of cancerous cell lines. They report 80% of colon cancer cell lines were susceptible to MBZ monotherapy whereas only 25% of lung cancer and 28% of renal cancer were found to be susceptible ($z < 2$ SD). This does bring into question whether benzimidazoles will show efficacy among many different cancer types, or whether their application will be more targeted to those cancers that are found to be more susceptible. To add confidence in the monotherapy anticancer effects, Patel et al. (2011) also showed inhibition of around 43% of SCLC and MM cell lines in-vitro ($p < 0.05$), whereas Zhu et al. (2022) report a decreased viability of 50% in melanoma cell lines compared to controls, respectively ($p < 0.001$). Of note, these are all percentages compared to zero inhibition of cells in controls which would represent a patient with conventional therapy and, in theory, could be represented as a patient population who was being treated with observation alone after failed therapy or who aren't able to partake in conventional therapies due to severe AEs associated with platinum-based therapies. This brings into question whether benzimidazoles would have a role in these specific cancer types, but further large-scale clinical trials would be needed to determine this.

Sensitizing and Synergism of Benzimidazoles

To determine further applications within a population of patients with cancer, many of these studies investigated the ability of benzimidazoles to sensitize cancer cells to conventional therapy, work synergistically with conventional antineoplastics, and their ability to overcome cisplatin resistance. All these applications would increase the spectrum of benzimidazoles within the role as an antineoplastic agent creating flexibility of when during treatment the therapy they could be utilized.

As previously seen in detail in the literature review, many of the studies showed efficacy in these applications. Huang et al. (2021) showed that MBZ was able to overcome cisplatin resistance ovarian cancer cell lines and inhibit cell viability as compared to the control which showed no inhibition with the treatment of cisplatin alone ($p < 0.01$). Interestingly, when combined with cisplatin therapy, Huang et al. report a significantly increased in cisplatin-based cellular toxicity when compared to cisplatin or MBZ alone ($p < 0.01 – 0.05$). These synergistic findings were also noted, although with Palbociclib, by Zhu et al. (2022) in melanoma cell lines showing increased efficacy as a combination therapy versus either ABZ or Palbociclib alone ($p < 0.05$). Similarly, Patel et al. (2011) reported, that in conjunction with radiation, ABZ co-therapy was able to sensitize the MM and SCLC cells to radiation as the radiation-only control group showed 61.6% cell survival compared to those treated with ABZ showing only 28.1 % survival ($p < 0.05$). Again, supporting these findings of increased radiation sensitization, Zhang et al. (2019) found that MBZ-treated cells significantly increased sensitivity to radiation in breast cancer cell lines with a lower surviving fraction compared to that of the radiation-only control ($p < 0.001$).

These findings, although non-clinical trials, give insight into benzimidazoles' future to be applied in various scenarios and cancer types. It is important to note that, while valuable

information, *in-vitro* studies can only theorize outcomes in patient populations. Many factors come into play once initiating preclinical and clinical trials that cannot be accounted for *in-vitro*. These studies appear to show that benzimidazoles have efficacy on the cell lines themselves in various settings in relation to their ability to act as sensitizers and work synergistically, but further large-scale clinical trials as we will discuss below are necessary to confirm these findings.

Benzimidazoles in the Clinical Setting

As apparent in the literature review, the clinical application of benzimidazoles is ongoing and in its infancy with one randomized, controlled, and double-blinded clinical trial having been published at the time of this writing. With the nature of introducing medications into the patient population, we will discuss some single-patient case studies currently published understanding that these provide anecdotal data with no statistical data available. The information obtained from these studies does provide important information to suggest whether the medication being studied should advance to further trials based on initial findings and is worth discussing and comparing to findings in the larger clinical trial that was recently published. To discuss findings, we will focus on three categories commonly seen throughout the studies including effects on biomarkers, tumor response, and quality of life measures.

Cancer biomarkers are commonly monitored as a sign of efficacy and response to therapy as well as indicating the action of the medication on known and presumed biomarkers for cancer pathways. While these are not as specific as CT imaging and nuclear medicine studies, they do provide insight as to what may be happening on a physiological level. As previously discussed in the effects of benzimidazoles on human tissue cancer cells, many anti-cancer pathways were found to have been affected by MBZ and ABZ administration. While only one clinical trial is

available that has studied this, Hegazy et al. (2022) report findings within the clinical setting that would suggest these early presumptions have merit. The study reported monitoring VEGF and CEA showed a significant decrease in the serum levels of these pro-cancer biomarkers within the MBZ group versus the control group ($p < 0.05$) (Hegazy et al., 2022). While there are no other clinical trials currently identified to compare these findings, it appears that studies on these cell lines have translated into the clinical population indicating that benzimidazoles can, at clinically attainable serum values, inhibit pro-cancer tumor markers such as VEGF and CEA as compared to control groups representing those undergoing conventional therapy and observation alone as these patients had all previously received conventional therapy. More studies within varied cancer-type populations appear needed to provide further evidence and to increase confidence in these initial clinical trial findings.

Tumor response to benzimidazoles appeared to be one of the most investigated categories of efficacy among the studies within the literature review. One of the first indications of the efficacy of benzimidazoles on tumors was published in a case study by Dobrosotskaya et al. 2011) as previously presented in the literature review. The study found that MBZ, following conventional therapies, was able to transition the 48-year-old patient's metastatic liver lesions to the stable category within the RECIST criteria and reported a 17-42% decrease in liver lesion size. Furthermore, the disease remained stable without significant fluctuations on serial CTs for 19 months up until the 24th month of treatment when metastatic disease returned. Similarly, Nygren and Larson (2014) followed this case study with findings consistent with Dobrosotskaya et al. reporting near complete remission of lung and lymph node involvement and partial remission of liver lesions per RECIST criteria of a 74-year-old's metastatic colon cancer, also monitored by comparative CT scans.

To expand on these findings, Hegazy et al. (2022) in their clinical trial reported data to suggest these previously discussed case studies' findings could indicate efficacy. The study reports monitoring ORR, which would be considered comparative to tumor response as both are monitoring serial CTs of metastatic lesions. Hegazy et al. investigated the ORR at 12 weeks and 12 months with varied results. The 12-week ORR control group had significantly more patients within the stable disease to progressive disease categories as compared to the MBZ group having the majority of patients within the complete or partial response categories with full breakdown available within the literature review ($p < 0.001$). The 12-month follow-up was much more mixed with both the MBZ and control group showing very similar disease states in patients scattered from a complete response to progressive disease, indicating less efficacy with a longer duration of treatment, although reported as not statistically significant ($p = 0.783$). To add to the mixed findings among clinical patients, Mansoori et al. (2021) evaluated eight patients in serial CT imaging following the administration of MBZ with very different findings than previously discussed. Mansoori et al. reported week 8 findings showing progressive disease had continued in seven out of eight of the patients. Of concern, four patients were further investigated for hyper-progressive disease indicating that not only was MBZ possibly not effective, but it may also have accelerated tumor progression. More evidence of this was reported with time to tumor progression being 103 days sooner in the MBZ group vs the control, although no statistical data was available for this study (Mansoori et al., 2021).

Overall, when comparing these case studies, pre-clinical, and clinical trials there appears to be data suggesting that benzimidazole may have efficacy in altering RECIST criteria, especially early during the treatment period. There is some question as to whether treatment with benzimidazoles provided longer-term benefits to prevent the progression of disease as well as if

some patients may have a hyper-progressive reaction to the therapy. While initial data suggest efficacy in tumor response, more large-scale clinical trials are indicated to further investigate the short and long-term efficacy and for paradoxical reactions to the therapy.

The final measures to discuss are those which deal with quality of life and its effects on the duration of survival. While these measures are usually reported in terms of one-year survival rate, overall survival, and progression-free survival, their presence is limited as these are commonly found in larger studies with long-term follow-up. To discuss what was presented, we can recall that Dobrosotskaya et al. (2011) reported their patient in the case study experienced 19 months of quality-of-life measures returning to pre-diagnosis levels, something the patient hadn't seen with any other forms of conventional treatment. To add to this, Hegazy et al. (2022) reported that their control group was found to have a 3-month PFS vs the MBZ group showing a PFS of 9.25 months indicating over 6 months of well-tolerated therapy being free of disease progression ($p < 0.01$). Considering these findings, along with the very well-tolerated medications compared to conventional therapies like platinum bases or monoclonal antibodies, data suggests that benzimidazoles appear to increase quality of life measures while extending post-treatment progression of disease, indicating very desired outcomes. To confirm this, large-scale clinical trials would be indicated to increase the confidence of these preliminary and singular clinical trial findings.

Conclusion

As is frequently evident in this document, the process of establishing a drug and its indication within a desired patient population is a monumental task and is not taken lightly as the outcome and safety of the patient are on the line. The question of risk versus benefit during the clinical trial phase becomes a difficult one, as data continues to be collected from previous

clinical trials and the choice of participation remains difficult as data is few and shows mixed results in the early clinical stages as trends are established. For this reason, the statement of more large-scale, controlled, and blinded clinical trials are required to determine the efficacy of this drug class against cancer is mentioned frequently within the discussion. With only one of these clinical trials available for examination at the time of this review, it is difficult to determine the relationship that benzimidazoles have on all the cancer markers discussed in this review.

Overall, the safety and toxicity profile of benzimidazoles is highly studied from previous research as an anthelmintic. This information was applied cautiously to drug repurposing among patients with cancer. The studies discussed above did show a bit more complicated picture as these immunosuppressed and metastatic patients did appear to have a tougher time with liver parameter monitoring, as well as adverse effects, requiring some dose adjustment due to toxicities associated with the administration of the drug. Overall, although limited studies, the safety profile does point toward being well tolerated as compared to other antineoplastics and would indicate continued clinical trials to determine efficacy as well as add confidence to the safety profile of benzimidazoles used within a patient population with cancer.

A bit less certain is the efficacy of benzimidazoles on cancer biomarkers, tumor progression, and quality of life. Much of the data reviewed, aside from one clinical study, was obtained from case studies and early-phase clinical trials. While this information is valuable and does indicate the hypothesized MOA of benzimidazoles may have a positive effect against cancer, more clinical trials are required to determine if these early findings will translate into an accurate trend in the future. During the writing of this literature review, the Hegazy et al (2022) trial was the only applicable ongoing clinical trial, which was completed in June of 2022. A search of clinicaltrials.gov in November 2022 showed there were four studies actively recruiting

or in progress researching benzimidazoles that would apply to use in cancer patient populations (National Institute of Health, n.d). Further monitoring of these studies, along with more studies like that of Hegazy et al. (2022) will be required to fully answer the initial question posed, In patients with cancer who have received conventional therapy, does the addition of adjuvant benzimidazoles versus those receiving conventional therapy or observation alone show improved safety and efficacy with increased antitumor tissue response, survival measures, and reduction in cancer biomarkers?

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