RISKS AND BENEFITS OF ADJUVANTS TO CANCER THERAPIES

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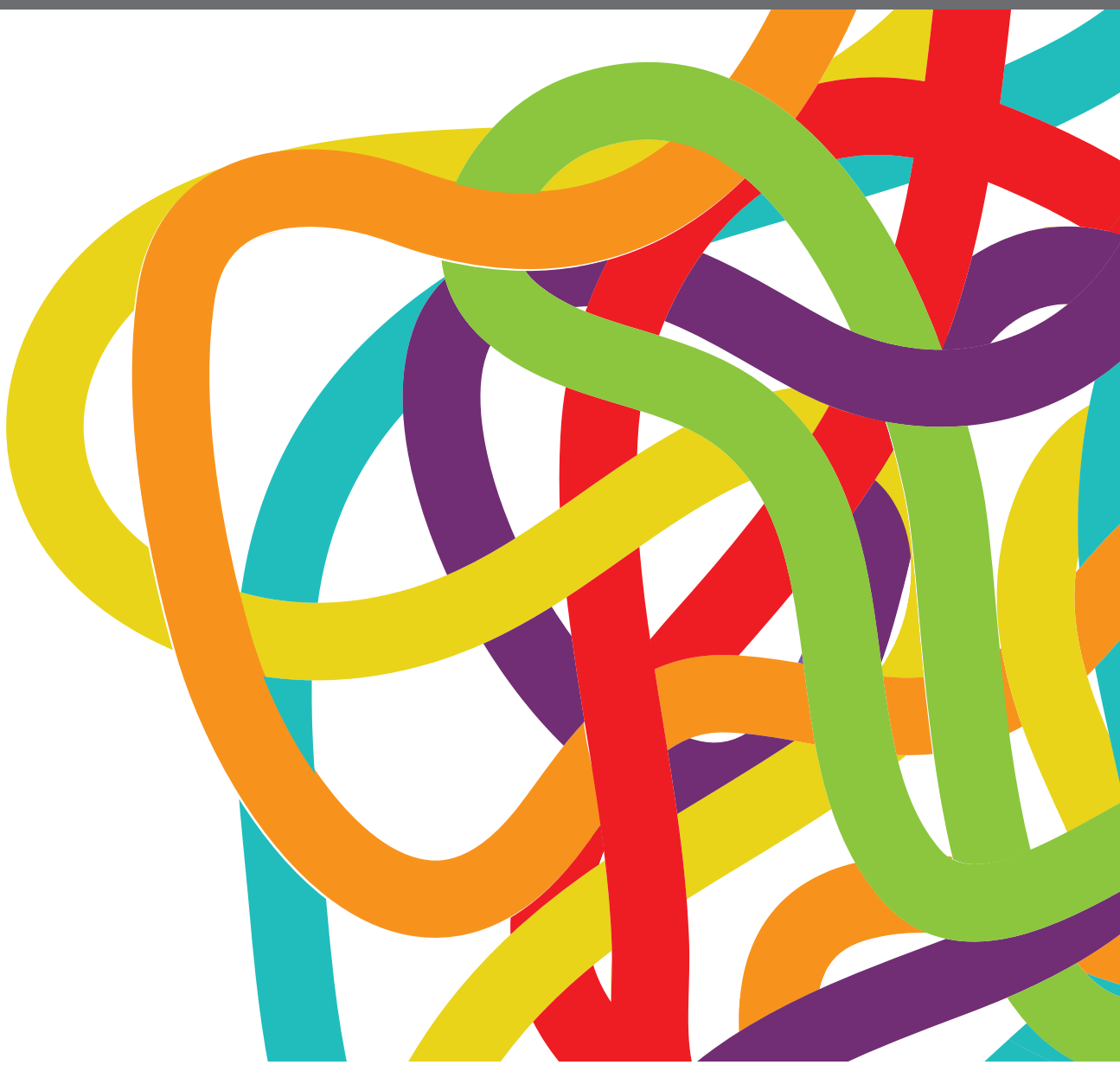
PUBLISHED IN: Frontiers in Oncology and Frontiers in Pharmacology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88976-269-9
DOI 10.3389/978-2-88976-269-9

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RISKS AND BENEFITS OF ADJUVANTS TO CANCER THERAPIES

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Citation: Dong, P., Gewirtz, D. A., eds. (2022). Risks and Benefits of Adjuvants to Cancer Therapies. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-269-9

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frontiers | Frontiers in Oncology

EDITORIAL
published: 16 May 2022
doi: 10.3389/fonc.2022.913626

Editorial: Risks and Benefits of Adjuvants to Cancer Therapies

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Keywords: Cancer therapy, adjuvant therapy, vitamin E, herbal, probiotics, curcumin, alantolactone, thalidomide

Editorial on the Research Topic

Risks and Benefits of Adjuvants to Cancer Therapies

Cancer patients may have minimal residual disease after completing primary treatment, which could be a source of subsequent early recurrence and metastasis. Adjuvant therapy (including chemotherapy, radiation therapy, hormone therapy, targeted therapy, and immunotherapy) has the potential to remove minimal residual disease and increase patient survival. In contrast, neoadjuvant therapy is utilized to shrink tumors prior to the primary treatment. Ongoing research in the fields of adjuvant and neoadjuvant cancer therapy is expected to yield new drugs and innovative approaches that can be combined with existing therapies to improve patient outcomes and prevent cancer recurrence.

The current Research Topic, titled "Risks and Benefits of Adjuvants to Cancer Therapies" includes 12 scientific studies (original research articles, reviews, and case reports).

Khallouki et al. established that vitamin E compounds, known as tocols (including tocopherols and tocotrienols), directly interact with estrogen receptors (ERs), thereby activating the transcription of an estrogen-responsive reporter gene in breast cancer cells. Tocopherols induce the proliferation of ER-positive breast cancer cells but not ER-negative breast cancer cells, while tocotrienols inhibit the proliferation of both ER-positive and ER-negative breast cancer cells. These studies indicate that tocopherols and tocotrienols have different roles in regulating cancer cell proliferation.

Zha et al. assessed the benefits and hazards of postoperative adjuvant chemotherapy versus surgery alone in patients with colorectal cancer. They found that patients with stage II/III colorectal cancer can benefit greatly from postoperative adjuvant chemotherapy, providing useful information for making decisions about the advantages and hazards of adjuvant chemotherapy in patients with colorectal cancer following resection.

Mei et al. investigated whether adjuvant treatment would benefit patients with pT2N0M0 gastric cancer, which is defined as tumors infiltrating the muscularis propria [T2], no regional lymph node metastases [N0], and no distant metastasis [M0]. Patients with pT2N0M0 gastric cancer who received adjuvant chemotherapy had higher 5-year overall survival and disease-specific survival rates. Thus, adjuvant chemotherapy may be considered for patients with pT2N0M0 gastric cancer.

Fasinu and Rapp reviewed the interaction between herbal and chemotherapeutic drugs. According to recent patient data, some of these supplements may interact with chemotherapy drugs. As a result, it would be prudent to avoid taking anti-cancer medications and natural products at the same time.

Lu et al. reviewed the effects of probiotics in preventing and treating cancer. As gastrointestinal discomfort is a common side effect of anti-tumor therapy, probiotics can help to improve the intestinal environment, increase the functionality of the intestinal mucosal barrier, and minimize the occurrence of diarrhea. The ability of probiotics to improve anti-cancer side effects has been also linked to innate immunity.

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: *06 April 2022*
Accepted: *25 April 2022*
Published: *16 May 2022*

Citation:
Dong P and Gewirtz DA (2022) Editorial: Risks and Benefits of Adjuvants to Cancer Therapies. Front. Oncol. 12:913626. doi: 10.3389/fonc.2022.913626

Xu et al. summarized the potential clinical applications of curcumin as an adjuvant to osteosarcoma treatment. Even though curcumin appears to have a high synergistic effect in other therapies (chemotherapy, immunotherapy, bone tissue engineering, and biomaterials), curcumin's properties such as hydrophobicity and low absorption, hinder its anticancer impact. Clearly, more research will be required to resolve these challenges.

Tian et al. discussed the advantages of carboplatin- and paclitaxel-based adjuvant and neoadjuvant chemotherapies in early triple-negative breast cancer. Their review shows that in both neoadjuvant and adjuvant contexts, the combination of carboplatin and paclitaxel resulted in a greater histological complete response in patients with early triple-negative breast cancer.

Cai et al. reviewed the molecular processes by which Alantolactone, a natural chemical isolated from the Chinese traditional medicine Inula helenium L, exerts anti-cancer effects and the potential of alantolactoneas as cancer therapeutic agents.

Xie et al. conducted a comprehensive assessment of the efficacy and safety of thalidomide in the treatment of chemotherapy-induced nausea and vomiting (CINV) in patients who had received highly emetogenic chemotherapy (HEC). The authors pointed out that thalidomide is effective and safe for preventing CINV in HEC patients, and that it has a considerable propensity to improve patients' quality of life.

Jiang et al. explored the connection between antibiotic use and the survival of cancer patients receiving immune checkpoint inhibitors. The authors proposed that antibiotic administration was significantly associated with worse progression-free survival and overall survival in these patients. Consequently, antibiotics should likely be used with caution in cancer patients who are being treated with immune checkpoint inhibitors.

In a case report by Money et al., the use of intravenous administration of magnesium before cisplatin was found to be the best practice to prevent cisplatin-induced acute kidney injury and hypomagnesemia. This is an intriguing observation that is likely deserving follow-up in the clinic.

Takayama et al. reported on a case of advanced malignant melanoma that developed prolonged anorexia and nausea after receiving nivolumab and was successfully treated with Kampo medications.

Overall, these papers in this Frontiers Research Topic touch on recent efforts to uncover novel adjuvant cancer medications, as well as new combinations of adjuvant therapies with existing treatments. For many malignancies, recommending adjuvant therapy and selecting the best medicines remains a challenge. Because human malignancies have such a wide range of remarkable biological diversity that affects treatment efficacy, optimal management or precision adjuvants will be required to generate effective therapeutic strategies where the anticipated benefits will have to be balanced with issues of tolerability. Promising prognostic and predictive biomarkers may help guide adjuvant therapy use (**Figure 1**).

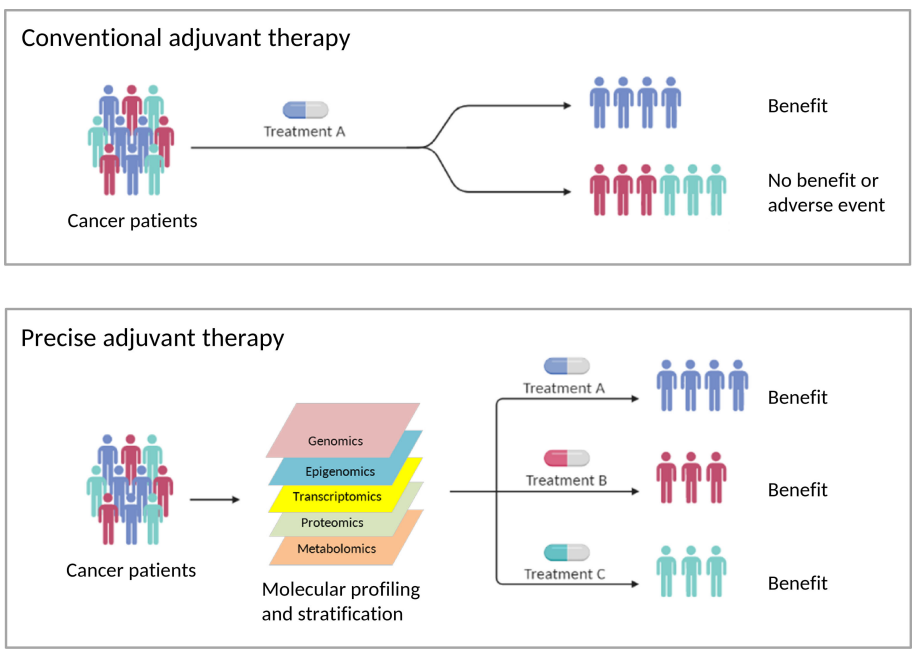

FIGURE 1 | The Risks and Benefits of Adjuvants to Cancer Therapies (created with BioRender.com). Conventional adjuvant therapies aim to provide treatments for the average cancer patient. As a result, this strategy is less effective in treating individual patients, and it is difficult to avoid side effects. To develop successful adjuvant therapies, optimal management or precise adjuvants will be necessary, where the predicted advantages must be balanced with concerns about tolerance. Promising prognostic and predictive biomarkers might help modify existing adjuvant cancer therapies or lead to the discovery of new adjuvant cancer therapies or adjuvant therapy combinations.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

PD was supported by a grant from JSPS Grant-in-Aid for Scientific Research (C) (19K09769; 22K09541 and 22K09634). Work in the Gewirtz laboratory is supported by NIH/NCI grants CA260819 and CA239706 and by Grant #W81XWH19-1-0490 from the Department of Defense Breast Cancer Research Program.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Oncology

ORIGINAL RESEARCH
published: 05 January 2016
doi: 10.3389/fonc.2015.00287

Molecular and Biochemical Analysis of the Estrogenic and Proliferative Properties of Vitamin E Compounds

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Tocols are vitamin E compounds that include tocopherols (TPs) and tocotrienols (TTs). These lipophilic compounds are phenolic antioxidants and are reportedly able to modulate estrogen receptor β (ERβ). We investigated the molecular determinants that control their estrogenicity and effects on the proliferation of breast cancer cells. Docking experiments highlighted the importance of the tocol phenolic groups for their interaction with the ERs. Binding experiments confirmed that they directly interact with both ERα and ERβ with their isoforms showing potencies in the following order: δ-tocols > γ-tocols > α-tocols. We also found that tocols activated the transcription of an estrogen-responsive reporter gene that had been stably transfected into cells expressing either ERα or ERβ. The role of the phenolic group in tocol–ER interaction was further established using δ-tocopherylquinone, the oxidized form of δ-TP, which had no ER affinity and did not induce ER-dependent transcriptional modulation. Tocol activity also required the AF1 transactivation domain of ER. We found that both δ-TP and δ-TT stimulated the expression of endogenous ER-dependent genes. However, whereas δ-TP induced the proliferation of ER-positive breast cancer cells but not ER-negative breast cancer cells, δ-TT inhibited the proliferation of both ER-positive and ER-negative breast cancer cells. These effects of δ-TT were found to act through the down regulation of HMG-CoA reductase (HMGR) activity, establishing that ERs are not involved in this effect. Altogether, these data show that the reduced form of δ-TP has estrogenic properties which are lost when it is oxidized, highlighting the importance of the redox status in its estrogenicity. Moreover, we have shown that δ-TT has antiproliferative effects on breast cancer cells independently of their ER status through the inhibition of HMGR. These data clearly show that TPs can be discriminated from TTs according to their structure.

Keywords: estrogen receptor alpha, estrogen receptor beta, vitamin E, molecular modeling, gene transcription, HMG-CoA reductase, breast cancer, proliferation

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: *12 October 2015*
Accepted: *06 December 2015*
Published: *05 January 2016*

Citation:
Khallouki F, de Medina P, Caze-Subra S, Bystricky K, Balaguer P, Poirot M and Silvente-Poirot S (2016) Molecular and Biochemical Analysis of the Estrogenic and Proliferative Properties of Vitamin E Compounds. Front. Oncol. 5:287. doi: 10.3389/fonc.2015.00287

INTRODUCTION

Vitamin E was first characterized in wheat germ oil and lettuce in 1922 (1). Vitamin E compounds are also known as tocols and include eight structurally related forms separated into two groups: tocopherols (TPs), in which the isoprenoid side chain is saturated, and tocotrienols (TTs), in which the side chain is unsaturated. The α-, β-, γ-, and δ-TP and -TT isomers are named according to the number of methyl groups on the chromanol ring at the 3, 5, and 7 positions (**Figure 1**). Vitamin E compounds have been extensively used in pharmacological studies due to their antioxidant properties; however, a major difference exists between TPs and TTs. TTs are potent down regulators of both HMG-CoA reductase (HMGR) and the isoprenoid-cholesterol biosynthesis pathway, and reduced cancer cell proliferation (2). In addition, TTs have been reported to induce cell cycle arrest and inhibit NFκB pathways and angiogenesis (3–6). Compared to TTs, much more is known about the effects of TPs. α-TP is quantitatively the major form of vitamin E found in humans and animals (7), whereas other TPs are present in various fat oils, such as palm oil (8) and argan oil (9). Many studies have focused their attention on vitamin E succinate (VES), a synthetic derivative of α-TP in which the hydroxyl phenol is esterified through succinylation. In contrast to α-TP, VES displays antiproliferative properties through an as-yet undefined mechanism *in vitro* and *in vivo* (10) but does not have antioxidant properties due to the esterification of the phenolic group. Vitamin E are fat-soluble antioxidants, and numerous studies have proposed that they can help in preventing or modulating diseases associated with oxidative stress, such as cardiovascular diseases (11, 12), neurodegenerative diseases (13), and cancers (14). Despite this, clinical trials have failed to establish any preventive effects of α-Toco on cardiovascular diseases and cancer (15–17). Recently, however, it was reported that dietary administration of δ- and γ-TP inhibited tumorigenesis in an animal model of estrogen receptor (ER)-positive but not human epidermal growth factor receptor (HER-2)-positive breast cancer (18). Parallel to this observation, a number of studies have shown that vitamin E, as an antioxidant, may interfere with the pharmacological action of some anticancer drugs, which rely on reactive oxygen species production as part of their mechanism of action (19). This is the case for the anticancer drug tamoxifen and other selective antiestrogen-binding site (AEBS) ligands such as tesmilifene, developed for the treatment of breast, lung, and prostate cancers (20–22), all of which have their antiproliferative and proapototic activities blocked by α-TP (23–27). α-TP has also been shown to inhibit the lipoperoxydation of cholesterol,

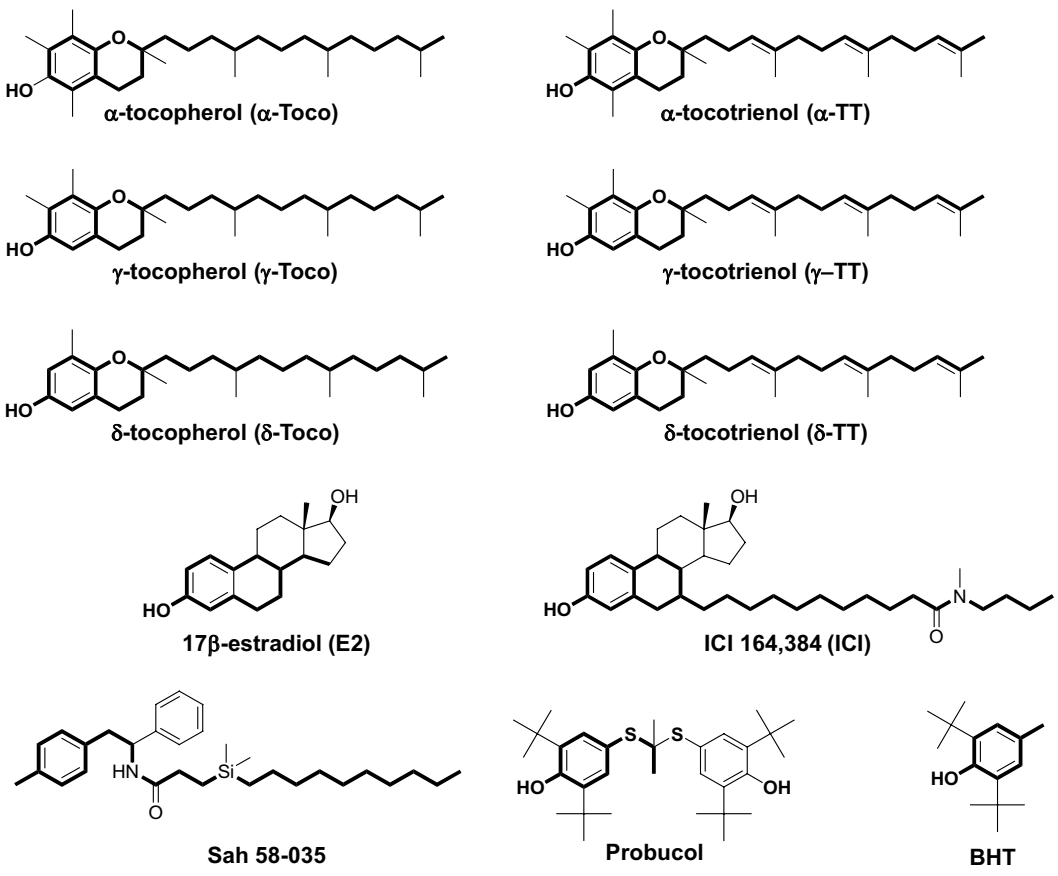

FIGURE 1 | Chemical structures of α-, γ-, and δ-tocopherol; α-, γ-, and δ-tocotrienol; 17β-estradiol; ICI 164,384; Sah 58-035; probucol; and butylated hydroxytoluene (BHT). Boldface type indicates the part of the molecules that are superimposable.

blocking the production of the prodifferentiation and proapototic cholesterol-5,6-epoxides that have been identified as mediators of tamoxifen activity in breast cancer cells (21, 26, 28, 29). These data suggest that the intake of α-TP during prophylactic or curative treatment could impair the clinical outcome of patients treated with tamoxifen. In fact, many patients undergoing breast cancer treatment are known to take antioxidant dietary supplements, which may have a negative impact on their clinical outcome (30).

Tocopherol and TT contain structural determinants such as a phenol group, a cyclic structure, and long hydrophobic side chains that make them possible ligands for ERs (31). One study reported them to be weak modulators of ERβ but curiously found that they did not affect ERα activity (32). ERs are nuclear receptors (NRs) that mediate the biological effects of estrogens. They influence many physiological processes, including not only reproductive functions but also hormone-dependent cancers, cardiovascular health, bone integrity, immunity, cognition, and behavior (33, 34). The present study aimed to reevaluate the impact of TP and TT on ER-dependent transcriptional activity and breast cancer cell proliferation.

MATERIALS AND METHODS

Chemicals

[^{3}H]-17β-estradiol and [^{14}C]-HMG-CoA were purchased from GE Healthcare (UK). ICI 182,780 was from Tocris (UK). TPs and TTs were from Merk-Millipore (USA) or were kindly provided by Dr. Abdul Gapor (Kuala Lumpur, Malaysia); other compounds and chemicals were from Sigma-Aldrich (USA). All solvents were from Prolabo (France).

Synthesis of δ-Tocopherylquinone

A solution of gold III chloride (0.28 g; 0.92 mmol) dissolved in water (1 ml) was added dropwise to a solution of δ-Toco (0.36 g; 0.89 mmol) dissolved in ethanol (9 ml). The mixture was stirred in the absence of light for 2 h at room temperature. The solution was then evaporated and the solid residue was resuspended in dichloromethane and filtered. The organic layer was washed three times with water, dried over magnesium sulfate, filtered, and evaporated to dryness. The orange oil was purified by reverse phase HPLC (Ultrasep ES 100 RP 18, 250 × 8 mm, 6.0 μm, using acetonitrile for 10 min, linear gradient of 100% acetonitrile to 100% MeOH for 60 min; flow rate = 1 ml/min) and yielded a pure colorless oil product. MS: DCI (NH_3), $MH^+ = 419$; TLC Silica: Rf ($CHCl_3$): 0.18; HPLC, Rt = 30 min; UV: $\lambda_{max} = 260$ nm.

Molecular Structure Analysis

Computational chemical calculations were performed on a Silicon Graphics Indigo workstation using Insight II version 2000 (Accelrys, San Diego, CA, USA). Minimal energy conformations were calculated using the Discover module (2.9.7/95.0/3.0.0) with the CVFF force field. Van der Waals volumes and van der Waals volume intersections were determined using the Search-Compare module version 95.0 (Accelrys). We first compared the structure of α-tocopherol (δ-TP) with that of ICI 164,384. Superimposition was carried out between the energy minimized structure of α-TP and ICI 164,384 in the conformations adopted in the crystallographic structure of ERβ-ICI 164,384 (35) (Protein Data Bank 1HJ1). Superimposition was conducted using the diphenylethane part of α-TP that was superimposed carbon to carbon onto the steroidal backbone of ICI 164,384. The van der Waals volumes of α-TP and ICI 164,384 were also compared and the percentage of superimposition was calculated by measuring the ratio of the intersection of the van der Waals volume of ICI 164,384 with the van der Waals volume of α-TP.

Estrogen Receptor-Binding Assay

Estrogen receptor-binding experiments with [^{3}H]17β-estradiol were conducted exactly as reported in a previously published paper using extracts from Cos-7 cells transfected with expression vectors encoding human ERα and ERβ (36).

Molecular Modeling with Estrogen Receptors

δ-tocotrienol (δ-TT), generated as described above, was prepositioned in the 4-hydroxytamoxifen (OHT)-ERα ligand-binding domain (LBD) crystal structure (Protein Data Bank 3ERT) (37) using the Search-Compare module of Insight II (Accelrys). The superimposition of OHT and δ-TT was carried out as described previously (38). Once prepositioned, OHT was unmerged from the OHT-ERα complex and deleted, and δ-TT was then merged to the receptor. The resulting complex was submitted to energy minimization using 250 steps of the steepest descent followed by a conjugated gradient until the root mean square gradient was <0.001 kcal/mol/Å. A distant-dependent dielectric term ($\epsilon = r$) and a 20-Å non-bonded cutoff distance were chosen, whereas the hydrogen bond involved in the conformation of the α helices was preserved by applying a generic distance constraint between the backbone oxygen atoms of residue i and the backbone nitrogen atoms of residue $i + 4$, excluding prolines. This was performed using the Discover calculation engine with the CVFF force field (Insight II version 2000.1; Accelrys). The minimized coordinates of the receptor were then used as the starting point for 100 ps at 300 k using the Verlet algorithm whereas the constraint used during minimization was maintained. The resulting conformation was then further minimized using 250 steps of the steepest descent followed by a conjugated gradient until the root mean square gradient was <0.001 kcal/mol/Å.

Reporter Cell Lines and Luciferase Assay

MELN cells were established by transfecting ER(+) MCF-7 cells with the ERE-β-globin-tk-Luc-SV-Neo plasmid (36). HELN cells were generated by transfection of ER(−) HeLa cells with this plasmid. The HELN-ERα, HELN-ERβ, HELN-ΔAB-ERα, and HELN-ΔAB-ERβ cell lines then underwent a second transfection with the corresponding pSG5-puro plasmids (pSG5-ERα-puro, pSG5-ERβ-puro, pSG5-ΔAB-ERα-puro, and pSG5-ΔAB-ERβ-puro, respectively) and expressed wild-type or mutated ERα or ERβ (39, 40). Mutated ERα or ERβ have been deleted for the AB domain which possesses a ligand-independent activation function (AF1). Comparison of the activities toward hERα and hERβ with the truncated ΔAB-ERα and ΔAB-ERβ provides a powerful model to identify partial ER agonists (requiring

ligand-independent AF-1 to induce maximal ER activation). MELN and HELN cells expressed luciferase in an estrogen-dependent manner. Cells were grown routinely in DMEM growth medium supplemented with 5% FBS (Gibco BRL, Life Technologies, Cergy pontoise, France). Cells were incubated at 37°C in a humidified 5% CO_2 incubator. For experiments, cells were grown for 5 days in phenol red-free medium, containing 6% dextran-coated charcoal-treated FCS (DCC-FCS) with penicillin–streptomycin. Medium was changed after 2 days. On day 5, cells were treated or not with the compounds, which were dissolved in ethanol. For each condition, 15×10^3 cells were seeded per well in 12-well plates and treated, as described above, for 8 h in a final volume of 0.5 ml. At the end of the treatment, cells were washed with PBS and lysed in 150 µl lysis buffer (Promega, Charbonnières, France). Luciferase activity was measured using the luciferase assay reagent (Promega), according to the manufacturer's instructions. Protein concentrations were measured using the Bradford technique (41) to normalize the luciferase activity data. For each condition, average luciferase activity was calculated from the data of three independent wells.

Cell Extracts and Western Blots

MCF-7 cells were grown in 12-well plates and treated as indicated, then washed with PBS, and collected by centrifugation. Total cell lysates were prepared by resuspending the cells from each well in 100 µl lysis buffer (50 mM Tris pH 6.8, 2% SDS, 5% glycerol, 2 mM EDTA, 1.25% β-mercaptoethanol, 0.004% Bromophenol blue). Samples were boiled for 20 min at 95°C and cleared by centrifugation at 12,000 × *g* for 10 min. Protein concentration was determined by the Amido schwartz assay when samples contained SDS. Samples were subjected to PAGE on a 10% SDS-polyacrylamide gel in 25 mM Tris–HCl, 200 mM glycine, pH 8.3, 0.1% SDS, and proteins were then transferred onto a nitrocellulose membrane. Western blot analysis was performed as previously described (42) using rabbit polyclonal ERα antibodies diluted to 1 µg/ml (HC20 or H-184 Santa Cruz Biotechnology, Inc.) and the mouse antihuman glyceraldehyde 3-phosphate dehydrogenase (1:1,000). Visualization was achieved with an Enhanced Chemiluminescence Plus kit (Perkin Elmer) and luminescence was measured by either autoradiography or using a PhosphorImager (Storm 840; GE Healthcare).

Cell Proliferation Assay

MCF-7 (ER(+)), T47D (ER(+)), and MDA-MB-231 (ER(−)) cell lines were from ATCC. Cell lines were maintained at 37°C in a humidified incubator in a 5% CO_2-enriched atmosphere in T-75 flasks. MCF-7, T47D, and MDA-MB-231 cells were grown routinely in phenol red RPMI 1640 medium supplemented with 5% FBS (Gibco BRL, Life Technologies, Cergy Pontoise, France) and with penicillin–streptomycin. Cells were grown for 24 h before treatment in phenol red-free medium containing 5% DCC-FCS. Cells were seeded into 96-well plates at 2000 cells/well. Treatment media (150 µl/well) was added on the following day and replaced at 48-h intervals until the end of the experiment. Cell density was measured via the sulforhodamine B method (43) after 0, 2, 4, 6, and 8 days. The absorbance of SRB was measured directly at 490 nm in the 96-well plates using a multiskan® multisoft reader from Labsystem.

Determination of HMG-CoA Reductase Activity in Cell Extracts

The microsomal fraction of MCF-7, T47D, and MDA-MB-231 cells was prepared as previously described (44). HMGR activity was determined using the procedure first described by Brown et al. (45): 100 µg of microsomal protein was suspended in 0.1M potassium phosphate buffer pH 7.5 containing 20 mM glucose-6-phosphate, 2.5 mM $NADP^+$, 1 unit of glucose-6-phosphate dehydrogenase, 5 mM dithiothreitol and 0.2 µCi [^{14}C]-HMG-CoA. The reaction was stopped after 3 h by the addition of 25 µl 6 N HCl. Mevalonate was converted to lactone by standing at 37°C for 30 min, then extracted into 5 ml ethyl acetate, and brought to dryness by evaporative centrifugation. The sample was dissolved in 50 µl ethyl acetate and fractionated by silica thin-layer chromatography with toluene:acetone (1/1). Mevalonolactone was identified by comigration with authentic mevalonolactone visualized by iodine vapor staining and quantified storm analysis.

Statistical Analysis

Values are the mean ± SEM of three independent experiments, each carried out in duplicate. Statistical analysis was made by two-way ANOVA, where appropriate (Prism 6, GraphPad Software, Inc., San Diego, CA, USA). $^{***}P < 0.001$; $^{**}P < 0.05$; $^{*}P < 0.01$; ns: not significant.

RESULTS

TP and TT Share Structural Similarities with Estrogen Receptor Ligands

In previous studies, we used a pharmacophore approach to identify new targets for known drugs to explain some of their pharmacological properties (36, 38, 42, 46–48). We applied this approach to vitamin E compounds. The secondary structures of TPs, TTs, 17β-estradiol, ICI-164,384, Sah 58-035, probucol, and butylated hydroxytoluene (BHT) are shown in **Figure 1**. Tocols are phenolic compounds with a long hydrophobic side chain that are similar to ER ligands, such as ICI 164,384 or Sah 58-035, when drawn in a two-dimensional representation (36) (**Figure 2A**). This similarity was confirmed by comparison of the active structure of ICI 164,384 cocrystallized with ER-β with a minimal energy conformation of α-TP in a three-dimensional representation (**Figure 2A**). The van der Waals volumes of α-TP and ICI 164,384 were 406.57 Å and 469 Å, respectively (**Figure 2A**). Superimposition of the compounds is shown in **Figure 2A** and reveals that ICI 164,384 and α-TP share a common volume of 254.07 Å, which represents 63% of the van der Waals volume of α-TP. The hydrophobic side chain of both compounds gives a perfect superimposition, with the exception of the ultimate ethyl group of the side chain of ICI 164,384. This shows that the molecular volume defined by α-TP lies within the ligand-accessible volume of the ER and that the orientation of

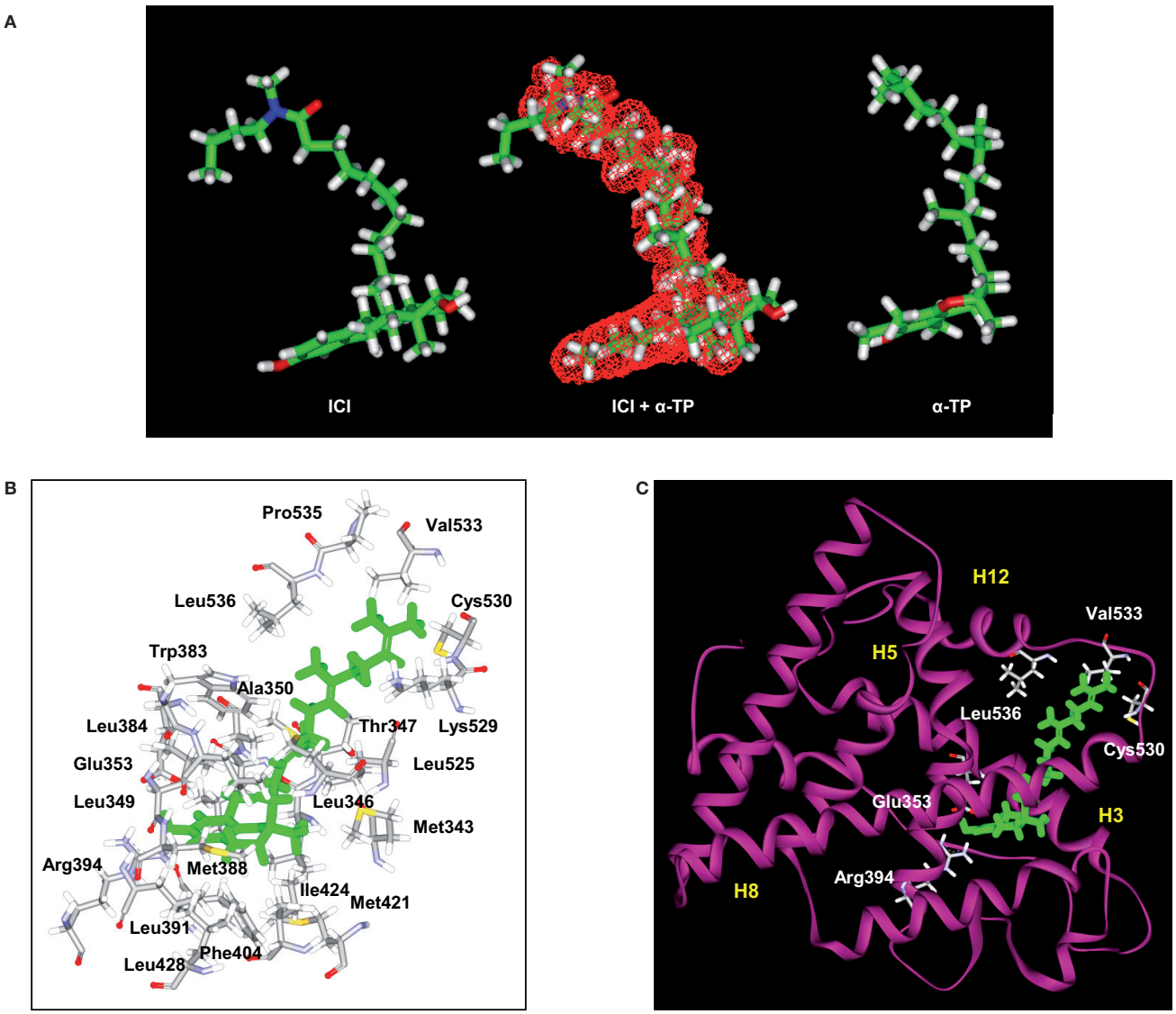

FIGURE 2 | Structural analyses of α-TP alone and δ-TP docked with ERα. **(A)** Three-dimensional structures of the conformations of ICI 164,384 (ICI) (left), taken in the crystallographic structure of rat ERβ-ICI 164,384, and the calculated minimal energy conformation of α-tocopherol (α-TP) (right). The calculated minimal energy was carried out using the Discover module of Insight II (version 2000), as described in Section "Materials and Methods." An overlay of α-TP and ICI (center), as well as van der Waals volume calculations and intersection measurements, was carried out using the Search-Compare module. The van der Waals volume intersection is depicted as the red grid and illustrates the structural similarities between α-TP and ICI. The van der Waals volumes of α-TP and ICI are 406.57 and 468.77 $Å^3$, respectively. Sixty-three percent of the van der Waals volume of α-TP is in common with that of ICI. **(B)** Amino acids that interact with δ-TT: cross-sectional view of δ-TT within the ERα ligand-binding domain. Green: δ-TT; gray: carbon atoms; white: hydrogen atoms; red: oxygen atoms; blue: nitrogen atoms; and yellow: sulfur atoms. **(C)** Ribbon representation of the molecular model of δ-TT bound to ERα. δ-TT is drawn in stick form and colored in green. Helical elements of the ER are numbered (H3, H5, H8, and H12) and colored in yellow.

the hydrophobic side chain of tocols corresponds to that of the aliphatic site chain of ICI 164,384 and Sah 58-035. Altogether, these data are consistent with a direct interaction of α-TP with the ER.

Tocols Are Ligands for ERα and ERβ

We next investigated whether α-, γ-, and δ-TPs and -TTs interact with the two human ER subtypes (ERα and ERβ) by conducting competition experiments with tritiated 17β-estradiol [^{3}H]-E2 (**Table 1**). The tocols bound to ERα and ERβ with the following order of affinity (highest to lowest): δ-tocols > γ-tocols > α-tocols. Thus, increasing the hindrance of the phenol group by increasing the number of methyl groups led to a decrease in affinity for both ERs. The oxidized product of δ-TP, δ-tocopherylquinone (δ-TPQuin), did not bind to the ERs, highlighting the importance of the phenol group in ER interaction (**Table 1**). The phenolic

TABLE 1 | ER binding experiments.

	ERα (IC_{50})	ERβ (IC_{50})
α-TP	453 ± 30 μM	431 ± 21 μM
γ-TP	227 ± 28 μM	215 ± 16 μM
δ-TP	118 ± 15 μM	98 ± 12 μM
α-TT	412 ± 22 μM	388 ± 32 μM
γ-TT	203 ± 25 μM	205 ± 18 μM
δ-TT	96 ± 6 μM	91 ± 7 μM
Probucol	N.M.	N.M.
BHT	N.M.	N.M.
δ-TPQuin	N.M.	N.M.

Extracts from cos-7 cells transfected with expression vectors encoding human ERα and ERβ were incubated with 2 nM [^{3}H]-E2 and different concentration of tocols ranging from 1 μM to 1 mM. IC_{50} values were determined using the iterative curve-fitting program GraphPad prism version 5 (GraphPad Software). N.M., not measurable.

antioxidants, BHT, and probucol, had no detectable affinity, probably because of the presence of two bulky tertiobutyl substituents adjacent to the hydroxyphenol group and because of the absence of a hydrophobic side chain. Interestingly, the IC_{50} values obtained for the TP corresponded to the concentrations they were tested on cell lines *in vitro* (100–500 μM) (23, 24, 26, 27, 49–53). These data show that tocols are ligands for both ERα and ERβ.

Molecular Modeling of the δ-TP-ERα Complex

The ability of tocols to interact with ERs raised the question of the molecular consequences of this interaction. In the absence of a crystal structure of the tocol–ER complex, we investigated this issue through molecular modeling. **Figures 2B,C** show the chemical interactions between δ-TP and ERα. Interestingly, the phenol group of δ-TP inhabited the LBD in a similar fashion as E2: the hydroxyl group interacted with Glu-353 and Arg-394. The phenyl part of the chromanol group produced a T-shaped interaction with the phenyl side chain of Phe-404 and had van der Waals contacts with the methyl groups of Leu-391 and Leu-384. These data show that the chromanol backbone of δ-TP can occupy the same cavity as E2 or diethylstilbestrol (37, 54). The side chain of δ-TP protruded into the 11β cavity of the LBD of ERα and produced multiple van der Waals interactions with hydrophobic amino acids, such as Ala-350, Leu-525, and Trp-383. The upper part of the side chain interacted with Val-533, Leu-536, Leu-539, Leu-540, and Met-543. These latter amino acids belong to helix H12, thus showing an interaction between the upper part of the side chain of δ-TP and helix H12 in this model, as was observed for Sah 58-035 (36). δ-TP established a van der Waals interaction with Met-421 but no interactions were detected with Leu-384, suggesting that they might not discriminate between the two ER subtypes, which are consistent with binding experiments. The docking of the more hindered α-tocols showed a loss of the interaction of the hydroxy phenolic group with Glu-353 and Arg-394, explaining their weaker affinity compared to δ-tocols. These data illustrate that tocols are accommodated well within the ER binding site in a similar way as that previously established with Sah 58-035 and auraptene (36, 42). This suggests that tocols can act as modulators of ERs rather than pure agonists.

Tocols Are Partial Agonists for ER-Mediated Transcription

The next set of experiments were designed to investigate whether tocols can modulate ER-dependent transcription, using MCF-7 cells stably transfected with a plasmid encoding an estrogen-responsive promoter fused to the luciferase gene (MELN cells) (36). **Figure 3A** shows that all the tocols tested stimulated luciferase transcription, with the best response obtained using 500 μM δ-TP and δ-TT, which resulted in 76.5 and 86.6% of the maximal ER-dependent response (taken as that obtained from treatment with 10 nM E2), respectively. Tocol-induced ER-dependent transcriptional activity was blocked in the presence of the ER antagonist ICI 164,384 (**Figure 3B**). As expected, compounds that were previously determined as non-ER ligands, such as δ-tocopherylquinone (δ-TPQuin), BHT, and probucol, did not stimulate the expression of luciferase (**Figure 3B**). We next established that δ-TP- and δ-TT-bound ERα was not degraded as was observed for ERα bound to E2 (**Figure 3C**). Thus, the effect of δ-TP and δ-TT on ER protein stability is similar to that of selective ER modulators, suggesting that tocols are not pure estrogens. δ-TP and δ-TT were also shown to activate ER-dependent luciferase activity through both ERα and ERβ, using HELN cells (HeLa cells transfected with the same plasmid as MELN cells, which encodes an estrogen-responsive promoter fused to the luciferase gene) (**Figure 3D**). In order to further characterize the agonistic properties of the δ-tocols, the HELN-ERα and -ERβ cell lines were used alongside the HELN-ΔAB-ERα and HELN-ΔAB-ERβ cell lines in which the N-terminal AF1 domain of the ERs (responsible for the majority of ER transactivation activity) is deleted (40). We observed that ERα-mediated transcriptional activation induced by the δ-tocols was strongly altered in the absence of the AF1 domain, whereas the loss of this domain did not significantly affect ERβ-mediated transcriptional activation induced by the δ-tocols (**Figure 3D**). To determine whether δ-TP and δ-TT can modulate the expression of endogenous E2-regulated genes as well as reporter genes, the expression of the progesterone receptor gene (PR), trefoil factor-1 (TFF1, Ps2), and transforming growth factor alpha (TGFα) was measured by quantitative RT-PCR in MCF-7 cells. Treatment of MCF-7 cells with δ-TP stimulated the transcription of TGFα (1.4-fold increase), PR (1.8-fold), and Ps2 (1.9-fold) (**Figure 3E**). The treatment of MCF-7 cells with δ-TT stimulated the transcription of TGFα (1.1-fold), PR (1.6-fold), and Ps2 (twofold). These results confirm that δ-TP and δ-TT can activate the transcription of endogenous genes that are known to be under the control of ERα.

The Effect of δ-Tocols on the Proliferation of ER(+) and ER(−) Breast Cancer Cells

To investigate the effects of δ-TP and δ-TT on cell growth, ER(+) human BC cell lines (MCF-7 and T47D) and an ER(−) BC cell line (MDA-MB-231) were used. As shown in **Figure 4A**, δ-TP

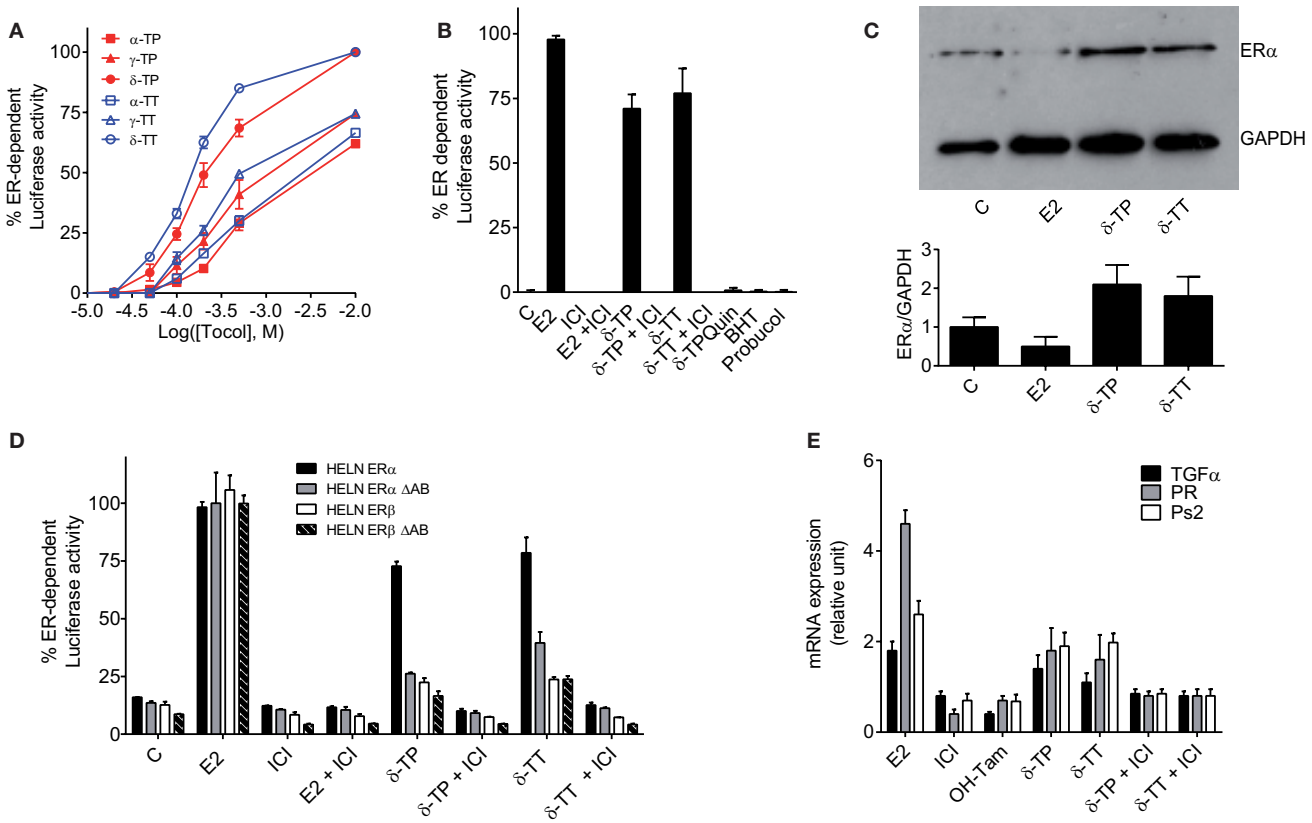

FIGURE 3 | Measurement of estrogenicity of tocols *in vitro*. **(A)** Dose–response curve of the effect of tocols on MCF-7 cells stably transfected with the ERE-β-globin-tk-Luc plasmid (MELN cells). Results are represented as the percentage of ER-dependent α-, γ-, and δ-TP and α-, γ-, and δ-TT luciferase activity obtained with 10 nM E2 and increasing concentrations of tocols ranging from 10 to 500 μM. **(B)** ER-dependent transcriptional modulatory activity of 500 μM δ-TP, δ-TT, δ-Tocopherolquinone (δ-TPQuin), butylated hydroxytoluene (BHT), and probucol in MELN cells. Cells were incubated with either 10 nM 17β estradiol (E2), 500 μM δ-TP or 500 μM δ-TT alone or in combination with 1 μM pure antiestrogen ICI 164,384 (ICI) or were incubated with 500 μM δ-TPQuin, 500 μM BHT or 500 μM probucol and assayed for luciferase activity. Data shown are the mean values ± SEM from three independent experiments. **(C)** δ-TP or δ-TT stabilized ER-α in MCF-7 cells. MCF-7 cells were cultured as described in Section "Materials and Methods" and treated with either solvent vehicle (EtOH), 100 nM E2, 500 μM δ-TP or 500 μM δ-TT for 3 h. MCF-7 extracts were analyzed for the presence of ERα by western blotting using GAPDH as a control. Visualization was achieved with an Enhanced Chemiluminescence Plus kit and fluorescence was measured by either autoradiography or using a PhosphorImager. The western blot shown is representative of three independent experiments. The ratio of ERα to GAPDH levels in each experiment was determined densitometrically and normalized to control value (taken to be 1). **(D)** Effect of δ-tocols on estrogen response element-dependent luciferase activity in HELN cells, which are HELA cells transfected with either fully functional ERα or ERβ or their mutated versions containing a deletion the AB domain (ΔAB). Cells were incubated with 10 nM 17β estradiol (E2), 500 μM δ-TP or 500 μM δ-TT alone or in combination with 1 μM pure antiestrogen ICI 164,384 (ICI) and assayed for luciferase activity. Data shown are the mean values ± SEM from three independent experiments. **(E)** δ-Tocols modulate the induction of endogenous genes under the control of ER. Cells were treated with solvent vehicle, 10 nM E2, 1 μM ICI, 1 μM OH-Tam. Cells were treated with 500 μM δ-tocols in the presence or in the absence of ICI. The relative expression of TGFα, PR, and Ps2 (TFF1) after 16 h was analyzed by quantitative RT-PCR. Data shown are the mean values ± SEM from three independent experiments.

(500 μM) induced a significant stimulation of MCF-7 and T47D cell proliferation over a 6-day period, albeit to a lesser extent than E2 (10 nM), and had no impact on ER(−) MDA-MB-231 cells. Both δ-TP- and E2-induced stimulation of proliferation was blocked by the ER antagonist ICI 164,384, consistent with an ER-mediated event (**Figure 4A**). In contrast, δ-TT inhibited the proliferation of cells, and these effects were amplified in the presence of E2 or ICI 164,384. Only cotreatment of cells with mevalonolactone (M) protected all three cell lines from the inhibitory effects of δ-TT (**Figure 4A**). Mevalonolactone is known to reverse the mevalonate-isoprenoide pathway when HMGR is inhibited suggesting that δ-TT inhibited HMGR in BC cells as observed in other cell lines (2, 55–58). We found a similar effect using when cells where treated with lovastatin, a prototypical inhibitor of HMGR, and as expected, the inhibition of cell proliferation was reversed by mevalonolactone (**Figure 4A**). These differential actions of δ-TP and δ-TT are consistent with an inhibition of HMGR activity that was observed downstream of δ-TT but not δ-TP in these cell lines (**Figure 4B**). Altogether, these data show that δ-TP stimulates cell proliferation in a similar way to that of ER agonists while δ-TT inhibits cell growth, consistent with its capacity to down regulate HMGR.

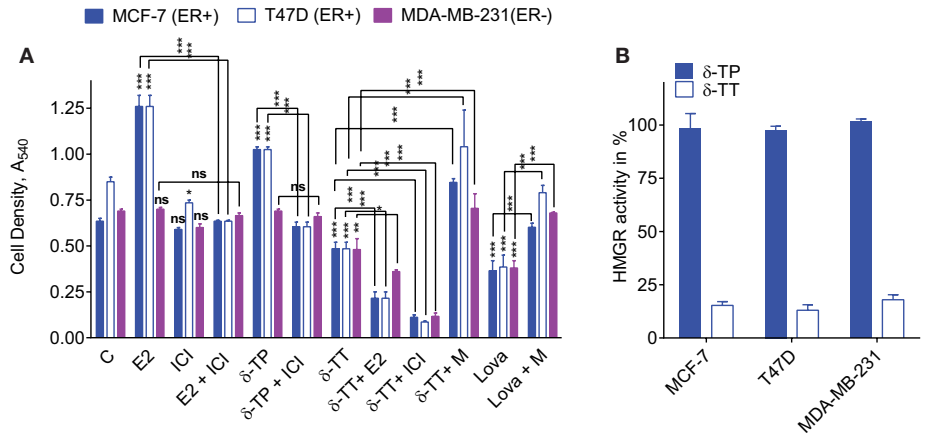

FIGURE 4 | Effect of tocol on ER(+) and ER(–) BC cell proliferation and HMG-CoA reductase activity. **(A)** Effect of δ-tocols on estrogen and non-estrogen-regulated growth of MCF-7, T47D, and MDA-MB-231 cells. Cells were treated with solvent vehicle (C), 1 nM 17β estradiol (E2), or 500 μM δ-TP in the presence or absence of 1 μM ICI. Alternatively, cells were treated with 500 μM δ-TT in the presence or absence of 100 nM E2, 1 μM ICI, or 5 mM mevalonolactone (M). Cells were also treated with 30 μM lovastatin (Lova) in the presence or absence of 5 mM M, as described in Section "Materials and Methods." Values are shown as means and vertical bars represent SEM. The data were analyzed by two-way ANOVA, followed by Bonferroni *post hoc* test. ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ in comparison with control or bare-linked specific control. Ns: not significant. **(B)** Effect of δ-TT on hydroxymethylglutaryl coenzyme A reductase (HMGR) activity in MCF-7, T47D, and MDA-MB-231 cells. Cells were treated with 500 μM δ-TT or δ-TP, as described in Section "Materials and Methods." Results are reported as the percentage of HMGR activity compared to solvent vehicle-treated cells. Data shown are the mean values ± SEM from three independent experiments performed in triplicate.

DISCUSSION

In this paper, we report the identification of a new molecular target of vitamin E compounds that sheds light on their pharmacological potency and the potential risks related to their specific substructures. Using a ligand-structure based approach, we found that TPs and TTs are ER ligands and behave like partial agonists in ER-mediated transcriptional regulation of synthetic and endogenous genes. Therefore, they are phytoestrogens. Consistent with this data, vitamin E has been previously reported to increase the expression of estrogenic markers in breast biopsies of patients (53). We found that both the effects of the tocol derivatives on transcription and their affinity for ERα decreased with the number of methyl groups present on the phenol ring of the compounds, the most potent phytoestrogens being δ-TP and δ-TT. These data emphasize the importance of the accessibility of the OH phenolic group in establishing a productive interaction with the Glu353 and Arg394 residues in ERα. Molecular modeling studies suggested that the aliphatic side chain of tocols can occupy the 11β-cavity of the LBD, as observed for the side chains of steroidal and non-steroidal ER ligands (59, 60). The tocol side chain enables their interaction with helix H12 on the NR box-binding site (**Figure 2B**), consistent with an agonistic activity. The use of AF1 deletion mutants also demonstrated the requirement of the AF1 transactivation domain for δ-tocol activity and revealed that they act differently than E2 on ERα since they do not induce receptor degradation upon binding. Other phenolic antioxidants, such as BHT or probucol, did not display any estrogenic effects, which supports the observation that the estrogenic action of δ-TP was peculiar in its ER-binding activity. The presence of a bulky tertiobutyl group in the ortho position from the hydroxyl of the phenols in these compounds may explain this effect. Furthermore, the oxidated form of δ-TP had no estrogenic activity as a consequence of its loss of affinity for binding to the ER. This established that ER binding and ER-dependent transcriptional stimulation of tocols are dependent upon their reduced form status.

It is noteworthy that δ-tocols were found to stimulate TGFα expression *in vitro* in human breast cancer cells. TGFα can activate mitogenic pathways; so, this finding highlights the potential risk that these compounds could promote tumor growth. However, dietary administration of δ-TP was shown to protect against *N*-methyl-*N*-nitrosourea hormone-dependent tumorigenesis in Sprague-Dawley rats (18); therefore, based on the present data, it is now important to determine whether this effect is observable in different rodent species because potential selective ER modulator activity has been shown to induce responses in animal models that are not seen in humans (61).

In this paper, we report that TPs and TTs are agonists for both ER subtypes. We show that δ-TP stimulated the proliferation of ER-expressing cells, whereas TTs were potent inhibitors of cell proliferation irrespective of the cell's ER status. This difference in activity could have resulted from the capacity of TTs to downregulate HMGR activity since it was reversed through addition of mevalonolactone, demonstrating the importance of the inhibition of the isoprenoide-cholesterol pathway in this effect (**Figure 4A**). Based on these data, we established that it is possible to distinguish between the action of TPs and TTs since although both TPs and TTs displayed antioxidant and ER stimulatory activity, and only TTs displayed an antiproliferative activity.

Altogether, these data have established that tocols are phytoestrogens and that their transcriptional modulation of ER must be taken into account to better understand their properties.

AUTHOR CONTRIBUTIONS

FK performed chemical, biochemical studies and analyzed data. PM performed biochemical studies and analyzed data; SC-S and KB performed biochemical and cellular experiments and analyzed the data; PB performed cellular experiments and analyzed the data; MP and SS-P designed the experiments, performed molecular modeling studies, analyzed the data, and wrote the paper.

ACKNOWLEDGMENTS

This study was supported by the Institut National de la Santé et de la Recherche Médicale, the Conseil Régional Midi-Pyrénées, and the Institut National du Cancer through the ResisTH network. FK was supported by a post-doctoral fellowship from the Institut National du Cancer.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

REVIEW
published: 03 December 2019
doi: 10.3389/fonc.2019.01356

Herbal Interaction With Chemotherapeutic Drugs—A Focus on Clinically Significant Findings

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One of the most consequential risks associated with the concomitant use of herbal products and chemotherapeutic agents is herb-drug interactions. The risk is higher in patients with chronic conditions taking multiple medications. Herb-drug interaction is particularly undesirable in cancer management because of the precipitous dose-effect relationship and toxicity of chemotherapeutic agents. The most common mechanism of herb-drug interaction is the herbal-mediated inhibition and/or induction of drug-metabolizing enzymes (DME) and/or transport proteins leading to the alteration in the pharmacokinetic disposition of the victim drug. Most mechanistic research has focused on laboratory-based studies, determining the effects of herbal products on DMEs and extrapolating findings to predict clinical relevance; however, not all DME/transporter protein inhibition/induction results in clinical herb-drug interaction. This study reviews relevant literature and identified six herbal products namely echinacea, garlic, ginseng, grapefruit juice, milk thistle, and St John's wort, which have shown interactions with chemotherapeutic agents in humans. This focus on clinically significant herb-drug interaction, should be of interest to the public including practitioners, researchers, and consumers of cancer chemotherapy.

Keywords: cancer, chemotherapy, complementary and alternative medicine, drug interaction, herb-drug interaction, pharmacokinetics

OPEN ACCESS

Edited by:
Hervé Emonard, Centre National de la Recherche Scientifique (CNRS), France

Reviewed by:
Nicholas Oberlies, University of North Carolina at Greensboro, United States
Tyler Nelson Graf, University of North Carolina at Greensboro, United States

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: *24 September 2019*
Accepted: *18 November 2019*
Published: *03 December 2019*

Citation:
Fasinu PS and Rapp GK (2019) Herbal Interaction With Chemotherapeutic Drugs—A Focus on Clinically Significant Findings. Front. Oncol. 9:1356. doi: 10.3389/fonc.2019.01356

INTRODUCTION

Like regular synthetic and natural drugs, phytochemicals are capable of altering physiologic processes and eliciting toxicity. Despite the scarcity of information on the safety or otherwise of herbal preparations, sales and use of medicinal herbs and complementary medicines have increased globally. In the United States, the passage of the Dietary Supplement Health and Education Act 25 years ago is believed to have further popularized herbal products and enhanced public confidence in the quality of commercial supplements. One of the major concerns in herbal supplementation is the concurrent use with prescription medicine. Based on the study conducted by Rashrash et al. (1) which relied on the data from the 2015 National Consumer Survey on the Medication Experience and Pharmacists' Roles, the practice of combining prescription medicine with herbal supplements among adults in the United States cuts across all disease states, with 38% of prescription drug users reporting concomitant use of herbal products. One of the most frequent users of herbal medicines, according to the study, are cancer patients (43.1%) surpassed only by stroke patients (48.7%). One study reported a 78% prevalence of herbal and supplementary medicine use among patients on

chemotherapy, with 27% assessed to be at a risk of deleterious herb-drug interaction (2). In another recent study, more than half of the respondents reported usage of dietary supplements (which include herbal products) along with chemotherapeutic agents (3).

While the benefit of concomitant herb-drug use may be uncertain, one of the known major clinical consequences of such practice is herb-drug interactions. Not well-known until the accidental discovery of the grapefruit juice-felodipine interaction, leading to a 2.8-fold increase in the oral bioavailability of felodipine (4), herb-drug interaction has become an important consideration in pharmacotherapy and is assuming a subcategory of research study on its own. A casual PubMed search with "herb-drug interaction" as a search term would yield no relevant result until after this grapefruit-felodipine phenomenon. Subsequently, the number of herb-drug interaction -related publications increased dramatically, remaining steady over the years (**Figure 1**) and leading to the introduction of herb-drug interaction as a Medical Subject Heading (MeSH) in 2004.

Herb-drug interactions occur when the pharmacological disposition and/or effect of a drug of interest is altered by the presence of a concurrently administered herbal product. In most cases, herb-drug interactions are mild and could be inconsequential. However, in several instances, therapeutic interventions have been warranted consequent to herb-drug interaction. Such herb-drug interactions include reported bleeding induced by garlic (*Allium sativum*) combined with warfarin, extrapyramidal effects precipitated by betel nuts (*Areca catechu*) in patients taking neuroleptic drugs, and induction of mania in patients taking antidepressants along with ginseng (*Panax ginseng*), among several other clinically significant reported herb-drug interactions (5–7).

Chemotherapeutic agents are generally toxic with an array of side effects. Some of the principal reasons cancer patients combine herbal products with their anti-cancer drugs are the need to manage the side effects associated with chemotherapy and to enhance a general well-being. The potential risk of herb-drug interaction from such herbal use outweighs any benefit. There are several reasons why herb-drug interactions are undesirable in chemotherapy. First, most chemotherapeutic agents have a narrow therapeutic window, thus any alteration in this steep dose-response relationship can lead to toxic manifestations (8). Secondly, plasma concentrations of some chemotherapeutic agents have been shown to be a poor predictor of safety and efficacy (9). Reliance on PK profile in dosage designs have shown a wide inter-individual variation in responses to chemotherapy (10). This is compounded by the variations in the measurable drug concentration in the plasma and the target sites of action. It is thus plausible that slight alteration in the disposition of a chemotherapeutic agent following a delicately established effective and safe dosing will not only be counter-productive but will lead to therapy failure or toxicity. Thirdly, some chemotherapeutic agents, such as ifosfamide and cyclophosphamide, are prodrugs whose efficacy depends on effective biotransformation by cytochrome P450 (CYP) enzymes. Since most herb-drug interactions result from the inhibitory/inductive effect of phytochemicals on these metabolic enzymes, such drugs can easily be rendered ineffective or toxic by herb-drug interaction. Finally, most cancer patients have co-morbidities necessitating the use of multiple drugs, aside from antiemetic agents and other chemotherapy-associated medications. This would increase their risk of experiencing an herb-drug interaction.

Some epidemiological studies have reported that about one-tenth of all general hospital admissions might be due to the effect of multiple drug use resulting in adverse drug interactions and reactions (11, 12). Drug interactions alter drug concentrations in the body, which is particularly undesirable with chemotherapeutic agents that are dosed close to their maximal tolerable levels. On one hand, drug interactions resulting in increased clearance of the cytotoxic drug can lead to subtherapeutic drug exposure, enhance the development of drug resistance, and/or lead to therapy failure. On the other hand, accumulation of cytotoxic drugs resulting from drug interactions can precipitate potentially life-threatening toxicities due to supratherapeutic drug concentrations. Cancer patients often take several medications concomitantly due to co-morbidities and other cancer-associated conditions. In addition to the high risk of drug-drug interactions in such patients, the use of herbal products and the additional risk of herb-drug interaction complicate therapeutic expectations. Finally, the inherent pharmacodynamic effects of the herbal products, including organ-specific effects, and long-term interactions with physiologic receptors may not be beneficial to cancer patients.

Several herbal products have been studied in different patient groups to assess for herb-drug interaction. Clinically relevant information on herb-drug interaction in oncology is generally sparse. Most predictions are based on *in vitro* and preclinical animal studies; however, a few case reports and studies in humans are available to provide perspectives on the risk of herb-drug

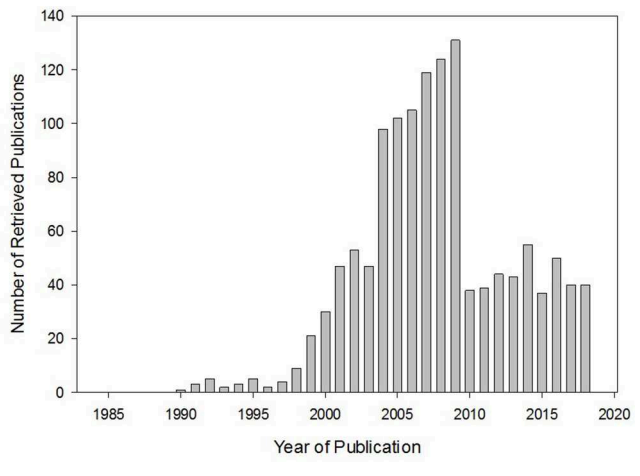

FIGURE 1 | Relevant publications retrieved from PubMed search using "herb-drug interaction" as a search term. The trend shows the introduction of and enhanced interest in herb-drug interaction. Interest has been maintained in this area over the years.

interactions in clinical settings. Therefore, the aim of this paper is to provide a review of the currently available literature evidence of herb-drug interaction in oncology, with emphasis on herbal products that have shown such interactions in human studies.

METHODS

This is a review conducted to provide an overview of herbal products capable of inducing clinically consequential herb-drug interaction in cancer chemotherapy. The review was systematically conducted by searching PubMed, Medline, Cochrane, Web of Knowledge, Scopus, and Google Scholar databases for original research, and case reports on herb-drug interaction using relevant search terms and the combinations thereof, including common herbal products, individual chemotherapeutic agents, "herbal interactions," and "herb-drug interactions." The reference lists of retrieved review papers/meta-analyses were also used to identify relevant publications. Inclusion was limited to publications available in English language and of studies performed in humans to evaluate interactions between herbal supplements and anti-cancer drugs. Searches were not limited by dates or place of publications.

RESULTS

A total of 345 publications were retrieved. The titles and abstracts were reviewed to determine if publications met inclusion criteria, and only 11 publications met the inclusion criteria. All of the databases searched, except Cochrane, returned the 11 clinically relevant studies. Cochrane did not have the clinical case reports. The included studies covered six herbal products—echinacea, garlic, ginseng, grapefruit juice, milk thistle, and St John's wort—which have been investigated in humans for potential interaction with chemotherapeutic agents. A summary of these studies is provided in **Table 1**. Subsequent subsections discuss these results. A highlight of the applicable mechanism of herb-drug interaction in cancer chemotherapy was also extracted and discussed below.

Applicable Mechanisms of Herb-Drug Interaction in Oncology

Understanding the mechanism of herb-drug interaction can help predict potentially harmful interactions. The mechanism of herb-drug interaction can broadly be categorized as pharmaceutical, pharmacodynamic, and pharmacokinetic. Pharmaceutical interactions usually arise from physicochemical incompatibility among drugs and formulations when they come in close proximity, such as in an IV bag. Information on pharmaceutical incompatibility of the various herbal formulations with

TABLE 1 | Studies of herbal interaction with chemotherapeutic agents conducted in human subjects.

Herbal product	Cancer drug	Study type and description	Findings	References
Echinacea	Etoposide	Case report	Taking echinacea with etoposide was found to significantly decrease the platelet nadir (16×10^3/L) when compared to the nadir of etoposide alone (44×10^3/L)	(13)
Echinecea	Docetaxel	Prospective study in 10 cancer patients	Echinacea did not cause significant alteration in the pharmacokinetics of docetaxel	(14)
Garlic	Docetaxel	Prospective, patient controlled, pharmacokinetic	Garlic was found to decrease docetaxel clearance. Although this decrease was non-statistically significant, it could potentially increase adverse effects due to accumulation of docetaxel	(15)
Ginseng	Imatinib	Case report	Patient taking imatinib for 7 years started having symptoms of hepatotoxicity after beginning to consume ginseng. Hepatotoxicity resolved upon discontinuation of ginseng	(16)
Grapefruit juice	Docetaxel	Case report	Grapefruit juice was found to increase the AUC and terminal half-life of docetaxel, while decreasing clearance of docetaxel	(17)
Grapefruit juice	Nilotinib	Open label, randomized, 2 period crossover	Grapefruit juice was found to increase the AUC and peak concentration of nilotinib but did not affect the elimination half-life	(18)
Milk thistle	Irinotecan	Pharmacokinetic study	Milk thistle was found to cause a statistically insignificant decrease in irinotecan clearance, making it unlikely to cause a clinical impact	(19)
St John's wort	Docetaxel	Pharmacokinetic study	St John's wort was found to cause a significant decrease in plasma docetaxel concentration	(20)
St John's wort	Irinotecan	Unblinded, randomized crossover study	St John's wort caused a decrease in plasma concentrations of active metabolite (SN-38) by 42%	(21)
St John's wort	Imatinib	Open label, crossover pharmacokinetic study	St John's wort decreased plasma concentration of imatinib by 32% and decreased the half-life of imatinib by 21%	(22)
St John's wort	Imatinib	2 period, open-label, fixed sequence study	St John's wort increased clearance of imatinib by 43%, and decreased its plasma concentration by 30%	(23)

prescription drugs is generally non-existent. This type of interaction is also not very likely with herbal products because little to no contact often exist with prescription drugs before any concomitant administration. Pharmacodynamic interactions are those involving the potentiation, additive, or antagonistic effect of a drug by the presence of an herbal product. To predict this, the biomolecular and pharmacological effect of the individual herbs and their phytoconstituents must be understood. Very little is known about the identity and biological effect of the active phytochemicals in the myriad other herbs used by patients that are not discussed here. However, the potential for pharmacodynamic herb-drug interaction is always present due to the ability of phytochemicals to interact with biological receptors. For example, the antidepressant effect of St John's wort may be expected to be additive in patients taking prescription drugs for the treatment of depression.

The most important category of herb-drug interaction has been identified as pharmacokinetic. The majority of clinically significant pharmacokinetic drug interactions occurs due to the inhibition or induction of the metabolism/clearance of one drug by another (24). This is molecularly mediated by drug metabolizing enzymes and transport proteins. Most anti-cancer drugs are substrates of CYPs and transport proteins (**Table 2**). Phytochemical compounds are capable of inhibiting and/or inducing drug-metabolizing enzymes, particularly the CYPs. CYP inhibition delays the clearance of CYP substrates, leading to drug accumulation. This is undesirable in cancer chemotherapy due to the narrow therapeutic window of many anti-cancer drugs. CYP inhibition is also deleterious for CYP-dependent prodrugs like ifosfamide and cyclophosphamide, whose biotransformation, once stalled, can lead to therapy failure. The induction of CYP enzymes lead to increased metabolic activity and reduced drug exposures. The resultant sub-therapeutic exposure can lead to treatment failure in the short term, and drug resistance in the long term. Enzyme inhibition/induction affect both the bioavailability and clearance of cancer drugs. Several herbal products including St John's wort, ginkgo, ginseng, licorice, kava, garlic, cranberry, grape seed, germander, goldenseal, valerian, and black cohosh, among others have been shown to inhibit or induce CYPs (24, 25). Similar inhibitory and inductive effects of herbal products on phase II enzymes have been variously reported (26–29). There can also be inhibition/induction of renal excretion and alteration of tissue distribution through displacement from protein binding.

Pharmacokinetic herb-drug interactions are also mediated by herbal interaction with transport proteins, principal among which is P-glycoprotein (P-gp). P-gp, also referred to as the multidrug resistance protein 1 (MDR1), or ATP-binding cassette sub-family B member 1 (ABCB1), is a 160-kD ATP-dependent efflux surface glycoprotein first identified in Chinese hamster ovary cells (30). P-gp is localized in various tumors expressing the

TABLE 2 | Several anti-cancer drugs are substrates of drug-metabolizing enzymes and transport proteins.

Metabolizing enzyme/transporter	Anti-cancer substrates
CYP1A1/1A2	Axitinib, bendamustine, bortezomib, dacarbazine, etoposide, exemestane, flutamide, pazopanib, pomalidomide, tegafur
CYP2A6	Cyclophosphamide, ifosfamide, letrozole, tegafur
CYP2B6	Busulfan, cyclophosphamide, docetaxel, doxorubicin, ifosfamide, procarbazine, thiotepa
CYP2C8	Anastrozole, dabrafenib, cyclophosphamide, enzalutamide, ifosfamide, imatinib, lapatinib, nilotinib, paclitaxel, pazopanib, tegafur
CYP2C9	Busulfan, ifosfamide, idarubicin, ruxolitinib, tamoxifen
CYP2C19	Axitinib, bortezomib, cyclophosphamide, ifosfamide, lapatinib, pomalidomide, tamoxifen, thalidomide
CYP2D6	Brentuximab, doxorubicin, gefetinib, idarubicin, pomalidomide, tamoxifen, vinblastine, vinorelbine
CYP2E1	Dacarbazine, etoposide, cisplatin, vinorelbine
CYP3A4/3A5	Anastrozole, axitinib, bortezomib, bositinib, brentuximab, cabazitaxel, cisplatin, crizotinib, cyclophosphamide, dabrafenib, dasatinib, docetaxel, doxorubicin, enzalutamide, etoposide, exemestane, gefetinib, imatinib, fulvestrant, ifosfamide, irinotecan, lapatinib, letrozole, mitoxantrone, nilotinib, olaparib, paclitaxel, pazopanib, pomalidomide, ponatinib, procarbazine, regorafenib, ruxolitinib, sorafenib, sunitinib, temsirolimus, teniposide, thiotepa, topotecan, trabectedin, vandetanib, vemurafenib, vinblastine, vincristine, vinorelbine
GSTs	Busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide, dactinomycin, daunorubicin, doxorubicin, etoposide, idarubicin, ifosfamide, mitomycin, mitoxantrone, oxaliplatin, tamoxifen, vinblastine, vincristine, vinorelbine
UGTs	Anastrozole, axitinib, bicalutamide, doxorubicin, epirubicin, etoposide, exemestane, irinotecan, sorafenib, regorafenib, tamoxifen, teniposide, topotecan
P-glycoprotein (ABCB-1, MDR-1)	Axitinib, bicalutamide, bosutinib, cytarabine, dactinomycin, dasatinib, daunorubicin, docetaxel, doxorubicin, epirubicin, etoposide, gefetinib, idarubicin, imatinib, irinotecan, methotrexate, mitoxantrone, paclitaxel, sunitinib, vincristine
MRP-1 (ABCC-1)	Chlorambucil, daunorubicin, doxorubicin, epirubicin, etoposide, idarubicin, irinotecan, melphalan, methotrexate, mitoxantrone, tenoposide, topotecan, vinblastine, vincristine
MRP-2 (ABCC-2)	Methotrexate, sulfinpyrazone, vinblastine
BCRP (ABCG-2, MXR)	Bicalutamide, dasatinib, docetaxel, daunorubicin, doxorubicin, epirubicin, gefetinib, idarubicin, imatinib, irinotecan, mitoxantrone, nilotinib, paclitaxel, sorafenib, sunitinib, topotecan

ABC, ATP-binding cassette; BCRP, breast cancer resistant protein; MDR, multidrug resistance gene; MRP, multidrug resistance-associated protein; MXR, mitoxantrone resistance-associated protein.

MDR phenotype. In normal cells, P-gp is expressed in the apical or luminal membranes of cells with excretory or barrier functions including the liver, kidney, intestines, and adrenal glands. P-gp is also a principal constituent of the physiologic blood-brain, blood-testes, and blood-ovary barriers. These anatomical and physiological positions of P-gp enhances its protective and detoxifying functions. In relation to drugs and other xenobiotics, the efflux activity of P-gp reduces cellular penetration and tissue distribution.

As a high-capacity transport protein, the activity of P-gp affects a wide range of structurally unrelated and pharmacologically diverse drugs, including chemotherapeutic agents, anti-retroviral drugs, immunosuppressants, cardio-active drugs, centrally-acting drugs, and several others. Numerous other drugs inhibit the activity of P-gp. Notable among these are verapamil and cyclosporine, used as standard controls in P-gp studies. Many other drugs, including ketoconazole, quinidine, ritonavir, etc., have caused adverse drug interactions through their inhibitory activity on P-gp. Herbal products and phytochemicals including silymarin and extracts from milk thistle, ginseng-derived ginsenosides, piperine, capsaicin, and several others have been reported to inhibit the activity of P-gp. Both the expression and activity of P-gp, like CYPs, can be induced (31–34). St John's wort is an example of a typical herbal P-gp inducer.

Herbal Products That Have Shown Clinical Interactions With Chemotherapeutic Drugs

Echinacea

Formulations of echinacea are globally popular for complementary treatment of respiratory infections and common cold. Among cancer patients, echinacea is popular as an immunomodulatory supplement (35, 36). Recent studies in animals have suggested that echinacea may have beneficial effect in abating some forms of cancer, like leukemia (37). The active constituents and the pharmacological mechanism of any beneficial effect is poorly understood. Echinacea is ranked one of the top widely sold herbal preparations in the United States (38). Most of the preparations of echinacea in the United States are made from one out of the nine common species—*Echinacea purpurea*. Several pre-clinical studies have suggested herb-drug interaction between echinacea and anti-cancer drugs. For example, extracts of echinacea induce P-gp and CYP3A4, two major enzyme/transporter combination that play major roles in the biotransformation and pharmacokinetics of anticancer drugs [**Table 1**; (39)]. Echinacea is also an inhibitor of CYP3A4 (40). This dual ability to inhibit and induce drug-metabolizing enzymes makes it difficult to predict clinically significant herb-drug interaction with the various CYP/P-gp drug substrates. In human studies, echinacea caused significant increase (34%) in the systemic clearance of midazolam, a CYP3A4 substrate (41). Therefore, there is a potential for herb-drug interaction between echinacea and anti-cancer drugs.

While preclinical studies have shown strong evidence of echinacea interacting with CYP and transport proteins, there is insufficient clinical data on herb-drug interaction with anti-cancer drugs. In a study in 10 cancer patients, echinacea did not cause significant alterations in the pharmacokinetics of docetaxel, which is a substrate of CYP3A4 and P-gp (14). The patients received an intravenous dose of docetaxel on day 1, and were then treated with echinacea supplementation (20 oral drops three times daily of a commercially available product) on days 7–21. They were then administered with another dose of docetaxel on day 22. No significant changes were observed in the pharmacokinetics of docetaxel with or without echinacea supplementation. However, with darunavir, an antiretroviral drug, echinacea caused a general decrease in concentration in the HIV/AIDS patient participants (42).

In a case report, echinacea caused a significant interaction in a cancer patient taking etoposide (13). The adult patient, who was newly diagnosed with squamous cell carcinoma of the lung received cisplatin and etoposide on the first day of treatment with a recorded normal bloodwork. However, by day 8 of this first cycle chemotherapy, his platelet count had dropped by over two-thirds, necessitating platelet transfusion. The discontinuation of echinacea in the cycle 2 chemotherapy helped the patient avoid any further need for platelet transfusion. No further incidence was reported until discharge after 20 days in the hospital. Patient was instructed to avoid taking any more herbal supplements. Etoposide, a cytotoxic agent, is a CYP substrate, whose dose-limiting toxicity is myelosuppression. This interaction is understood to have been as a result of echinacea-induced CYP inhibition, leading to etoposide accumulation and the resultant thrombocytopenia.

Garlic

Garlic (*Allium sativum*) is one of the most popular herbal products used to supplement the treatment of infection, diabetes, and heart diseases (43). Its use is common among people with chronic diseases, such as cancer. The major bioactive component of garlic is allicin (diallyl thiosulfinate). Whole garlic extracts have been shown to inhibit the CYP3A4-dependent formation of 6β-hydroxytestosterone from testosterone through *in vitro* liver microsomal incubations (44).

In a study to assess the effect of garlic supplementation on the pharmacokinetics of docetaxel, Cox and co-workers administered docetaxel to women with metastatic breast cancer weekly for 3–4 weeks. A 12-day supplementation with twice-daily 600 mg garlic was commenced on the participants 3 days after the initial dose of docetaxel (15). By Day 15 of the study, garlic supplementation reduced the clearance of docetaxel by 36% (from 30.8 to 20.0 L/h/m^2. Although, changes in the other pharmacokinetic parameters were reported to be insignificant, the decrease in docetaxel clearance in the presence of garlic may pose significant risk of toxicity due to docetaxel accumulation. This interaction is also consistent with the ability of the phytochemicals in garlic to inhibit CYP enzymes, which are responsible for the metabolism of docetaxel.

Ginseng

Ginseng is one of the most popular herbal products sold globally and especially in the United States. Commercial

ginseng products are made mainly from three of the several species of ginseng—*Panax ginseng* (Asian ginseng), *Panax quinquefolius* (American ginseng), and *Panax japonicus* (Japanese ginseng). Most therapeutic claims including energy boosting, immunomodulation, enhancement of sexual desire, and pain management are anecdotal. Pharmacological activity of ginseng is generally attributed to ginsenosides, a group of steroidal saponins, which forms the primary phytochemical constituents. Anti-oxidant and cardiovascular protective effect of ginseng have been reported (45, 46). Other reported pharmacological activity of ginseng include immunomodulatory and anticarcinogenic effects, neurotransmitter modulation, and antimitogenic activity (47–49).

There have been mixed findings on the effect of ginseng on drug-metabolizing enzymes and P-gp. In *in vitro* studies, some studies reported no inhibitory activity on CYPs, contrary to others which found inhibitory activity against DMEs (50–54). In a study involving eight healthy volunteers, the effect of the extracts of *P. ginseng* on the pharmacokinetics of midazolam and fexofenadine—substrates of CYP3A4 and P-gp, respectively, was evaluated. Results showed a significant reduction in the AUC and C_{max} of midazolam, which the authors attributed to the inductive effect of ginseng on CYP3A4/5 (55).

As a popular herbal supplement among cancer patients, ginseng has the potential to mediate clinically significant interactions with chemotherapeutic agents. In a case report, an onset of imatinib-induced hepatoxicity was reported in a patient who was being treated with imatinib for chronic myelogenous leukemia (CML). Having used imatinib for 7 years, the patient developed liver dysfunction (confirmed by abnormal liver function test results showing elevated alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, and albumin; as well as liver biopsy) only after concurrent use with a *P. ginseng*-containing energy drink for 3 months (16). The symptoms of hepatotoxicity were resolved after the discontinuation of the energy drink. At high blood levels, and in some patients, imatinib may induce hepatotoxicity within the first 2 years of therapy. Thus, the patient was believed to tolerate the drug before consuming the energy drink, having used it for 7 years; however, the multicomponent nature of the energy drink raises questions on the singularity of responsibility of ginseng.

Grapefruit Juice

Grapefruit (*Citrus paradisi*) is not a regular herbal supplement used for medicinal purposes. As a drink, it has been well-reported to influence the pharmacokinetics of a variety of drugs when consumed together. Phytochemical constituents of grapefruit juice are potent inhibitors of CYPs and P-gp. Various comprehensive reviews have been published on the interaction between grapefruit juice (GFJ) and prescription drugs (56, 57).

In a study in 21 healthy human volunteers, concomitant intake of grapefruit juice and nilotinib caused a 60% increase in the peak concentration of nilotinib, along with a 29% increase in the AUC (18). Participants received 400 mg nilotinib with either 250 mL double strength GFJ or water in a cross-over study of two periods separated by 10-day washout period. This was attributed to the inhibitory actions of the phytochemical constituents of grapefruit juice on CYPs.

In a case report published by Valenzuela et al., a patient diagnosed with esophageal squamous cell carcinoma, had taken 250 mL of GFJ daily for more than 3 months while on docetaxel and had shown unusual pharmacokinetics of docetaxel relative to dose (17). The elimination of docetaxel had been observed to be slow in the patient, with an estimated plasma clearance of 13.2 L/h compared to the typical plasma clearance of docetaxel of 36.7 L/h. After reviewing the patient's medication records, the authors reported suspecting that GFJ might be influencing the pharmacokinetics of docetaxel in the patient. A 60% reduction in the AUC (to infinity), with a 36% increase in plasma clearance and a 10% decrease in the terminal half-life of docetaxel were observed following GFJ discontinuation. This further confirmed that GFJ suppressed the clearance of docetaxel, most likely through inhibitory activity of CYP enzymes which are responsible for the metabolism of docetaxel.

Milk Thistle

Milk thistle (*Silybum marianum*) is another popular herbal product used as complementary medicine in cancer patients and to boost immunity in HIV/AIDS patients. It is also used for the treatment and prevention of liver diseases. Silymarin, a mixture of biologically active flavonolignans, is the active constituent of milk thistle and generally expressed in the leaves, seeds, and fruit of the plant. Commercially available products of Milk thistle are usually provided as silymarin, a complex mixture of flavonolignans and a flavonoid. A fraction of this mixture called silibinin (containing silybin A and silybin B) have also been made commercially available. A recent publication provides a comprehensive review of these phytochemical components of Milk thistle, and their nomenclature (58). Silymarin has been clinically investigated for its anticancer activity with promising results (59, 60). Silymarin has been shown through *in vitro* studies to inhibit the activity of CYP and phase 2 enzymes (61, 62). This potential for herb-drug interaction has been shown in clinical studies, where silymarin significantly reduced the CYP2C9-mediated metabolism of losartan (63).

Due to the preponderance of use of milk thistle product among cancer patients, the potential for herb-drug interaction is a major clinical concern; however, clinical data on this is sparse. A study was conducted to determine if the inhibitory activity of milk thistle extract on CYP3A4 will translate to the alteration of the pharmacokinetics of irinotecan, a CYP3A4 substrate, in humans when taken together. The study in six cancer patients who were being treated with once-a-week irinotecan, in the course of which thrice-daily milk thistle was administered for 12 days assessed the pharmacokinetics of irinotecan and its metabolites. Authors reported that neither the short-term (4 days) nor prolonged use of milk thistle (12 days) resulted in any significant alteration in the pharmacokinetics of irinotecan. Only a slight and insignificant drop in clearance was observed with 31.2, 25.4, and 25.6 L/h in the first, second and third week, respectively, reported (19). According to the authors, potential for clinically significant interaction between silymarin and CYP3A4 substrate may not be very strong because the C_{max}

of silibinin, at the usual dose, is reported to range from 0.0249 to 0.257 μM, a concentration that may be too low for CYP/P-gp inhibition (64).

This notwithstanding, in the absence of further proof, the risk of clinically significant herb-drug interaction between milk thistle and chemotherapeutic agents may still be present due to variations in silymarin concentrations in different commercially available milk thistle formulations.

St John's Wort

St John's wort (*Hypericum perforatum*) is a common herbal supplement widely used for the treatment of depression, anxiety, sleep disorders, and nervousness (65). Official guidelines in multiple countries have recommended St John's wort for the treatment of depression, which has increased the popularity and consumption of St John's wort among various patient groups (66). Other popular uses of St John's wort include in the treatment of premenstrual syndrome, alcohol withdrawal, and somatoform disorders (67–70). Several active phytochemical constituents including naphthodianthrones (like hypericin), phloroglucinols (like hyperforin), and flavonol glycoside (like hyperosides) have been isolated and characterized from St John's wort (71). The antidepressant activity of St John's wort has been attributed to hyperforin, the constituent with the most potent ability to inhibit the synaptic reuptake of central neurotransmitters such as dopamine, noradrenaline, and serotonin.

Several *in vitro* studies have demonstrated the ability of the extracts of St John's wort to modulate the activity of CYP and major drug transporters. For example, St John's wort has been shown as a potent inducer of CYP2B6, CYP2C19, CYP2E1, and CYP3A4. Hyperforin, in addition to its inductive effects on several CYP isoforms, is a potent inhibitor of CYP2C9 and CYP2D6. Other constituents of St John's wort have shown inhibitory activities against CYPs. For example, biapigenin, a flavonoid from St John's wort, is a potent inhibitor of CYP1A2, CYP2C9, and CYP3A4 whereas hypericin is a competitive inhibitor of CYP2C9, CYP2D6, and CYP3A4 (72). Mechanistic studies in cell lines and in animal models have demonstrated the herb-drug interaction potential of St John's wort. The effect of concomitant administration of St John's wort and several clinically important substrates of these CYPs and transporters have been investigated in humans. In some instances, clinical case reports have been published showing significant herb-drug interaction between St John's wort and prescription medicine.

In human studies and clinical case reports, St John's wort has been shown to alter the pharmacokinetics of various substrates of CYP3A4 and P-gp including omeprazole, simvastatin, cyclosporine, indinavir, verapamil, and tacrolimus (73–78).

Four clinically relevant studies retrieved from the literature, show the influence of concomitantly administered St John's wort on the pharmacokinetics of anti-cancer drugs. The influence of St John's wort on the pharmacokinetics of docetaxel, a CYP3A4 substrate, was evaluated in 10 cancer patients. Subjects were intravenously administered with 135 mg docetaxel on day 1 of the study followed by blood withdrawal and pharmacokinetic analysis. From day 7 to 21, participants were treated with commercially available 300 mg tablets of St John's wort extracts (Hyperiplant®), three-times-daily. The mean AUC∞ of docetaxel was decreased by 12%, and the total clearance increased by 14% due to the pre-supplementation with St John's wort. In addition, the C_{max} and $T_{1/2}$ of docetaxel was decreased, non-significantly. The study also found a lower incidence of docetaxel-related adverse effects due to St John's wort supplementation (20). These observations are consistent with the mechanistic ability of St John's wort to induce CYP3A4 and accelerate the metabolism of its substrates.

In another study, the effect of St John's wort on the metabolism of irinotecan was assessed. Five cancer patients recruited for the study were treated with irinotecan with or without St John's wort supplementation for 18 days in an unblinded randomized cross-over study. St John's wort decreased the plasma level of the active metabolite, SN-38, by 42% (21). Authors also reported a mean decrease in leucocyte counts of 63% when irinotecan alone was used compared to a 4.3% decrease count when combined with St John's wort. The reduced incidence of myelosuppression was attributed to increased metabolism of irinotecan, brought about by the inductive effects of St John's wort on the metabolism of irinotecan and SN-38. The St John's wort -irinotecan combination has also been reported to mitigate against hematologic and gastrointestinal toxicities associated with irinotecan (79).

Smith and co-workers conducted an open-label cross-over study to determine the influence of St John's wort on the pharmacokinetics (PK) of imatinib in 10 healthy adult subjects (22). PK parameters were compared following a single administration of 400 mg imatinib before and after a 2-week St John's wort treatment. St John's wort reduced the median AUC of imatinib by 32%, and the observed C_{max} by 29%. This significant St John's wort -induced reduction in imatinib exposure, alongside decreased plasma half-life, occurred in all 10 participants. Additionally, the C_{max} in the presence of St John's wort was diminished in all participants but one.

In a similar study, using a 2-period design for an open-label, fixed-sequence study in 12 healthy volunteers, Frye and co-workers reported a 43% increased clearance of imatinib with a 30% reduction in imatinib exposure (23). Each of the volunteers had received 400 mg of imatinib orally on days 1 and 15, while also receiving three-times-daily 300 mg of St John's wort from days 4 to 17. Plasma imatinib were analyzed over 72 h after each imatinib administration. In addition to the increased total clearance and the reduced total exposure, St John's wort caused a 31% decrease in the plasma half-life (from 12.8 to 9 h) and a 20% decrease in the plasma C_{max} of imatinib in the subjects. All the pharmacokinetic changes were observed in all 12 participants. These effects are significant and may pose a risk for therapeutic failure in cancer patients who take St John's wort along with their therapeutic agents.

DISCUSSION

Despite the scarcity of data on therapeutic benefit of herbal supplements in cancer, the use of herbal products is very common among cancer patients. Studies have reported figures as high as

50–66% use of one or more complementary/alternative medicine, the majority of which are herbal preparations, concurrently with conventional cancer therapy (80, 81). This review identified six herbal products—echinacea, garlic, ginseng, grapefruit juice, milk thistle, and St John's wort—which have shown clinically relevant interactions with specific chemotherapeutic agent. Several other herbal products are commonly used among cancer patients for which there are currently no clinically relevant herb-drug interaction data, but with strong potential for interactions based on laboratory-based results. These include green tea (*Camellia sinensis*), mistletoe (*Viscum album*), evening primrose (*Oenothera paradoxa*), parsley (*Petroselinum crispum*), goldenseal (*Hydrastis canadensis*), kava (*Piper methysticum*), aloe vera (*Aloe barbadensis*), wild yam (*Dioscorea villosa*), valerian (*Valeriana officinalis*), golden root (*Rhodiola rosea*), medicinal mushrooms (including species of *Ganoderma*, *Grifola,* and *Trametes*), agaricus (*Agaricus campestris*), and rooibos (*Aspalathus linearis*) (82).

As highlighted earlier, herb-drug combination is particularly undesirable in cancer patients because of herb-drug interaction risks. Most herb-drug interactions are pharmacokinetic in mechanism and are brought about by either the induction or inhibition of drug-metabolizing enzymes and transport proteins. Since echinacea can inhibit and induce CYP enzymes, it is difficult to predict what effect it will have on a patient's therapy. Current data is sparse and showed conflicting outcomes as to enhancing or decreasing the effect of chemotherapy. Ginseng is another inducer that may place a patient at higher risk for adverse effects if taken along with chemotherapy. Based on the case study found, it is unclear if ginseng was the definite cause of hepatotoxicity; however, since there is evidence to suggest that ginseng induces CYP enzymes, the patient's hepatotoxicity is thought to be due to the ginseng component of the energy drink. Further studies and reports are needed to assess the interaction between ginseng and chemotherapeutic agents. By inhibiting CYP enzymes, garlic and milk thistle can effectively inhibit the metabolism of certain chemotherapeutic agents. Based on the available literature, both can clinically influence the pharmacokinetics of chemotherapeutic drugs. Interaction of grapefruit juice is unique in that most people consume grapefruit juice for non-medicinal purposes. Current literature shows that grapefruit juice caused the accumulation of CYP/P-gp substrates due to inhibition, placing patients at increased risk for adverse effects from chemotherapy. This interaction is important because it highlights the importance of diet during chemotherapy treatment.

To ensure effective care, providers should have open conversations with their patients in order to document their herb-drug use and provide necessary counseling. Patients need education on the potential beneficial and harmful effects of herbal products in cancer. Such education should include the lack of sufficient supportive data and the liberal marketing strategies employed in the sale of herbal products. Importantly, patients should understand the potential for herb-drug interaction and the attendant toxicity or therapy failure.

CONCLUSION

While the beneficial effects of the commonly consumed herbal products by cancer patients is uncertain, data from human studies suggest that some of these supplements are capable of interacting with chemotherapeutic agents. It is therefore prudent and advisable to avoid the concomitant use of anti-cancer drugs and herbal products, especially echinacea, garlic, ginseng, grapefruit juice, milk thistle, and St John's wort. Clinicians and practitioners need to be vigilant in monitoring for any herb-anticancer combination.

AUTHOR CONTRIBUTIONS

PF and GR conceptualized the research, contributed equally to the literature search, synthesis, writing of the manuscript, and agree on the final version of the manuscript.

FUNDING

This research was supported by a departmental grant from the Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Campbell University.

ACKNOWLEDGMENTS

We would like to thank our colleagues at the Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Campbell University.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

CASE REPORT
published: 26 February 2021
doi: 10.3389/fonc.2021.607574

Case Report and Supporting Documentation: Acute Kidney Injury Manifested as Oliguria Is Reduced by Intravenous Magnesium Before Cisplatin

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OPEN ACCESS

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: 17 September 2020
Accepted: 22 January 2021
Published: 26 February 2021

Citation:
Money ME, Hamroun A, Shu Y, Matthews C, Ahmed Eltayeb S, Ciarimboli G and Metz CN (2021) Case Report and Supporting Documentation: Acute Kidney Injury Manifested as Oliguria Is Reduced by Intravenous Magnesium Before Cisplatin. Front. Oncol. 11:607574. doi: 10.3389/fonc.2021.607574

After more than four decades of post-approval, cisplatin is still an important treatment for numerous cancers. However, acute kidney injury (AKI), defined as significant impairment of renal filtration as discussed below, is the major limiting side effect of cisplatin, occurring in approximately 30% of patients (25–33% after the first course). Cisplatin also damages the kidneys' ability to reabsorb magnesium in 40–100% of patients, with collateral health risks due to subsequent hypomagnesemia. Multiple methods and drugs have been proposed for preventing cisplatin-induced AKI, including saline infusion with or without mannitol, which has not always prevented AKI and has been found to activate a cellular stress response in renal tubular cells. While numerous reports and trials, as well as the National Comprehensive Cancer Network (NCCN), support premedication with magnesium and hydration, this practice has not been universally accepted. Many clinics administer intravenous magnesium (IV) only after identification of hypomagnesemia post-cisplatin treatment, thus placing patients at risk for AKI and chronic renal loss of magnesium. We present the following case report and additional supporting evidence identifying the immediate effect of IV magnesium prior to intraperitoneal cisplatin for cycle 4 because of documented hypomagnesemia resulting in normalization of oliguria, which had been experienced for the first three cycles. The patient subsequently requested and received IV magnesium before cisplatin for the next two cycles with continuation of normal urinary output. The effect of pretreatment with IV magnesium on urine output following cisplatin has not been previously reported and further supports pre-cisplatin administration. In addition, two recent meta-analyses of clinical trials and pre-clinical research are reviewed that demonstrate effectiveness of magnesium pretreatment to preventing AKI without reducing its chemotherapeutic

efficacy. This case report with additional evidence supports the adoption of administration of 1–3 g IV magnesium before cisplatin as best practice to prevent cisplatin induced AKI and hypomagnesemia regardless of patient baseline serum magnesium levels.

Keywords: intravenous magnesium, cisplatin, acute kidney damage, oliguria, hypomagnesemia, nephrotoxicity, ovarian cancer, intra-peritoneal chemotherapy

INTRODUCTION

Oncologists now have numerous therapeutic agents for various cancers, but cisplatin, the first platinum compound the FDA approved in 1978, continues to be one of the most effective treatments against numerous cancers (1, 2). Cisplatin is highly effective in damaging cancer cell DNA, but its use is restrained by dose-limiting side effects, including AKI, considered to be the most serious toxicity, occurring in approximately one-third of patients (3). Even a single injection of cisplatin may result in a transient episode of AKI in 20–30% of patients (4, 5), which can be missed when measuring only the serum creatinine and blood urea nitrogen. Multiple reviews have discussed the molecular mechanism of AKI induced by cisplatin, which is beyond the scope of this case report (2, 6). Providers in outpatient clinics may not appreciate oliguria as a sign of AKI as manifested by the case report discussed below. Thus, identifying an agent that will prevent or ameliorate this irreversible side effect has been a priority.

The most recognized and followed recommendation to prevent AKI is fluid administration before and after cisplatin, typically with or without mannitol or furosemide (2, 7, 8). Magnesium administered concomitantly with cisplatin has been recommended by Vokes (9) since 1990 to prevent secondary hypomagnesemia due to distal tubular damage (10) and by multiple other clinicians to prevent AKI, (3, 11–16) but has not been established as a standard protocol and was not followed initially for this patient. This review will identify the benefits of this therapy for patients receiving cisplatin, regardless of serum magnesium levels, proposing the adoption of magnesium administration before cisplatin as best practice protocol.

CASE REPORT

The patient is a 71-year-old board-certified internist who received intraperitoneal (IP) cisplatin and paclitaxel for recurrent ovarian cancer in December 2014.

Background: Stage 3C, poorly differentiated serous adenocarcinoma of the fallopian tube was diagnosed by exploratory surgery at Mercy Medical Center, Baltimore, MD by Dr. Neil Rosenshein in September 2012. Debulking was complete except for 1.5 cm tumor implant on the diaphragm. Chemotherapy with paclitaxel and carboplatin resulted in complete remission by 2013. Background medical history for patient includes: family history for breast cancer (mother and maternal grandmother); non-smoker, history of A–V dura fistula treated conservatively, primary hypothyroidism, allergic rhinitis; BP 112/78, pulse 72, BMI 20.9. Patient was entirely asymptomatic but CA 125 had increased from eight to 19 on 10/20/14 and a PET scan showed 1 cm implant on the right kidney on 10/31/14. Rather than accepting standard chemotherapy, the patient underwent exploratory laparotomy by Dr. Robert Edwards at Magee-Women's hospital, UPMC, Pittsburgh, PA. No tumor implant was identified, but cell washings were atypical and an intraperitoneal port was placed for IP therapy.

Baseline serum lab included: blood urea nitrogen (BUN) 16, creatinine (Cr) 0.7, and magnesium 2.0 mg/dL (normal range: 1.7–2.2) on 12/26/14. Cisplatin and paclitaxel were administered *via* IP infusion every 21 days for six cycles beginning on 12/29/14 (day 1). Paclitaxel 135 mg/m^2 in 500 ml of normal saline intravenous (IV) over 3 h was administered on day 1. After 1 L of normal saline IV, cisplatin at 75 mg/m^2 was given in 1 L of normal saline IP followed by a second liter of normal saline IP, if tolerated. Another liter of normal saline IV was subsequently administered, too. Significant oliguria (concentrated, dark urine estimated at <30 cc h) was observed within 3 h after IP cisplatin. This oliguria and abdominal distention with pain continued for the next 36–48 h despite >2,000 cc oral intake of liquids and an additional liter of normal saline IV the following day.

Because the patient (an internist) recognized the disproportional oliguria, the on-call provider was consulted that evening within 6 h of cisplatin administration, but no action was recommended since the patient still had some urine output. AKI, which manifested as oliguria, was not recognized and stat renal function studies were not ordered. Diuresis had occurred by 1/3/15 when the following tests were obtained prior to having urgent surgery for a fractured wrist due to a fall on ice: BUN 20, Cr 0.74, and magnesium 1.3 mg/dL.

Oral magnesium oxide >500 mg was consumed daily. Intravenous magnesium was not administered unless serum magnesium dropped below 1.5 mg/dL, which was only checked immediately before each cycle of chemotherapy and not in between. On 2/9/15, prior to the next cycle, which had been delayed for 3 weeks due to a wrist fracture, BUN was 22, Cr 0.8, and magnesium 1.8.

The patient continued to experience severe oliguria immediately following cisplatin IP administration, lasting for approximately 48–72 h for the next two cycles, but on day 1 of cycle 4 on 3/25/15, 4 g (32.48 mEq) magnesium sulfate was administered IV prior to chemotherapy for a serum magnesium of 1.0 mg/dL. (Serum magnesium had been 1.6 on 3/2/15). The patient immediately observed normal urinary output on day 2 post-cisplatin and thereafter. In addition, abdomen distention and pain were significantly less than during the prior cycles.

Recognizing this response, the physician-patient completed a literature search regarding the effect of magnesium on cisplatin toxicity and consulted with a scientist who had recently published studies in rodents demonstrating the benefits of pretreating with magnesium to protect against cisplatin-induced AKI (17). Therefore, the patient subsequently requested and received 2 g of magnesium sulfate IV pre-cisplatin (on day 2) for cycles 5 and 6 even though serum magnesium was 1.7 mg/dL. To clearly demonstrate the effectiveness, she recorded the fluid intake and output for day 2, cycle 6: 4,800 cc combined IV, IP, and oral route, with a concomitant urine output of 4,950 cc in 24 h. The patient continued to demonstrate improved tolerance to the IP treatment with the administration of IV magnesium preceding cisplatin.

After completion of the six cycles, on 6/29/15, relevant values were: BUN 19, serum Cr 0.9, and magnesium 1.9. Patient at that time was receiving 1–2 g of IV magnesium weekly and has continued to suffer from persistent hypomagnesemia requiring 600 mg of oral magnesium threonate in divided doses daily to prevent tetany. These effects are presumed due to the irreversible AKI from cycles 1–3 prior to pretreatment with magnesium in subsequent cisplatin cycles. Glomerular filtration rate from 2015 until 2020 has been greater than 59 ml/min and current renal function on 8/27/20 was: BUN 23, Cr 1.04. As a physician, the patient strongly supports this case report to prevent AKI, hypermagnesemia, and the discomfort associated with intraperitoneal cisplatin that was diminished with magnesium preceding cisplatin.

DISCUSSION

Overview of Acute Kidney Injury by Cisplatin

According to the Kidney Disease Improving Global Outcomes (KDIGO), AKI is defined as ≥0.3 mg/dL increase in serum creatinine or a 0.5 ml/kg/h decrease in urine output within 48 h; whereas Common Toxicity Criteria for Adverse Events Version 4.0 (CTCAE v4.0) agrees on serum creatinine increase ≥0.3 mg/dL but with no time consideration (5).

Using CTCAE v4.0 criteria, 26.5% of patients experienced AKI after the first round of cisplatin-based chemotherapy in one retrospective study for urothelial cancer, resulting in >40% being unable to receive the planned second round, and 50% reduction in 3-year survival (5). Consequently, mortality is increased among patients with AKI, frequently due to inability to continue chemotherapy as scheduled (18, 19).

Risk factors for renal damage include people who are older, female, African American (20), smokers, have hypoalbuminemia, prior kidney damage, hypomagnesemia, dehydration, and/or have concomitant medical conditions such as diabetes, liver disease and use of angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, diuretic therapy, and non-steroidal anti-inflammatory drugs (16–18).

Cisplatin is freely filtered at the glomeruli but is subsequently absorbed by the proximal tubule cell where it becomes a more potent toxin by multiple enzymatic pathways including gamma glutamyl transpeptidase (GGT), which has the highest activity in the kidneys (2, 6, 21).

However, a consistent amount of cisplatin is secreted from the blood into the urine through the cells of proximal tubules (22). Here, cisplatin uptake is accomplished by the human copper transport protein 1 (Ctr1) and the organic cation transporter 2 (OCT2) (23) located on the basolateral side of the proximal (24) and distal (25) renal tubules, as well as by passive diffusion (26). Concentration in the proximal tubule may be five times greater than the blood (27), with intrinsic damage of the proximal and distal tubules resulting in renal tubular cells death, affecting renal tubular blood flow, decreasing glomerular filtration rate, and preventing reabsorption of magnesium and other electrolytes (2). Excretion from the tubular cells is dependent on multidrug extrusion transporters (MATEs) (6, 28). Although renal tubular cells may recover, fibrotic scarring may occur resulting in chronic kidney disease (CKD) (29).

Bunel et al. (29) in a small human study, identified an increase in urinary biomarkers for acute renal damage within 3 h after cisplatin administration, but a diagnostic rise in plasma creatinine in each patient with AKI was delayed until 3–6 days post-administration, by which time urinary biomarkers had normalized.

SUMMARY OF STUDIES DEMONSTRATING EFFECTIVENESS OF MAGNESIUM IN HUMANS

In 2019, two systematic reviews and meta-analysis of therapies directed at prevention of cisplatin AKI were published, both suggesting a benefit with pre-administration of IV magnesium (30, 31) (**Table 1**). Casanova reviewed all placebo-controlled trials published up to 2017 (22 met their criteria), and concluded that 1 g (8 mEq) of IV magnesium before cisplatin reduced AKI (30).

Hamroun et al. (31) searched Pubmed, Embase, and Web of Science from January 1, 1978, to June 1, 2018, assessing cisplatin AKI as defined by the 2012 AKI-KDIGO classification, which identified stage 1 as either serum creatinine 1.5–1.9 times OR ≥0.3 mg/dL (≥26.5 mmol/L) increase above baseline, or urine output decrease to 0.5 ml/kg/h for 6–12 h; stage 2: serum creatinine 2–2.9 × baseline, or decrease in urine output <0.5 ml/kg/h for × 12 h; stage 3: serum creatinine 3 × baseline or urine output <0.3 ml/kg/h (32). Of 4,520 eligible studies reviewed, 51 articles fulfilled the authors' selection criteria, which included evaluating 21 different prevention methods. A meta-analysis was only performed on those studies that used magnesium at the same time as the first dose of cisplatin (15 observational involving 1,841 patients), and demonstrated a significant AKI protection for all grades of injury (31). Based upon analysis of the data regarding stage 1 AKI, "25 mEq of magnesium was

TABLE 1 | Published results on the association between cisplatin-induced AKI* and magnesium administration.

Study	Intervention and outcome(s)		Results	P-value
Hamroun et al. (31)	**Risk of cisplatin-induced AKI***		**Odds Ratio** [95% Confidence Interval]	
Drugs	Magnesium dosage	All dosages confounded	0.24 [0.19; 0.32]	<0.001
		8 mEq	0.23 [0.16; 0.34]	<0.001
		20 mEq	0.13 [0.06; 0.29]	<0.001
		25 mEq	0.28 [0.14; 0.54]	<0.001
Casanova et al. (30)	**Risk of cisplatin-induced AKI***		**Odds Ratio** *[95% Confidence Interval]*	
Eur J Clin Pharmacol	Magnesium dosage	All dosages confounded	0.22 [0.15; 0.33]	<0.001
	Modification in serum creatinine levels		**Mean difference of serum creatinine** *(mg/dL) [95% Confidence Interval]*	
	Magnesium dosage	All dosages confounded	–0.19 [–0.34; –0.05]	< 0.001

**AKI, acute kidney injury defined by the 2012 KDIGO-AKI classification.*

associated with a significant nephron-protective effect (OR 0.20 [0.12–0.31], with a positive trend test (p = 0.002)" (31).

MAGNESIUM PREVENTS RENAL TOXICITY IN ANIMAL STUDIES: POTENTIAL MECHANISMS

Like humans, laboratory animals exhibit hypomagnesemia and AKI following serial cisplatin doses. Cisplatin specifically targets the proximal tubules, which comprise a significant portion of the kidneys. The proximal tubules contain a high density of epithelial cells with a large number of mitochondria necessary for providing the critical regulatory (pH balance, absorption, and secretion) and endocrine functions of the kidneys. In mice, organic cation transporters 1 and 2 (OCT 1 and OCT2) located on proximal tubule epithelial cells are considered to be the main transporters involved in cisplatin uptake by the renal proximal tubules (33, 34). Thus, this segment of the nephron is most susceptible to cisplatin-induced AKI (35, 36). Once taken up by the renal epithelial cell, cisplatin mediates its acute toxic effects by enhancing inflammation [*via* the Extracellular Signal Regulated Kinase (ERK) and Signal Transducer and Activator of Transcription 3 (STAT3) signaling and subsequent cytokine production], increasing oxidative stress and by inducing cell death (apoptosis, necrosis, and autophagy).

Animal studies also demonstrate that cisplatin treatment lowers serum magnesium levels (37, 38) and that poor magnesium status enhances cisplatin-induced AKI (17, 37, 39, 40). Although the exact mechanism(s) involved are not completely understood, there is some evidence showing that magnesium absorption is impaired by cisplatin-mediated renal damage, suggesting that magnesium deficiency augments cisplatin uptake (probably *via* OCT2 and Ctr1) and reduces elimination (*via* MATE1) by kidney epithelial cells. Most importantly, the nephrotoxicity of cisplatin can be blocked by early and sustained magnesium supplementation in magnesium-deficient animals, preventing irreversible kidney injury (17, 39). Additional data support that the host's magnesium status regulates multiple pathways associated with cisplatin-induced AKI, including oxidative stress, inflammation and apoptosis, and early magnesium supplementation protects against cisplatin-induced kidney damage through modulating these pathways (17, 40). Finally, while magnesium deficiency was associated with significantly larger tumors in mice and reduced cisplatin-mediated tumor killing *in vivo*, early magnesium supplementation was shown to protect the kidneys against cisplatin-mediated damage without compromising cisplatin anti-tumor efficacy while additionally potentiating the cytotoxic effect (39, 40). Together, these data strongly support that early magnesium supplementation exerts kidney-protective effects and may improve the anti-tumor efficacy of cisplatin.

IMPORTANCE OF MAGNESIUM HOMEOSTASIS AND HEALTH RISKS ASSOCIATED WITH HYPOMAGNESEMIA

Over 300 biological enzymes are dependent on magnesium, the second most abundant intracellular cation, and fourth most abundant cation in the body (41). The normal body contains 22–26 g of magnesium; 52.9% in the bone, 27% in the muscle, 19.3 in soft tissue, 0.5% in red blood cells, and 0.3% in the serum (42).

Although magnesium deficiency is almost always asymptomatic (43), it may lead to long-term health problems including but not limited to: affecting cardiac electrical activity, including sudden death; association with insulin resistance, inhibiting acute phase of insulin release in hyperglycemia; contributing to progression of atherosclerosis by affecting lipid concentrations; hypertension; osteoporosis; increase frequency in renal calculi; reactive airway disease; muscle weakness; and multiple non-specific complaints including: fatigue, anorexia, fibromyalgia, tendonopathy, tetany, and mood alterations (41).

Measurement of the serum magnesium level is not an accurate reflection of total body stores. Renal excretion is predominantly responsible for maintaining serum balance with 70–80% of non-protein bound magnesium being filtered at the glomerulus, 95% of the magnesium in plasma is reabsorbed by the kidneys (60% at the ascending loop of Henle and 10% in the distal tubule, resulting in a loss of only 100 mg) (41, 42, 44).

Although not labeled AKI, the most common evidence of early renal damage is hypomagnesemia, first identified in 1979 (45). Hypomagnesemia may enhance the severity of nephrotoxicity (46). In addition, observational studies in humans, similar to those in animals, have demonstrated that premedication with magnesium prior to cisplatin may reduce the nephrotoxicity of magnesium loss. In 1990, Vokes (9) reported a randomized study of 23 patients treated with cisplatin for head and neck cancer using oral magnesium aspartate hydrochloride either by continuous oral magnesium, with dosage being increased or magnesium supplemented intravenously if unable to be tolerated *versus* intermittent administration only if the serum magnesium level dropped to ≤1.4 mg/dL. All patients receiving intermittent magnesium required magnesium at some point in the study, but 80% of patients receiving continuous magnesium never developed hypomagnesemia in a given cycle. Likewise, Martin et al. (47) demonstrated in 1992 that both intravenous (3 g before each cycle) and oral magnesium supplementation (2 g orally every 8 h, days 2–21 of each cycle) appeared effective in prevention of cisplatin-induced hypomagnesemia in the majority of patients with only mild gastrointestinal side effects observed with the oral group. Vokes concluded that "preventive administration of a magnesium supplement can ameliorate, if not completely eradicate, cisplatin-induced hypomagnesaemia." (9) Hodgkinson, in 2006, also recommended routine supplementation of magnesium with each cycle of cisplatin to prevent cisplatin-induced hypomagnesemia (48). Most recently, a recent review by Duan supports magnesium administration to prevent AKI in elderly patients receiving cisplatin along with short hydration and amifostine (49).

Intravenous magnesium has been shown to be safe (50) and effective in multiple other medical conditions. It has been used to prevent AKI in contrast-induced nephropathy in primary percutaneous coronary intervention (51) and when administered intraoperatively with major laparoscopic abdominal surgery (52). Magnesium sulfate has been demonstrated to be more effective than anticonvulsants in acute eclampsia and reduces the risk of eclampsia by 50% in pre-eclampsia (41, 53). It has been used for status asthmaticus, torsades de pointes, and a higher concentration of magnesium has been correlated with better survival in chronic kidney disease (54). Lactate clearance has been shown to decrease in critically ill patients with severe sepsis with magnesium supplementation achieving a serum magnesium level near the upper limit of normal.

How exactly IV magnesium prevents AKI when given prior to cisplatin in humans is currently unknown and possibly involves numerous pathways as discussed previously. Although magnesium downregulated the OCT2 transporter and upregulated the MATE transporter, preventing AKI in rats, (55) this was not observed with acute exposure in recent experiments using cells expressing human OCT2 performed by Dr. Ciarimboli (see **Figure 1 A–C**). Therefore, further studies will be necessary to completely understand how magnesium prevents AKI in humans. Other drugs have demonstrated a downregulation of human OCT2 (hOCT2),

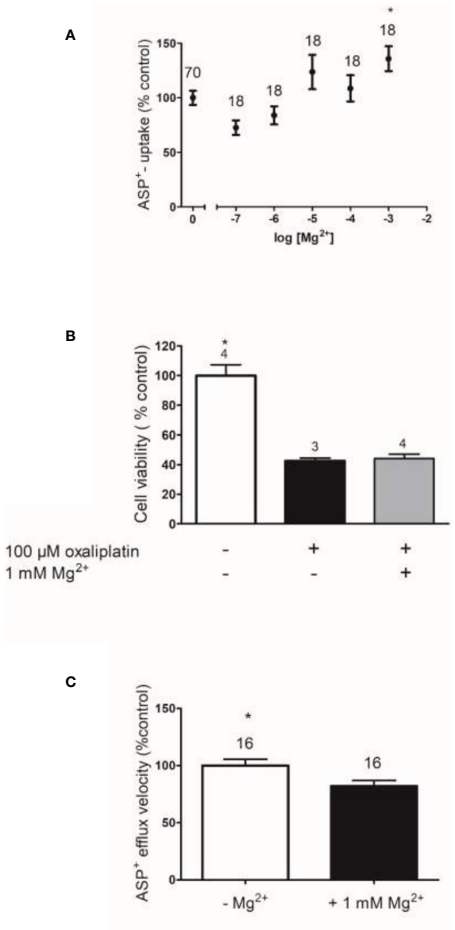

FIGURE 1 | The effects of Mg^{2+} on hOCT2 function **(A)**, on hOCT2-mediated Oxaliplatin toxicity **(B)**, and on hMATE1 function **(C)**. Function of the transporters was measured as uptake (hOCT2) or efflux (hMATE1) of the fluorescent organic cation 4-[4-(dimethylamino)styryl]-N-methylpyridinium (ASP^+) as described in Wilde et al. (56) and Kantauskaite et al. (57) Panel **(A)** shows the changes of ASP^+ uptake by hOCT2 stably expressed in Human Embryonic Kidney (HEK) cells in dependence from extracellular Mg^{2+} concentration compared to what was measured in Mg^{2+} absence (mean ± SEM). Only 1 mM Mg^{2+} significantly increased ASP^+ uptake by hOCT2 (Kruskal–Wallis test with Dunn's multiple comparison test). The presence of Mg^{2+} stimulates the activity of hOCT2, a transporter mediating nephrotoxicity of platinum derivatives, therefore regulation of hOCT2 by Mg^{2+} cannot explain Mg^{2+} protective effects against cisplatin nephrotoxicity. **(B)** shows the effects of Mg^{2+} supplementation (1 mM) on toxicity of 100 µM Oxaliplatin measured with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in hOCT2 overexpressing HEK cells (mean ± SEM). The presence of 1 mM Mg^{2+} does not protect the cells against hOCT2-mediated Oxaliplatin toxicity. Finally, **(C)** shows the changes of ASP^+ efflux by hMATE1 stably expressed in HEK cells in dependence from extracellular Mg^{2+} concentration compared to what measured in Mg^{2+} absence (mean ± SEM). The extracellular presence of 1 mM Mg^{2+} slightly but significantly decreases the function of hMATE1 (Mann–Whitney-test), which is an efflux transporter for platinum derivatives. Therefore, also a regulation of hMATE1 by Mg^{2+} cannot explain Mg^{2+} protective effects against cisplatin nephrotoxicity. In **(A, C)** the numbers above the columns represent the number of replicates measured in at least three independent experiments. In **(B)** they represent the number of independent experiments.

protecting against AKI, such as carvedilol, (58) while metformin and cimetidine have been competitive substrates for hOCT2. Research has shown the renal protective effects of both of these agents from cisplatin toxicity (59, 60). However, two meta-analyses have both identified that IV magnesium prevents AKI from cisplatin and recommend it over all other agents at this time.

CONCLUSION

Currently, there is no dispute regarding the renal toxicity associated with cisplatin. AKI, based on elevation of serum creatinine or decreased urine output, occurs in approximately one-third of all patients receiving cisplatin. Hypomagnesemia occurs in 40–100% of patients following cisplatin and may persist long after chemotherapy completion, reflecting irreversible cisplatin-mediated kidney damage. What has been controversial is the administration of IV magnesium prior to each dose of cisplatin, rather than after inevitable hypomagnesemia is subsequently identified. Compelling recent reviews of human trials and animal studies clearly support the pre-administration of IV magnesium for ameliorating both AKI and hypomagnesemia. Acute cisplatin-induced nephrotoxicity (including AKI and hypomagnesemia) may cause persistent and irreversible kidney impairment, resulting in further health complications.

Hesitancy regarding IV magnesium prior to cisplatin may have originated from the early termination of the Combined Oxaliplatin Neurotoxicity Prevention (CONcePT) in 2007, in which calcium/magnesium was infused before oxaliplatin chemotherapy for colon cancer to prevent neurotoxicity. Although initial data suggested a 52% reduction in oxaliplatin's killing efficacy following calcium/magnesium administration, (61) this was subsequently reversed in 2008 and Wu, in 2012, published a systematic review concluding that IV calcium/magnesium does not impair oxaliplatin effectiveness (56). In addition, there has been no evidence in any of the trials analyzed that magnesium affected the chemotherapeutic effect of cisplatin, and a higher magnesium level actually potentiated cisplatin chemotherapeutic effect in mice (40). Finally, the OCT2 transporter that is involved in the cisplatin/oxaliplatin uptake and subsequent AKI has not been identified in tumors.

This case report along with supporting documentation provided by both clinical and pre-clinical studies clearly demonstrate the effectiveness of IV magnesium before cisplatin in preventing acute kidney injury manifested by oliguria. We recommend that at least 2 g of magnesium be administered prior to cisplatin since a recent study by Uhm found that 1 g still resulted in 33% of patients developing hypomagnesium (57), while a study by Hase supported the use of 20 mEq/L or approximately 2.5 g. (62)

Therefore, if we are to follow our oath to do no harm, it is imperative that IV magnesium administration with cisplatin become a "best practice" guideline at all oncology centers.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MM: concept for paper and case report. AH: review of human studies and comparison of recent meta-analyses, and development of table comparing these. CM: editing and review of paper. YS: review of renal receptors and effect of magnesium on them. CNM: summary of research in animals and editing of manuscript. SE: assistance in experiments using magnesium with human renal transporters GC: development and analysis of experiments using magnesium with human renal transporters. All authors contributed to the article and approved the submitted version.

FUNDING

Source for funding: Medical Research Fund Community Foundation of Washington County, Inc 37 S. Potomac Street Hagerstown, MD 21740.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

REVIEW
published: 12 March 2021
doi: 10.3389/fonc.2021.638148

Probiotics in Cancer

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In recent years, the consumption of over-the-counter probiotics to promote health has grown rapidly worldwide and become an independent industry. In medicine, various studies have demonstrated that probiotics can help improve the immune system and intestinal health. They are usually safe, but in some rare cases, they may cause concerning adverse reactions. Although the use of probiotics has been widely popularized in the public, the results of many probiotic clinical trials are contradictory. Particularly in cancer patients, the feasibility of probiotic management providing benefits by targeting cancer and lessening anticancer side effects requires further investigation. This review summarizes the interactions between probiotics and the host as well as current knowledge on the pros and cons of utilizing probiotics in cancer patients.

Keywords: probiotics, cancer, safety, clinical trials, treatment

OPEN ACCESS

Edited by:
Guido Bocci,
University of Pisa, Italy

Reviewed by:
Luca Antonioli,
University of Pisa, Italy
Sandra Donnini,
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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: *05 December 2020*
Accepted: *01 February 2021*
Published: *12 March 2021*

Citation:
Lu K, Dong S, Wu X, Jin R and Chen H (2021) Probiotics in Cancer. Front. Oncol. 11:638148. doi: 10.3389/fonc.2021.638148

INTRODUCTION

In the human intestine, there are more than 100 trillion symbiotic bacteria, far exceeding the number of host cells, which together constitute the intestinal flora (1). They affect multiple functions of the host, and the stability of the intestinal flora is essential for preventing pathogen infection and disease (2). The history of human consumption of probiotics can be traced back as early as 1907 (3). After more than a century of screening, lactic acid bacteria and Bifidobacteria have dominated the market. Among them, Bifidobacterium (adolescentis, animalis, bifidum, breve, and longum) and Lactobacillus (acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, plantarum, rhamnosus, and salivarius) are the most commonly used species on the market (3). At the same time, several other strains seem promising for human health, such as Roseburia spp., Akkermansia spp., and Faecalibacterium spp., which are worthy of in-depth investigation (4).

In recent years, studies on the use of probiotics for the prevention and treatment of human diseases have been performed globally (1). At present, a variety of beneficial mechanisms have been identified, including regulating intestinal flora, enhancing intestinal barrier function, protecting intestinal epithelium from invasion by pathogens and strengthening immune function (5, 6).

Cancer patients have compromised immunity caused by primary diseases, chemotherapy and radiotherapy. The effects of probiotics in this population may differ from those of healthy people and raise several critical concerns (7). Therefore, this article reviews whether cancer patients can take probiotics as well as their pros and cons (**Figure 1**).

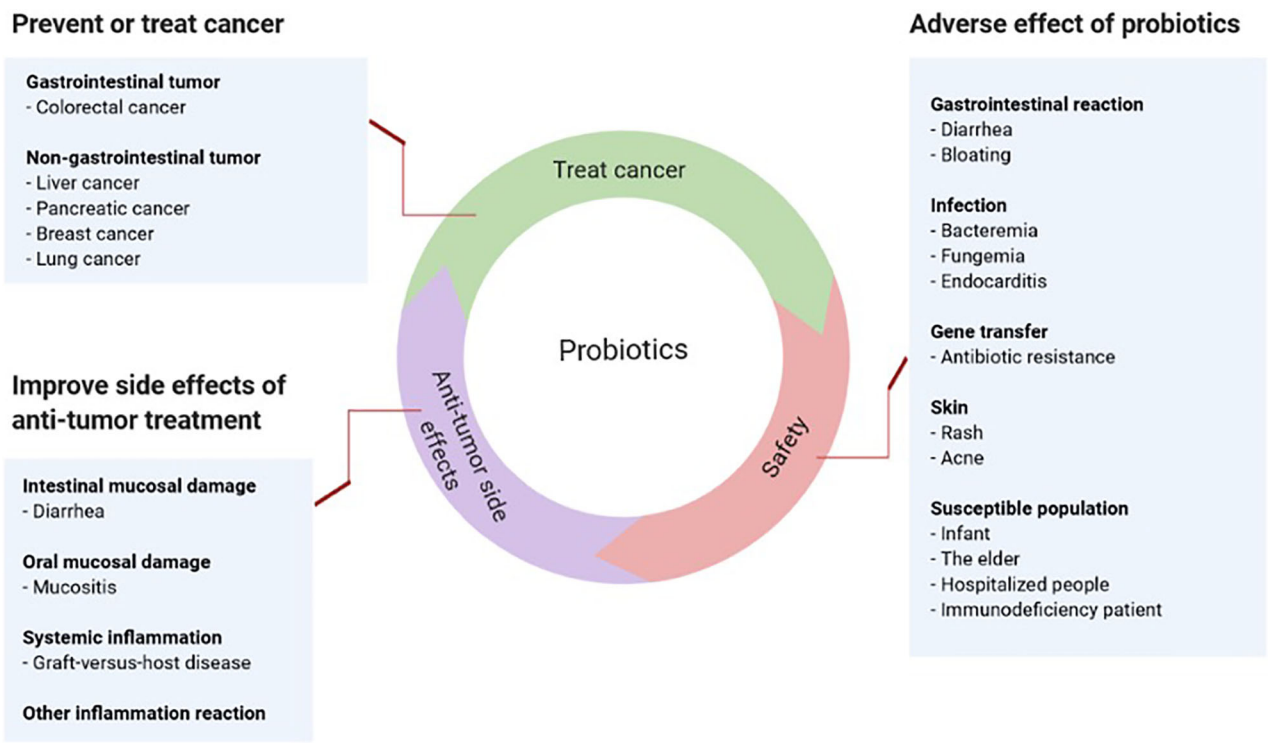

FIGURE 1 | Pros and cons of probiotics in cancer.

THE EFFECT OF PROBIOTICS ON THE HOST

Studies have confirmed that probiotics can exert a variety of beneficial effects on the host. In addition, probiotic metabolites, such as short-chain fatty acids (SCFAs) and lactic acid, also play a significant role (4). Using forward chemical genetic screening, a recent study found that multiple probiotic metabolites modulate host physiology by activating G protein-coupled receptors (GPCRs) (8). Based on the contribution of probiotics to intestinal health, it is currently believed that the core benefit of probiotic management is to maintain healthy intestinal flora and support a healthy immune system through nonspecific and specific physiological effects, respectively (8) (**Figure 2**).

Nonspecific Physiological Effects

Regulation of intestinal flora: probiotics can maintain a healthy balance of intestinal flora. By studying fecal specimens, it was found that supplementation with probiotics may increase the count of specific bacterial strains in healthy adults, suggesting that probiotics may cause changes in the total number, diversity and composition of intestinal flora (9). In the past, this has been used as an evaluation standard, but considering that fecal flora only reflect part of the intestinal flora information, a great deal of information is missing when evaluating fecal samples only (10). The closer the sampling site is to the end of the rectum, the less it reflects the structure of the upper flora. In a large-scale genomic analysis, fermented foods were indeed an important source of intestinal lactic acid bacteria, providing unprecedented evidence that food-derived probiotics are closely related to the composition of intestinal microorganisms (10).

Stabilizing the intestinal epithelial cell barrier: probiotics regulate the cytoskeleton to stabilize the mucosal barrier and promote mucin secretion to prevent the colonization of pathogens in the epithelium (11). They can induce expression and distribution of tight junction proteins (12). By sealing the top epithelium and endothelium, an increase in epithelial permeability and damage to the epithelial structure are prevented. Probiotics could also restore abnormal transepithelial resistance caused by pathogenic lipopolysaccharide (LPS), thereby reducing the inflammatory response and excessive apoptosis (12). In addition, certain probiotic strains regulate the polarization of T helper 17 (Th17) cells and effectively induce secretion of IL-17α, which triggers type 3 innate lymphocytes (ILC3s) to produce IL-22 (6). IL-22 is a key immune defense cytokine that plays an important role in maintaining intestinal homeostasis and promoting healing and tissue regeneration. Animal experiments have revealed that mice lacking these cytokines are prone to experimental colitis due to defects in defensin secretion and damaged epithelial tight junctions (13).

Inhibiting pathogens: There are primarily two distinct mechanisms of inhibiting pathogens. One belongs to the physical defense system. The infection of pathogens starts from

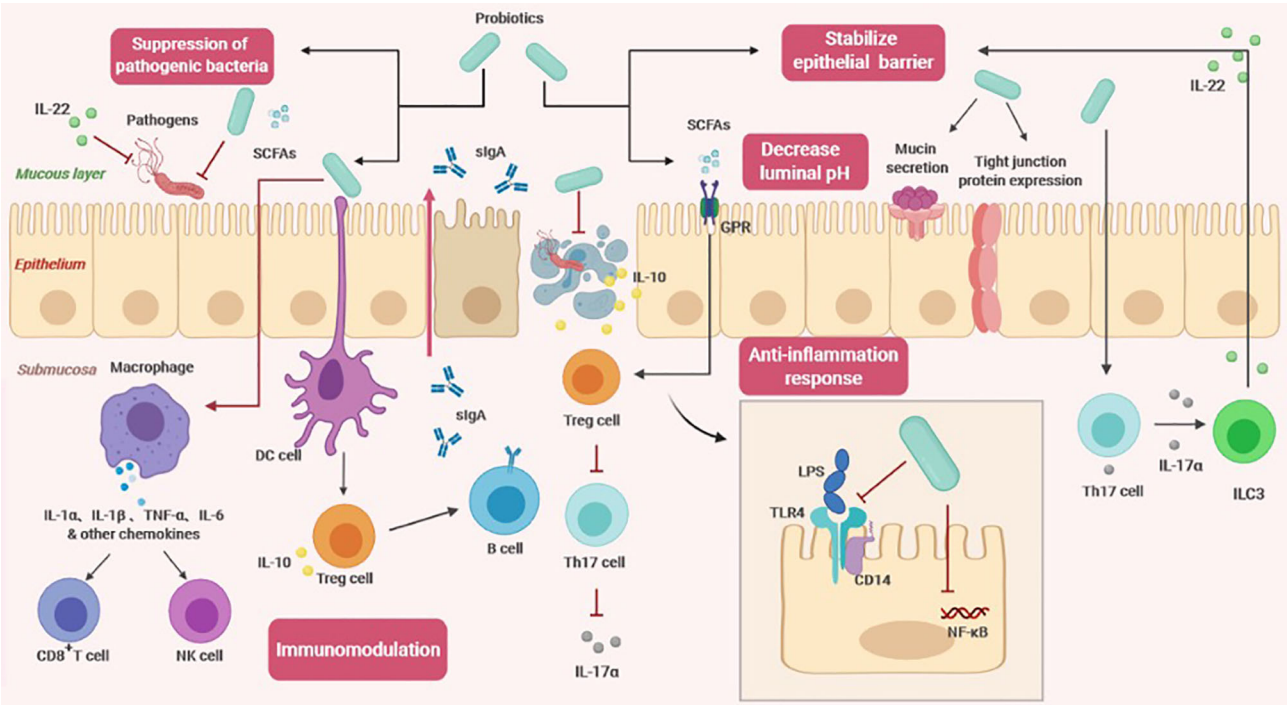

FIGURE 2 | The effects of probiotics on the host (SCFAs, short-chain fatty acids; sIgA, soluble IgA; GPR, G protein coupled free fatty acid receptor; DC cell, dendritic cell; Treg cell, regulatory T cell; Th17, T helper cell 17; ILC3, Type 3 innate lymphocyte; NK cell, Natural killer cell; LPS, lipopolysaccharide; TLR4, Toll-like receptor 4; NF-κβ, nuclear factor-κB).

colonization on the surface of the intestinal mucosa, causing tissue damage. When probiotics completely occupy the space of the intestinal wall, there is no available space for pathogens, and probiotics can further inhibit the adhesion of pathogenic bacteria by obtaining more nutrients (7). The other mechanism is related to the antagonistic properties of probiotics, which can reduce the microenvironment pH by producing SCFAs (11). Some studies have found that SCFAs are primarily produced by utilization of undigested carbohydrates by colon anaerobic bacteria, mainly acetic acid, propionic acid, and butyric acid. The high concentration of SCFAs that accumulate in the intestinal tract can quickly lower the pH (14). Compared to pathogens, probiotics are more able to adapt to lower pH environments and therefore have a better survival rate. In addition to changing the pH value, probiotics also antagonize pathogen adhesion and transport through other mechanisms (7). A new study showed that IL-22 derived from the intestinal flora regulated mucosal glycosylation modification, promoted the growth of the symbiotic bacterium Phascolarctobacterium, and competed with Clostridioides difficile for succinate, preventing Clostridioides difficile infection (15).

Specific Physiological Effects

Immune regulation: Probiotics can regulate humoral immunity, innate immunity and cellular immunity through distinct mechanisms (11). Despite some commonalities between probiotic and pathogenic surface molecules, intestinal epithelial cells can perceive and distinguish between symbiotic and pathogenic bacteria through cytokine production and signal transduction (16). After probiotics come into contact with intestinal epithelial cells, host dendritic cells (DCs) accurately recognize probiotic surfaces and effector molecules through pattern recognition receptors and coreceptors and then present antigens to regulatory T cells (Tregs) after processing (17). The increase in the number of Tregs promotes the transformation of B cell antibody classes and the secretion of large amounts of sIgA (17). Recent studies have shown that in addition to T cell-dependent pathways, sIgA production is also regulated through T cell-independent pathways (18). This process is mediated by metabolite-sensing free fatty acid receptors (18). After SCFAs bind to fatty acid receptors, they induce dendritic cells to express class 1A acetaldehyde dehydrogenase (Aldh1a), which converts vitamin A into retinoid acid, thereby assisting in the production of sIgA (18). In addition, probiotics activate macrophages to secrete cytokines and subsequently activate host natural killer cells and cytotoxic T cells, which participate in the immune response to clear pathogens (16). SCFA-mediated G protein-coupled free fatty acid receptor 43 (GPR43) signaling also causes NLRP3 inflammasome activation and secretion of IL-18 to further limit pathogen invasion (19).

Anti-inflammatory response: There are reports of probiotics inducing both anti-inflammatory and pro-inflammatory responses. Although this may seem contradictory at first glance, it indicates that probiotics have an important balancing

effect on intestinal homeostasis in different contexts (20). Through multiple signaling pathways, probiotics can regulate the expression of cytokines, chemokines, and antimicrobial peptides, including the nuclear factor-κB (NF-κβ) and mitogen-activated protein kinase (MAPK) pathways (16). The role of probiotics in the anti-inflammatory response is related to their ability to regulate Toll-like receptors (TLRs) and GPRs. Probiotics could stimulate negative regulatory factors (A20, Bcl-3, and MKP-1) to attenuate LPS-induced TLR4 activation (21). They can also inhibit binding of LPS to the CD14 receptor, reducing the overall activation of NF-κβ (22). After SCFAs bind to GPR, the regulatory function of $Foxp3^+$ Treg cells is enhanced, increasing IL-10 production. Tregs recognize protection in various inflammatory diseases, so SCFA signaling reduce sensitivity to chronic inflammation (22). Another study indicated that GPR109A on the surface of dendritic cells and macrophages recognizes butyrate, promotes Treg development and inhibits proliferation of proinflammatory Th17 cells (19).

EFFECTS OF THE HOST ON PROBIOTICS

It has been reported that the same strain has differential effects on host physiology. Distinct from medicines, the efficacy of probiotics varies greatly from individual to individual. Age, physical condition, intestinal microbial composition, colonization permission and diet of the host all contribute to the heterogeneity of the effect (23). In infants and young children whose immune function is not yet fully developed, during the first month after birth, the development of intestinal flora is essential for the balanced development of the baby's immune system. Bifidobacterium in breast milk is not only noncytotoxic but also has good immunostimulatory ability, but there is insufficient evidence to show that supplementation with probiotics is beneficial to infant health (14). In an observational study, although probiotic supplementation increased infant sIgA response, the incidence of mucosa-related diseases was higher in early childhood (24). Compared to healthy adults, the beneficial effects of probiotic exposure in infancy were not only limited but were also related to increased infections later in life (24).

In cancer patients, after undergoing treatments, such as chemotherapy, radiotherapy or surgical eradication, underlying medical conditions, such as cachexia combined with treatment-related side effects, and the microenvironment are more complicated, and can directly lead to intestinal mucosal barrier destruction and immune system dysfunction. The above changes are not conducive to the colonization of beneficial probiotics in the colon (25). In individuals with colorectal cancer, a reduction in the number of probiotics was observed (26). Zmora, N. et al. found that host local intestinal microbes also played a central role in the colonization of probiotics, and the useful function of probiotics was dependent on the support of the intestinal flora (27). These results indicate that even if the probiotics used are beneficial, the colonization barrier will greatly affect the therapeutic effect. There is an urgent need to elucidate the effects of probiotics in specific populations, such as cancer patients.

The intestinal microecology is composed of intestinal flora, prebiotics and enteral nutrition, which complement one another. Therefore, probiotics need a suitable environment to function. A variety of foods has been added to maintain healthy flora (28). For example, fermentable carbohydrates support the colonization and growth of beneficial bacteria in the intestine (29). Dietary fiber stimulates the growth and activity of beneficial bacteria and can reduce stomach acid to protect probiotics, allowing them to pass smoothly into the intestine. Polyunsaturated fatty acids regulate the adhesion of probiotics (9). For cancer patients, in addition to individual factors, dietary difficulties and the occurrence of malnutrition accelerates the collapse of intestinal homeostasis caused by cancer. In this vicious cycle, the therapeutic effect of probiotics is greatly reduced (30).

PROBIOTICS TO PREVENT AND TREAT CANCER

The results of many *in vitro* studies have shown that probiotics have beneficial properties in regulating proliferation and apoptosis of cancer cells (31). For example, it has been demonstrated that in mouse colon cancer HGC-27 and human colon cancer Caco-2, DLD- 1, and HT-29 cells that Lactobacillus rhamnosus GG strain inhibits proliferation and induces apoptosis (32).

In preclinical experiments, potential antitumor products include probiotics and their metabolites, such as butyrate and pyridoxine. SCFAs are the energy source of colon cells, maintaining the acidic environment of the intestine, inhibiting the formation of high levels of secondary bile acids, and promoting acidosis and apoptosis of cancer cells (33). Among them, butyric acid helps to balance proliferation, division and apoptosis of colon cells. Approximately 70%–90% of butyrate is produced by colon cell metabolism, and compared to healthy people, there is an obvious reduction in this type of acid in the stool of patients with colorectal cancer (34). Although SCFAs are derived from the intestinal flora, due to individual differences, the amount produced may not be sufficient to inhibit the development of colorectal cancer. Therefore, the consumption of probiotics can help increase the daily production of SCFAs. The presence of SCFAs can inhibit the growth of pathogens. In *in vitro* experiments, propionic acid and butyric acid inhibited expression of invasive genes encoded by Salmonella typhimurium, thereby preventing its attack on healthy cells (35).

In addition, SCFAs can also regulate local intestinal immunity and the systemic immune response. SCFAs induce intestinal epithelial cells to produce antibacterial peptides and enhance the expression of tight junctions to stabilize intestinal barrier function. SCFAs affect inflammation by interacting with G protein-coupled receptors in the intestine and balancing the immune response (36). Conjugated linoleic acid (CLA) is an isomer of linoleic acid (LA), and both isomers can induce expression of apoptosis genes, including Bcl-2, caspase 3, and caspase 9, inhibiting the spread of colon cancer cells (**Figure 3**). Previous studies have reported

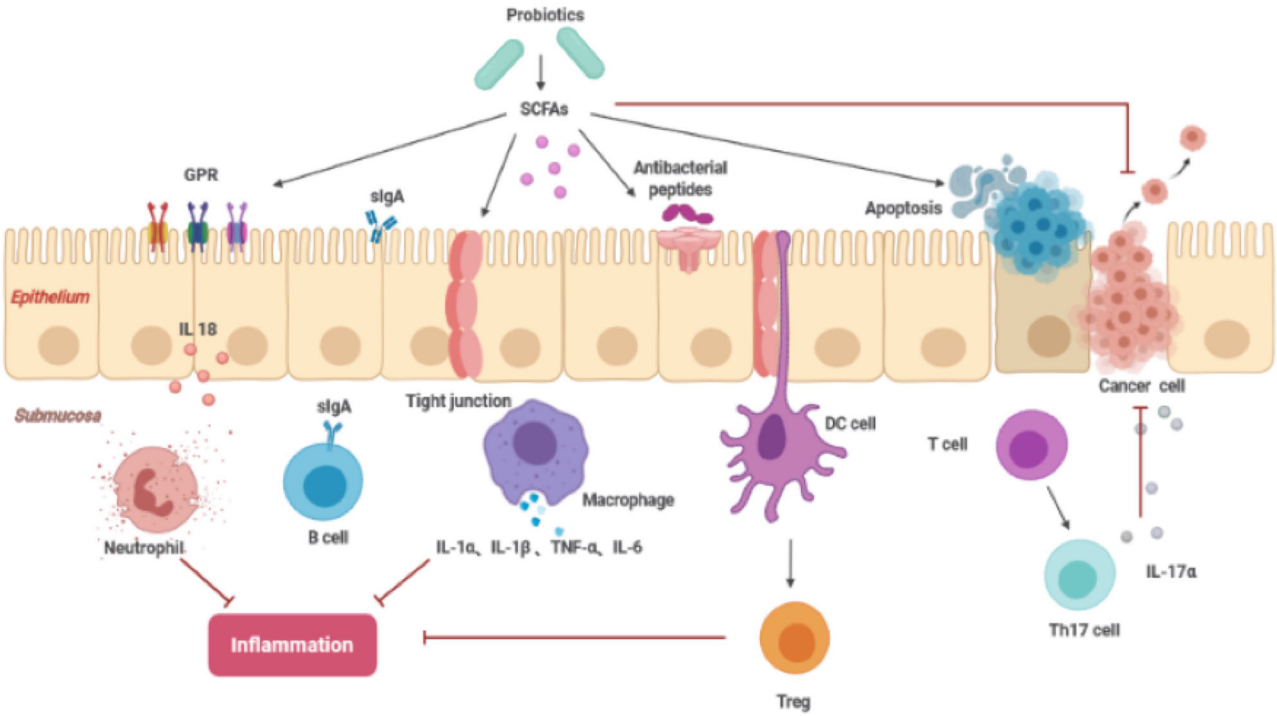

FIGURE 3 | The function of SCFAs inhibiting cancer (SCFAs, short-chain fatty acids; sIgA, soluble IgA; GPR, G protein coupled free fatty acid receptor; DC cell, dendritic cell; Treg cell, regulatory T cell; Th17, T helper cell 17).

that Lactobacillus, Bifidobacterium, Streptococcus salivarius, and Propionibacterium freudenreichii subspecies can produce CLA in the terminal ileum, which can be absorbed by colonic cells or interact with it to exert its beneficial effects (31).

These specific microbial strains can be used either alone or in combination with cancer treatment agents. The goal of treatment was achieved by activating immune surveillance against cancer (19). For example, Shi L et al. found that combined treatment with TGF-β receptor blockers and probiotics could enhance the antitumor immune response, thereby inhibiting the growth of tumors (37).

Studies have indicated that the anticancer mechanisms of probiotics primarily include positive regulation of intestinal flora, changes in metabolic activity, the binding and degradation of carcinogenic compounds, immunomodulation to improve chronic inflammation, lowering intestinal pH and the inhibition of enzymes that produce potential carcinogenic compounds (26, 38) (**Figure 4**). The positive role of probiotics in the treatment of tumors has been confirmed, at least in animal models (39, 40).

Abnormal composition of the intestinal flora is a high-risk factor for colorectal cancer (41). The intestinal flora of patients with colorectal cancer usually contains a greater proportion of bacteria that cause gastrointestinal inflammatory diseases and bacteria that can produce toxins and carcinogenic metabolites (42). In contrast, SCFA-producing bacteria and potentially beneficial probiotics exhibit a decreasing trend (26). Chronic inflammation can make individuals susceptible to cancer (26). Studies found that under the mucus layer of the colon, Clostridium spp. were in direct contact with colon cells, invading the submucosa of the colon and causing persistent local inflammation (38). In addition, increased Clostridium spp. were found in colorectal cancer tissues, and they exhibited a profile of inflammation-related genes and proteins, such as COX-2, NF-κB, TNF-α, IL-6, IL-8 and IL-12, and matrix metalloproteinases 3 and 9, all of which contributed to tumor occurrence and transfer (26). Chandel D et al. found that the use of Lactobacillus rhamnosus GG, Lactobacillus acidophilus, or combination with celecoxib in a colorectal cancer animal model reduced NF-κB, COX-2, β-catenin, and K-ras carcinogenic biomarkers (43).

Compared to noncancer patients, the microbial structure of sample tissues from colorectal cancer patients was significantly different, and the diversity was lower (43). Treatment with probiotics increased the number and diversity of mucosal microorganisms and improved microbial structure (44). Pyrosequencing also revealed that probiotics could significantly reduce the abundance of the Fusibacter genus, which was previously suggested to be a contributing factor to tumorigenesis (45). Another preclinical study claimed that Bifidobacterium bifidum and L. acidophilus could be used as biotherapeutic agents to inhibit colon cancer by modifying intestinal bacteria (39). In people who are highly susceptible to colorectal cancer, probiotics might be used as an alternative biological therapy to prevent or even treat cancer (39).

In addition to gastrointestinal tumors, abnormal changes in the composition and function of intestinal microbes could also

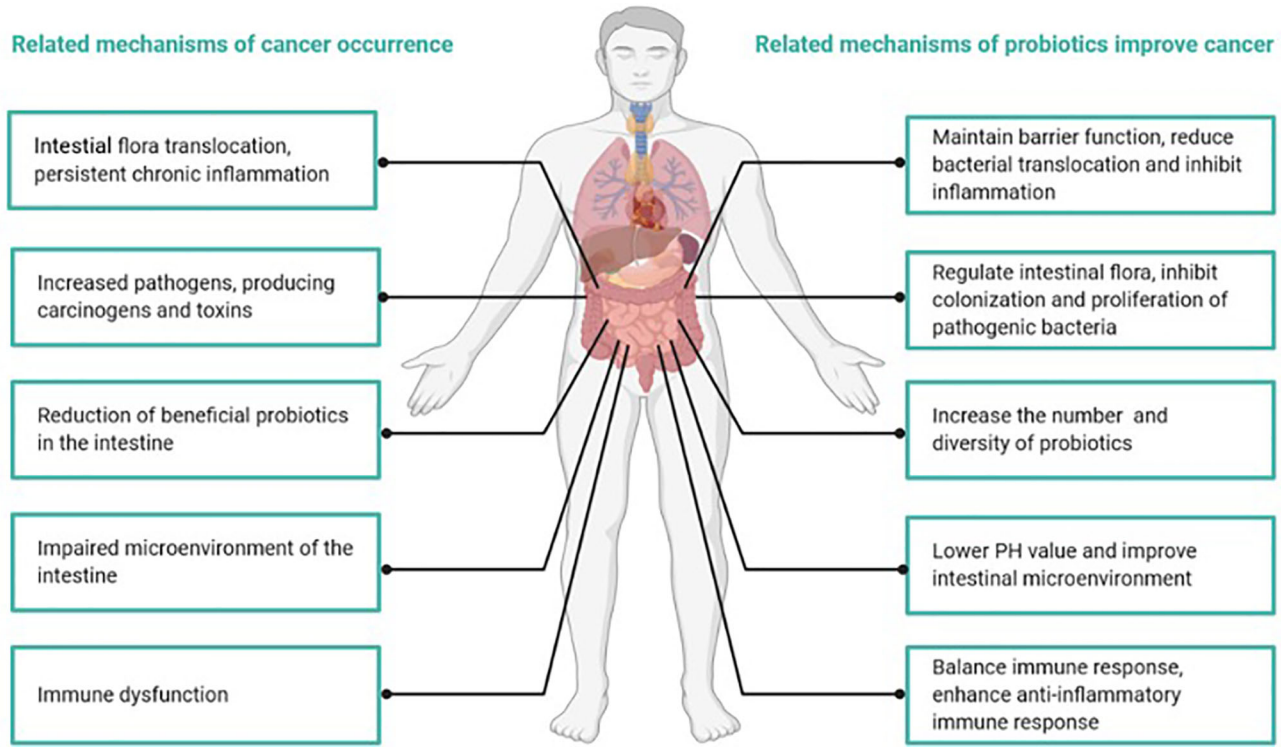

FIGURE 4 | Mechanisms of cancer occurrence and how probiotics attenuate cancer.

affect nongastrointestinal tumors, including liver cancer, pancreatic cancer and even breast cancer (25, 46). Through the portal venous system, the liver is uniquely exposed to intestinal bacteria and their metabolites, which may cause inflammatory changes and hepatotoxicity and ultimately directly lead to cancer. It has also been widely recognized that disturbance of the intestinal flora may cause liver cancer (47). For example, Hemophilus is a common pathogenic bacterium colonizing the colonic mucosa that has also been detected in human liver cancer tissues (47). Studies indicate that Hemophilus produces a lethal dilatant toxin after translocation to the liver and activates Wnt/β-catenin, NF-κB, p21, and Ki67 signaling in liver cells to induce liver cancer (19).

By constructing a mouse liver cancer model, Li J et al. confirmed that treatment with the probiotic E. coli Nissle 1917 enhanced the antitumor immune response, inhibiting tumor progression (40). The specific mechanism included Th17 cells and their product IL-17 being reduced in tumor tissues, while differentiation of Treg/Tr1 cells was enhanced, which affected expression of vascular growth factors and suppressed the progression of liver tumors through inflammatory and angiogenic mechanisms (40).

A recent study by Le Noci V et al. showed that probiotic aerosol therapy was beneficial for inhibiting lung melanoma metastasis (48). The lung microenvironment has high immune tolerance, and this feature prevents excessive inflammation caused by inhaled air particles (49). However, it also provides conditions for lung metastasis of various tumors (49). Lactobacillus rhamnosus induces the maturation of resident antigen-presenting cells, further activating lung T cells and NK cells and improving the immune suppression state, enhancing the antitumor immune effect (48). When used in combination with the chemotherapeutic drug dacarbazine, treatment efficacy was significantly enhanced. Probiotic aerosol therapy has become a new clinical therapy to prevent lung metastasis in high-risk melanoma patients (48).

Abnormal intestinal flora not only affects the pathogenesis of cancer but also participates in the therapeutic effect of anticancer treatment. Research in the past two years has emphasized the relationship between the microbiome and immunotherapy based on immune checkpoint inhibitors, such as PD-1/PD-L1 (50). Several research teams have discovered that the number, type and composition of the intestinal flora of cancer patients are closely related to the efficacy and survival of patients receiving PD-1 inhibitor therapy. The possible mechanism is that the interacting flora participates in the anticancer natural immune response (51, 52).

Routy et al. reported the response of patients with lung cancer, kidney cancer and bladder cancer to immunotherapeutic PD-1 blockade. They found that if patients had used broad-spectrum antibiotics before and after immunotherapy (two months before treatment and one month after the start of treatment), the bacteria in the body, including the intestinal flora, were disordered, and the immunotherapy effect was very poor. Both progression-free

survival and the overall survival were significantly lower compared to patients who did not use broad-spectrum antibiotics. The bacteria Akkermansia muciniphila, enriched in the intestine, was the reason for some patients responding to PD-1 blockade (53).

In another clinical study, Gopalakrishnan et al. also found that the response of melanoma patients to anti-PD1 immunotherapy was related to the diversity and composition of trillions of beneficial and harmful bacteria in the digestive tract. Based on the analysis of patient stool samples, it was found that compared to patients who did not respond to PD1 checkpoint inhibitor treatment, patients who did respond to PD1 checkpoint inhibitor treatment had a more diverse intestinal flora, and the content of Clostridium order was increased. There are a large number of Bacteroides bacteria in the intestine of melanoma patients who have not responded to treatment, and their bacterial diversity is far less than that of melanoma patients who have responded to treatment. By detecting the presence of important immune system cells in the patient's tumor, patients who respond to anti-PD1 immunotherapy were found to have higher levels of immune infiltration, including $CD8^+$ killer T cells related to specific bacteria (52).

The intestinal flora is not a necessary condition for the antitumor effect of chemotherapeutic drugs, and experiments have found that the survival rate of sterile or flora-depleted mice was significantly reduced (52). After treatment with lactic acid bacteria, the anticancer effect of chemotherapeutic drugs was restored. These results indicate that the flora might facilitate the chemotherapy effect through a flora-dependent mechanism (54).

In conclusion, *in vitro* studies have found that probiotics induce tumor cell apoptosis and inhibit tumor cell proliferation and metastasis. In animal models, probiotics improve tumor conditions. This positive effect provides a basis for clinical trials. However, considering that most of the current research on probiotics and cancer is limited to gastrointestinal tumors, the specific mechanism of probiotics against tumors has not been fully elucidated. Even in animal experiments, because most tumors are induced by chemical drugs, they are different from the complex pathogenesis of human tumors, so the therapeutic effects of probiotics must be carefully considered.

THE ROLE OF PROBIOTICS IN THE TREATMENT OF ANTITUMOR SIDE EFFECTS

Gastrointestinal discomfort is a common side effect of antitumor therapy. Radiochemotherapy directly kills intestinal cells, and the stress response it causes leads to destruction of the intestinal mucosal barrier. In the case of increased permeability of the intestinal mucosa, intestinal flora and endotoxins enter extraintestinal tissues and organs, causing uncontrolled systemic inflammation and multiple organ failure (55, 56). Surgery may result in impaired physiological gastrointestinal function. Diarrhea can be caused by a significant reduction in the transit time of food through the intestines and excessive bacterial growth (57). Antibiotics are often used during treatment, which can also affect the microbiome (58). Probiotics based on Bifidobacterium and Lactobacillus can effectively resist the growth of harmful bacteria through biological action (59). Supplementing with probiotics can improve the intestinal environment, enhance intestinal mucosal barrier function, and reduce the occurrence of diarrhea (57, 59). Recent studies revealed that the improvement of antitumor side effects by probiotics was also related to innate immunity. For example, probiotic cell wall acyl dipeptides alleviate mucosal damage caused by antibiotic chemotherapeutics by stimulating intracellular pattern recognition receptors (NOD2) (57, 59). In general, probiotics may have a beneficial effect by improving diarrhea caused by radiochemotherapy or surgery and rarely cause side effects.

In addition to restoring the intestinal mucosal barrier, probiotics can also attenuate oral mucosal damage induced by chemotherapy. In clinical treatment, more than 70% of hematological patients receiving high-dose chemotherapy and hematopoietic stem cell transplantation (HSCT) may develop grade III or IV oral mucositis, which causes great pain. Atul Sharma et al. analyzed the efficacy of Lactobacillus CD2 in preventing grade III/IV mucositis in patients receiving HSCT (60). Only 19% of patients developed grade III or IV mucositis. The median time to onset and recovery were 6 days and 8 days, and throughout the observation process, no adverse reactions related to probiotics were observed (60).

Probiotics also help in systemic inflammation, such as graft-versus-host disease (GVHD). Donor-derived T cells, proinflammatory cytokines, and LPS are the primary triggers of GVHD, in which the intestine is one of the organs most affected by GVHD and a key determinant of GVHD severity. The occurrence of GVHD greatly limits the feasibility and efficacy of HSCT (61). An intact intestinal barrier plays an important role in the development of GVHD, and LPS can enter the circulatory system through the damaged mucosal barrier to induce GVHD (62). In animal experiments, oral administration of L. rhamnosus GG before and after transplantation improved the survival rate of mice, especially between 7 and 14 days after transplantation, and the reduction in mortality was even more pronounced (63). Probiotic administration in patients receiving HSCT may also reduce the incidence of stage III-IV acute GVHD. One ongoing study showed that probiotic supplementation therapy reduced the bacterial translocation of mesenteric lymphoid tissue and the reduction of terminal ileal histological inflammation, indicating that probiotics can indeed attenuate GVHD (64).

Emerging data indicate that there is a strong correlation between abnormal microbiota composition and intestinal manifestations of acute GVHD (65). Although it has been observed that probiotics can improve GVHD in animal models, the mechanism is poorly understood. There are reports that SCFAs directly act on intestinal epithelial cells to promote recovery (65). Studies have also shown that IL-22 plays an important role in mediating the recovery of intestinal stem cells in GVHD, which might be related to its function of promoting Paneth cells to secrete antimicrobial peptides and mediating epithelial regeneration (65).

Similarly, probiotic metabolites may also ameliorate GVHD. Indole or indole derivatives metabolized by tryptophan in the

intestinal flora can limit intestinal inflammation caused by various stressors (66). Indole-3-carbaldehyde (ICA), an indole derivative, reduced intestinal bacterial translocation and inflammatory cytokine production in mice through type I IFN signaling (66). In mice lacking type I IFN signaling, the protective effect of ICA was eliminated after radiation exposure (66). These data indicate that indole could assist in limiting acute GVHD-related damage while retaining the antitumor response (66). In general, intestinal GVHD is characterized by the destruction of the integrity of the intestinal epithelial barrier and the disorder of flora. Therefore, probiotics and their production, which remodel the microbial community, inhibit pathogens, reduce inflammation and restore the intestinal epithelial barrier, might represent a good treatment strategy for GVHD in the future (67).

Compared to the lack of clinical data for probiotics to treat tumors, there are more clinical trial results demonstrating that probiotics have certain benefits in attenuating antitumor-related side effects (**Table 1**).

SAFETY ASSESSMENT OF PROBIOTICS

As additional supplementary active microorganisms, the adverse reactions of probiotics, primarily including systemic infections, gastrointestinal side effects, skin reaction, access to antibiotic resistance genes, harmful effects of probiotic metabolites and abnormal stimulation of the immune system, must be considered. The population at highest risk includes infants, the elderly, hospitalized patients, and patients with immunodeficiency due to genetic or acquired diseases (68). Studies have shown that the incidence of bacteremia in patients using yeast is approximately 1/5.6 million and for lactic acid bacteria is less than 1/1 million (69). The results of another large-scale epidemiological study indicated that infections caused by Lactobacillus and Bifidobacteria were extremely rare, accounting for 0.05%–0.4% of the total cases of infective endocarditis and bacteremia, and most patients had severe underlying diseases (70). In addition to being related to individual factors, the risk of infection was also related to the type and dose of the probiotics. It was reported that compared to Bifidobacterium, Lactobacillus was more likely to cause infection (71, 72).

One of the most important theoretical issues in the clinical use of probiotics is bacteremia, while fungal infections caused by yeast are even more difficult to treat. Compromised intestinal integrity and probiotic translocation are the main causes (73). Genomics data confirmed that these adverse reactions were indeed related to ingested probiotics rather than colonized probiotics in the intestine (74). It was found that for patients with impaired immune function, the risk of infection was far higher. Redman et al. conducted a systematic retrospective study and found that five of 1530 patients reported probiotic-related bacteremia, although probiotic management did indeed improve the severity and frequency of diarrhea in these cancer patients (75). Therefore, in cancer patients, the serious invasive disease caused by probiotics deserves vigilance (**Table 2**) (76–80).

In another systematic retrospective study, currently managed probiotic strains (primarily Bifidobacterium and Lactobacillus),

TABLE 1 | Clinical trials using probiotics to improve the side effects of anticancer therapy.

Malignancy	Case number	Treatment strategy	Objective	Intervention	Outcome	Side-effect	Reference
Cervical cancer	54	Radiotherapy	Improve diarrhea	From day 1 to the end of radiotherapy, receive 3 capsules per day, each containing 1.75 billion live bacteria (Lactobacillus acidophilus LA-5 and Bifidobacterium animalis subsp. BB-12)	The incidence of diarrhea in the probiotic group was lower than placebo group (53.8 and 82.1%, p <0.05), and the use rate of the anti-diarrhea drug loperamide was significantly reduced (p <0.01)	No probiotics-related toxicity reported	(46)
Colorectal cancer	150	Postoperative chemotherapy	Improve diarrhea	1-2×10^{10}Lactobacillus rhamnosus GG supplements daily	Patients receiving probiotics had mild diarrhea, and the incidence of grade 3 or 4 diarrhea (experimental group vs control group: 22% vs 37%, P = 0.027)	No probiotics-related toxicity reported	(45)
Lungcancer	41	Chemotherapy	Improve diarrhea	Starting one day before chemotherapy, take C. butyrate 3 times a day (420 mg/tablet) for 3 weeks	The incidence of grade I diarrhea was lower in the probiotic group (20% and 42.86%)	No probiotics-related toxicity reported	(59)
Gastric cancer	120	Surgery	Improve diarrhea	Nutrient formula food rich in fiber and probiotics, providing enteral nutrition for 7 consecutive days after surgery	Diarrhea cases decreased in combination of fiber and probiotics group	No probiotics-related toxicity reported	(49)
Head and neck cancer	200	Radiotherapy and chemotherapy	Improve oral mucositis	From the first day of treatment to 1 week after the last treatment, Lactobacillus brevis CD2 tablets (not less than 2 × 10^{9}), 6 times a day	The incidence of grade III and IV mucositis in the probiotics was lower than placebo group (52% and 77%, P <0.001), the completion rate of anticancer treatment in probiotic group was significantly improved (92% and 70%, P = 0.001)	No probiotics-related toxicity reported	(60)
Colorectal cancer	52	Surgery	Improve inflammation	Starting 4 weeks after surgery, oral administration of 30 billion probiotic mixed preparations twice a day for 6 months	Inflammatory cytokines in probiotics group were significantly reduced, including TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C, and IL-22 (P<0.05)	No probiotics-related toxicity reported	(61)

TABLE 2 | Five reported cases of probiotic-related bacteremia.

Age/Gender	Malignancy	Treatment strategy	Objective	Probiotics strains	Neutropenia	Side-effect	Outcome	Reference
8-month old baby	Acute myeloid leukemia	Intensive treatment with high-dose idarubicin, cytarabine, and etoposide (ICE)	Prevention of chemotherapy-related diarrhea	Saccharomyces boulardii	Yes	Saccharomyces cerevisiae strain isolated from blood culture	Anti-fungal treatment was performed for 14 days until full recovery from neutropenia. The patient eventually undergoes HLA-matched sibling donor bone marrow transplantation	(76)
65-year-old male	Oropharyngeal carcinoma, T3 N2 M0	Chemotherapy plus radiotherapy, including cisplatin and 5-fluorouracil plus external radiation (60 Gy)	Treat aseptic diarrhea	Saccharomyces boulardii	Not report	Saccharomyces cerevisiae strain found in blood culture	Amphotericin B, 60 mg/day for 4 weeks, fever decreased. Evaluation after 6 months showed partial remission of the tumor with no signs of residual infection	(77)
38-year-old male	Stage IV Hodgkin lymphoma with AIDS	Chemotherapy (specifically unknown)	Unknown	Lactobacillus acidophilus	Not report	Lactobacillus acidophilus found in blood culture	Clindamycin combined with gentamicin treatment, on day 3, blood culture was negative. On the 10th day, he was discharged from the hospital and received home care	(78)
69-year-old male	Stage IIIA mantle cell lymphoma	4 cycles of alternating Rituxan-Hyper CVAD Part A (rituximab, CL, VCR, doxorubicin and dexamethasone) and Part B (Ara-C and MTX) chemotherapy, after 4 months of chemotherapy, hematopoietic stem cell transplantation	Improve mucositis	6–8 cups of yogurt on the market rich in probiotics	Not report	Lactobacillus acidophilus found in blood culture	Antibiotic treatment (specifically unknown), symptoms relieved and discharged after 1 week	(79)
73-year-old male	Chronic lymphocytic leukemia	Unknown	Unknown	Bacillus subtilis	Not report	Bacillus subtilis found in blood culture	From day 1 to 16, imipenem treatment, day 16 later combined with antibiotic treatment (ceftazidime, amikacin and vancomycin) and intravenous immunoglobulin, fever quickly reduced. Death on day 25, may be due to central nervous system involvement	(80)

dosage (daily supplemental doses did not exceed 5.0×10^{10} CFU/day, median was 2.0×10^{9} CFU/day), and there were no serious adverse reactions caused by the probiotics. The results showed that it was safe to use probiotics in patients with impaired immune function, including very severe patients. However, most of the studies focused on the efficacy of probiotics rather than safety, and large-scale clinical studies are needed to further determine their true safety (81).

HSCT has become the standard treatment for many adult and childhood malignant tumor diseases, but the side effects caused by the treatment cannot be underestimated (82). Increasing evidence shows that the diversity of the microbiome is disturbed during treatment, often leading to abnormal systemic immune responses, pathogen colonization and mucosal invasion. There were also studies showing that the loss of microbial diversity was an independent risk factor for death after allogeneic HSCT (83).

Probiotics protect the microbiome and can minimize the risk of gut-mediated diseases. However, their safety has not been fully evaluated in the case of HSCT. Recently, Ladas et al. evaluated the safety and feasibility of probiotics in 30 children and adolescents who had undergone allogeneic HSCT (84). In the time range that coincided with intestinal mucosal damage and accompanying neutropenia, no cases of probiotic bacteremia (0% (0/30), 95% CI 0-12%) were observed, and there were no other unexpected adverse events. Although new infections of C. difficile were found in 20% of participants, studies confirmed that they were not related to probiotic management (84). Their research provides preliminary evidence that use of probiotics is safe and feasible in children and adolescents undergoing HSCT (84). Another study showed that for patients who received unrelated cord blood transplantation, early-stage yogurt supplementation was safe and feasible, and no unexpected adverse events caused by probiotics were observed (85). Therefore, in patients receiving HSCT, probiotics may have a positive role in maintaining the health of the intestinal flora and improving the patient prognosis.

However, in one clinical study, it was believed that probiotics did not benefit patients with acute myeloid leukemia undergoing intensive treatment or bone marrow transplantation (86). Instead, the probiotic treatment group exhibited a higher incidence of infection, especially blood infection (86). The researchers concluded that in patients with a long-term risk of neutropenia, without other indications for using probiotics, it was not recommended for such patients to use probiotics (86).

CONCLUSIONS

As a dietary supplement, probiotics lack strict standards for efficacy and safety certification. Although the efficacy of several strains has been experimentally supported, the health-promoting effects of most probiotics have not been proven. Relevant publicity of probiotic products rarely mentions the potential risks.

In a number of trials evaluating the protective effects of probiotic therapy on antitumor treatment-related side effects, combined use of probiotic strains did have a positive protective effect for patients with respect to certain immune functions (47). However, for patients with severely impaired immune function, especially patients with neutropenia, careful consideration is required (87). Due to the complex pathogenesis of tumors, different patients receive different treatment options, and different strains will affect the results, so large-scale clinical trials are urgently needed.

Identifying the most beneficial strains for the prevention and treatment of different types of cancer requires a very extensive human database, and it is necessary to carefully analyze correlations between different strains and clinical responses. Once we have identified a beneficial flora for cancer prevention and treatment, the next challenge is how to use probiotics and their products to regulate patient flora. At the same time, we can use the intestinal flora as a new cancer biomarker based on its response to changes in the pathophysiological environment. The ultimate goal is to identify specific strains or combinations of strains that can both reduce the side effects of cancer treatment and boost anticancer treatment (88). Therefore, for cancer and other diseases, the regulation of targeted human flora is likely to become a new field of precision and personalized medicine in the future.

AUTHOR CONTRIBUTIONS

KL and SD prepared the original draft. XW and RJ reviewed and edited the draft. HC supervised and finalized the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by National Natural Science Foundation of China (No. 31701207 to HC).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

REVIEW
published: 20 May 2021
doi: 10.3389/fonc.2021.672490

Curcumin in Osteosarcoma Therapy: Combining With Immunotherapy, Chemotherapeutics, Bone Tissue Engineering Materials and Potential Synergism With Photodynamic Therapy

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OPEN ACCESS

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: 25 February 2021
Accepted: 26 April 2021
Published: 20 May 2021

Citation:
Xu C, Wang M, Guo W, Sun W and Liu Y (2021) Curcumin in Osteosarcoma Therapy: Combining With Immunotherapy, Chemotherapeutics, Bone Tissue Engineering Materials and Potential Synergism With Photodynamic Therapy. Front. Oncol. 11:672490. doi: 10.3389/fonc.2021.672490

Osteosarcoma is a dominating malignant bone tumor with high mortality due to pulmonary metastases. Furthermore, because of the cancer cell erosion and surgery resection, osteosarcoma always causes bone defects, which means dysfunction and disfigurement are seldom inevitable. Although various advanced treatments (e.g. chemotherapy, immunotherapy, radiotherapy) are coming up, the 5-year survival rate for osteosarcoma with metastases is still dismal. In line with this, the more potent treatments for osteosarcoma are in high demand. Curcumin, a perennial herb, has been reportedly applied in the therapy of various types of tumors via different mechanisms. *In vitro*, it has also been reported that curcumin can inhibit the proliferation of osteosarcoma cell lines and can be used to repair bone defects. This seems curcumin is a promising candidate in osteosarcoma treatment. However, due to its congenital property like hydrophobicity, and low bioavailability, affecting its anticancer effect, clinical applications of curcumin are highly limited. To enhance its performance in cancer therapies, some synergist approaches with curcumin have emerged. The present review presents some prospective ones (i.e. combinations with immunotherapy, chemotherapeutics, bone tissue engineering, and biomaterials) applied in osteosarcoma treatment. Additionally, with the advancements of photodynamic therapy in cancer therapy, this review also prospects the combination of curcumin with photodynamic therapy in osteosarcoma treatment.

Keywords: osteosarcoma, curcumin, immunotherapy, chemotherapy, bone tissue engineering, biomaterials, photodynamic therapy

INTRODUCTION

Osteosarcoma (OS) originating from mesenchymal stem cells is the main primary malignant bone tumor (1), making up for *ca*. 35% of all bone carcinomas (2); it is usually diagnosed in children and adolescents (3). The principal cause of death in patients suffering from OS is pulmonary metastases (4). More than 90% of these patients died from this before the introduction of polychemotherapy (5). Another reason for the high mortality may refer to the rapid tumor development: frustratingly, once diagnosed, OS has most been in stage IIb or III (6). Furthermore, bone metastases are also common in OS, causing bone defects and followed by potential dysfunction and disfigurement (7, 8). However, to date, it is still hard to identify a targeted treatment for OS, as it is with a high frequency of gene and chromosome mutations (9). Currently, the prevailing remedies for OS are surgery, neoadjuvant and adjuvant chemotherapy. Conventionally, OS is indicated to be resistant to radiotherapy, nonetheless, it is implied that it is beneficial for those who have received chemotherapy but are unable to undergo complete resection (10). With these modern systemic therapies, the 5-year survival rate has improved, while this rate of those with metastases is still dismal—less than 30% (8). On the other hand, in the latest decades, therapeutic approaches for OS have not developed. Regarding this, more efficient therapies are still in urgent need.

Curcumin also named 1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, a natural polyphenol, is isolated from the rhizome of *Curcuma longa* (11). Although curcumin is isolated from herbs, its chemical structure has been identified (**Figure 1**). Structurally, there are 3 reactive sites in curcumin: metal chelator, Michael acceptor, and hydrogen atom donor, which bestows versatile abilities on curcumin to fight against diseases. It has been reported that curcumin possesses not only anti-inflammatory, anti-oxidative but also anti-tumor potential through targeting various molecules (12–16). In cancer treatments, curcumin suppresses tumor progression *via* various mechanisms (**Table 1**); commercial curcumin products have been used to evaluate the anti-cancer effect *in vitro* and *in vivo* (31). As a capable phytochemical, it has identified curcumin inhibits the proliferation of osteosarcoma cell lines and induces their apoptosis (24, 32, 33). Moreover, curcumin is with the potential to repair bone defects owing to tumor erosion or surgery (34–37). Taken together, curcumin seems to be an outstanding candidate that can be used in osteosarcoma treatment with the "one stone two birds" effect: inhibiting OS progression and repairing the bone defects simultaneously. Nevertheless, due to its poor aqueous solubility—about 11 ng/ml in water (38), rapid metabolism, and rapid system elimination, contributing to the low bioavailability (39), its clinical applications are not common currently. Based on some research, it has been demonstrated that the IC50 of curcumin for most cancer cells is 15–30 μM, whereas, the highest concentration of curcumin in the human body is just in the nanomolar range (40). Hence, to improve its anti-cancer efficacy, synergistic approaches have been carried out. We herein summarize combinations of free curcumin with other therapeutic strategies to enhance its anticancer effect on OS treatments.

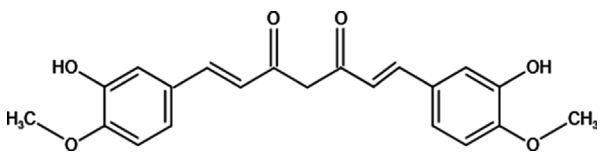

FIGURE 1 | Chemical structure of curcumin.

TABLE 1 | Targets of curcumin in anticancer treatments.

	Targets of Curcumin
Breast Cancer	NF-κB (17), Nrf2 (18), MMPs, VEGF (19), Akt (20)
Lung Cancer	PI3K/Akt/mTOR (21), EGFR and TLR4/MyD88 (22)
Osteosarcoma	p-JAK2/p-STAT3 (23), Notch-1 (24), miR-138 (25)
Head & Neck Cancer	IL-6/p-STAT3 (26), NF-κB, cyclin D1, and Bcl-2 (27)
Gastric Cancer	PI3K and P53 (28), ROS (29), Wnt/β-catenin (30)

INHIBITION EFFECT OF CURCUMIN ON TUMORS

Apoptosis or programmed cell death (PCD) plays a potent role in tumorigenesis. In physiological conditions, it can eliminate the precancerous cells, thereby preventing normal cells from being malignant; in turn, anticancer agents will induce cancer cells apoptosis to cure cancers. Generally, there are two canonical apoptotic pathways: extrinsic and intrinsic pathways (**Figure 2**). For the former, apoptosis initiates after the bond between some extracellular cytokines or growth factors and their receptors, death receptors, on the cytomembrane, which will activate caspase 8 followed by the activation of caspase 3 finally. The well-known death receptor couples are TNF-TNFR1 and FasL-Fas (41). For the intrinsic pathway, apoptosis is mainly induced by the mitochondria dysfunction attributed to some stress conditions. With increased mitochondria membrane potential, some molecules (mainly cytochrome c) released from mitochondria will initiate the process of apoptosis.

Curcumin can inhibit cancer development via various mechanisms: inducing apoptosis and some miRNA expression, dampening angiogenesis, metastasis, etc. Curcumin is identified to induce neoplasm apoptosis through extrinsic and intrinsic pathways *via* various targets such as Bax, Bcl-2, Fas, p53 (42–44). It is also found to suppress non-small cell lung cancer by upregulating miR192-5p (45) and in leukemic cells, curcumin can upregulate miR-15a and miR-16-1, which will decrease WT-1expression, thereby suppressing the proliferation of leukemic cells (46).

For osteosarcoma, several researchers have successfully proven that curcumin can induce the MG63, U2OS, and HOS cell line apoptosis based on different signal pathways (32, 33, 47–50). Besides, curcumin has also been identified to suppress the proliferation, invasion, and metastasis of osteosarcoma (23, 24, 51, 52). Thence, curcumin is a promising agent with multifaced roles it plays in the treatment of osteosarcoma. However, due to

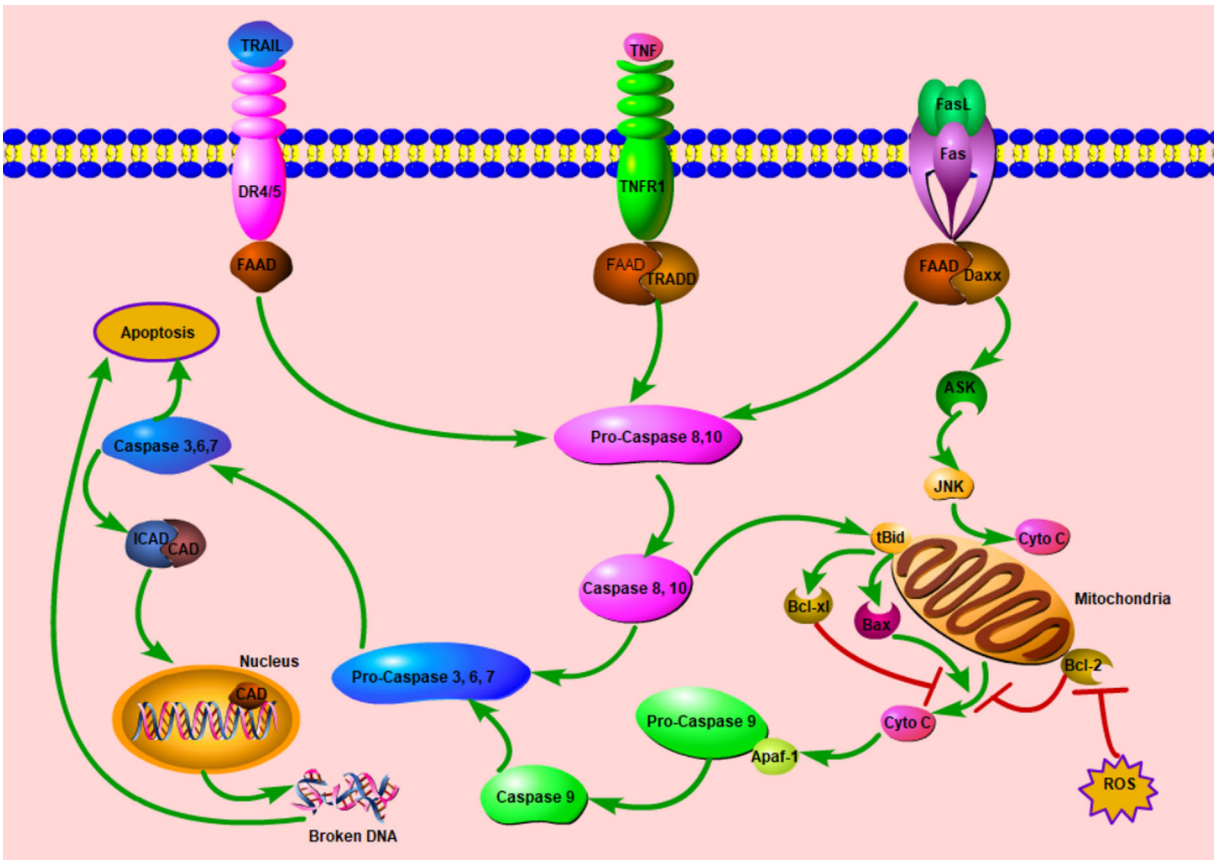

FIGURE 2 | Schematic diagram of cell apoptosis. Once the extracellular cytokines bond to relative death receptors (DRs), DRs recruit FADD intracellularly, this complex initiates the cascade of apoptosis. In some stress conditions, mitochondrial permeability transition will increase, afterwards, Cyto C will be released. With Apaf-1, Cyto C will start the activation cascade from Pro-Caspase 9. In these two ways, caspase 3 is activated finally and exerts the apoptosis process with various mechanisms including the DNA fragmentation in the nucleus. The extrinsic pathway can modulate the intrinsic pathway by the tBid, ASK, and JNK. ASK, apoptosis signal-regulating kinase; Apaf-1, apoptotic protease activating factor-1; CAD, caspase-activated deoxyribonuclease; Cyto C; cytochrome C; Daxx, death domain associated protein; DR, death receptor; FAAD, Fas-associated death domain protein; ICAD, inhibitor of caspase-activated deoxyribonuclease; JNK, c-Jun N-terminal kinase; tBid, truncated Bid; ROS, reactive oxygen species; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TRADD, Tumor necrosis factor receptor type 1-associated death domain protein. TRAIL, tumor necrosis factor related apoptosis inducing ligand; ┤, Inhibition; →, Promotion.

poor bioavailability, the administration of curcumin in cancer treatment is not common. To overcome this issue and improve its efficiency in tumor therapy, synergistic approaches are carried out.

SYNERGISTIC APPROACHES

Combination With Immunotherapy

The immune system is vital for the human to defect various pathogens causing infections or tumors with the cooperation of immune cells and some cytokines. As tumor immunotherapy has achieved great success in clinical, the importance of cancer immunotherapy has been gradually acknowledged in these decades. In 2018, the Nobel prize for physiology or medicine was awarded to the Nobel Laureates who found two immune checkpoints: cytotoxic T-lymphocyte associated protein (CTLA-4) and programmed cell death protein 1 (PD-1) and its ligand (PD-L1) (53) that are responsible for the tumor immune evasion. Currently, some tumor immunotherapy agents applied in the treatment of melanoma, lung cancer, head and neck squamous cell cancer have been approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) (54). However, due to the complicacy of the immune response in the tumor microenvironment (**Figure 3**), further and more studies still should be carried out.

Immunotherapy is a practical strategy to treat osteosarcoma. Immunotherapy enables an increase in the survival rate of patients suffering from osteosarcoma. Back in 1891, Coley's research manifested that around 10% of patients with bone and soft tissue sarcomas got benefit from the stimulated immune system by the injection of two kinds of heat-inactivated bacteria (55); in a randomized phase III study, Mifamurtide with chemotherapy performed better than monotherapy. In this study, Mifamurtide was used to activate some innate immune cells (e.g. monocytes and macrophages) to

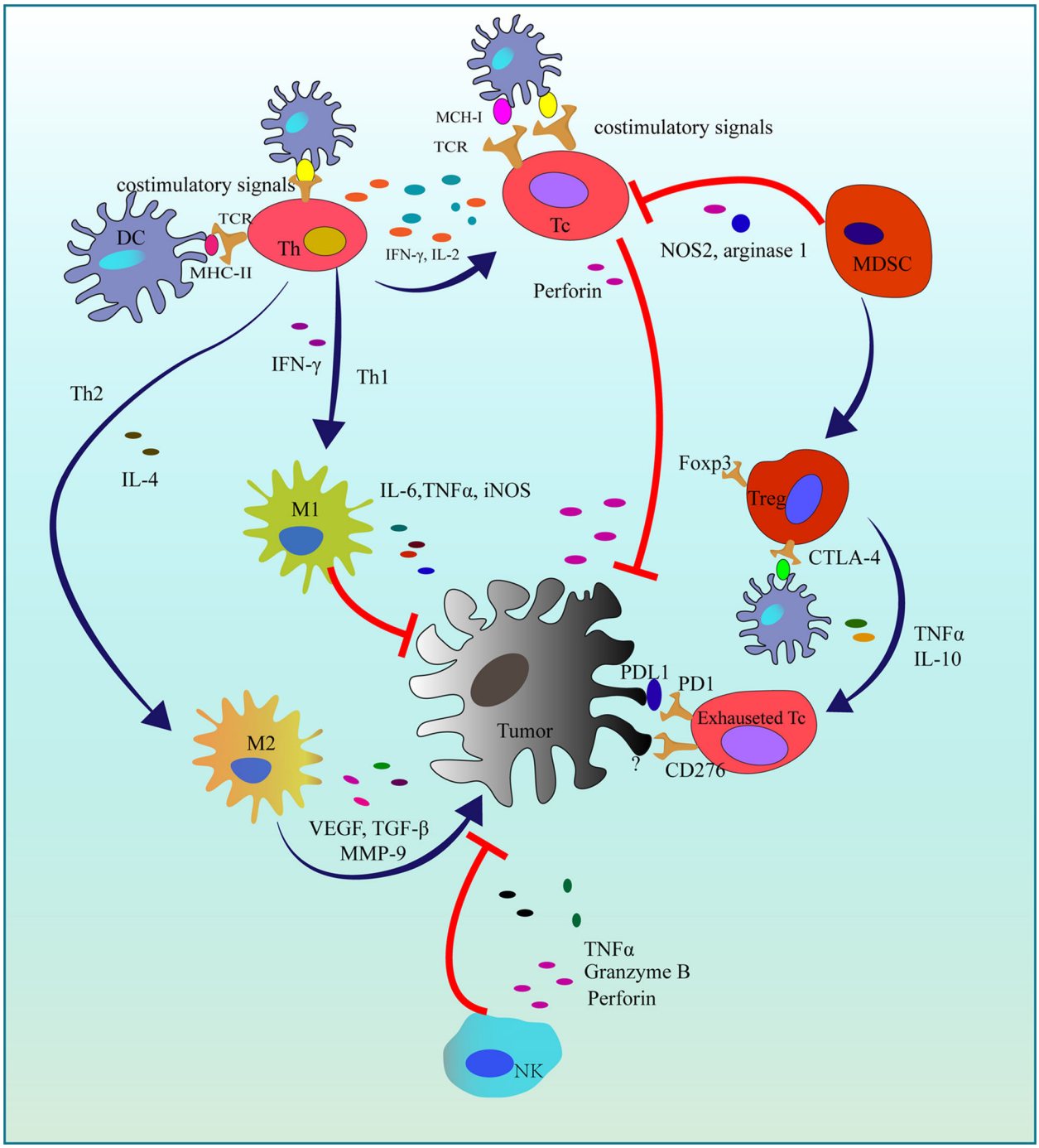

FIGURE 3 | Immune responses in the tumor microenvironment. In the initiation of tumorigenesis, activated Tc and NK cells suppress tumor proliferation. While in the immune evasion, Th2 recruits MDSC and promotes macrophages polarization (M1 to M2). Finally, survived tumor cells exhaust Tc cells by some immune checkpoints. DC, Dendritic cell; MCH-1, Major histocompatibility complex class I; MCH-II, Major histocompatibility complex class II; TCR, T cell receptor; Th, T helper cell; Th1, T helper 1 cell; Th2, T helper 2 cell; Tc, Cytotoxic T cell; IFN-γ, Interferon-gamma; Treg, Regulatory T cell; Foxp3, Forkhead box Protein 3; MDSC, Myeloid-derived suppressor cell; NOS2, Nitric Oxide Synthase 2; TNFα, Tumor necrosis factor-alpha; IL-2, Interleukin 2; IL-6, Interleukin 6; iNOS, Inducible nitric oxide synthase; M1, Macrophage 1; M2, Macrophage 2; PD-1, Programmed cell death protein 1; PD-L1, Programmed cell death protein ligand 1; CTLA-4, Cytotoxic T-lymphocyte antigen 4; NK, Natureal kill cell; TGF-β, Transforming growth factor-beta; MMP-9, Matrix metallo proteinase-9; VEGF, Vascular endothelial growth factor; ┤,Inhibition; →, Promotion.

control the tumor development as it is an analog of bacteria cell walls (56). Furthermore, adoptive T cell therapy in osteosarcoma also worked well (57, 58). Tumor-infiltrating lymphocytes (TIL) are detected in osteosarcoma by an immunohistochemical study, and among those TILs, CD8+ T-lymphocytes dwarf others (59, 60). Similarly, Tsukahara and his colleagues also found CD8+ T-lymphocytes play a pivotal role in the suppression response to osteosarcoma (61).

Curcumin can improve tumor immunotherapy targeting PD-1/PD-L1 and CTLA-4. Traditionally, researchers focus on the anti-cancer effect of curcumin on various signal pathways in cancer cells, however, an increasing body of literature has indicated that curcumin can elevate no matter the innate or adaptive immune response to cancer (62) by the modulation of T cells, macrophages, dendritic cells (DC), natural killer cells (NK), cytokines, etc. (63, 64). Curcumin can promote T cells quantitively and functionally (62, 65–67). The potential mechanism may include the downregulation of Treg and the expression of some immune checkpoints (e.g. PD-1/PD-L1, CTLA-4). It is well-known that Foxp-3$^+$ Treg can suppress cytotoxic T lymphocytes (CTLs) (68), while curcumin has been found to inhibit the activity of Treg *via* the decrease of IL-2 (69). On the other hand, the overexpression of PD-1/PD-L1 and CTLA-4 is responsible for the exhaustion of CTLs, which leads to tumor immune evasion finally. In some previous research, CTLA-4 and PD-1/PD-L1 are identified to overexpress in osteosarcoma and negatively correlate to the prognosis (70–75). Blockade of PD-1 or CTLA-4 can contribute to the inhibition of osteosarcoma, but, in a phase II trial, only 5% of patients with osteosarcoma were relieved by PD-1 inhibitor-pembrolizumab (76). Reassuringly, Taeko et al. found curcumin can enhance the PD-1 blockade therapy (77). Similarly, Paul also found curcumin can improve anti-PD1 efficacy *in vivo* (78). This means combining curcumin with immune checkpoints blockade is a potential promising clinical approach in the treatment of osteosarcoma.

Except for T lymphocytes, some innate immune cells are also of great importance for immunotherapy. Dendritic cell (DC) is a professional antigen presentence cell (APC). With this property, it can activate lymphocytes, not only T cells but also NK, thereby fighting against tumor cells (79). The application of DCs to inhibit some pediatric solid tumors including osteosarcoma has been reported in a clinical study (80). Kawano et al. found DCs pulsed with tumor lysate cannot enhance IFN-γ level in serum and the accumulation of CTLs in metastatic areas (81). What's more, they also found combining with CTLA-4 blockade in a mouse osteosarcoma model, the anticancer effect had been enhanced: more CTLs, less Treg, prolonged survival, etc. (82). Another immune checkpoint PD-L1 also expresses on DCs and can attenuate T cell activation (83). It has been identified that PD-1 inhibitor combing with DCs vaccines has improved anticancer effect (84, 85). As mentioned above, curcumin may affect the expression of PD-1/PD-L1, according to this, curcumin combing with DCs may also be a promising therapeutic strategy. Interestingly, PD-1 inhibitors also can induce macrophage polarization from M2 (pro-tumor) to M1 (anti-tumor) in an osteosarcoma model (73). In line with this, curcumin may also be able to inhibit osteosarcoma *via* the polarization of macrophages from M2 to M1. Nevertheless, these trials have not been conducted widely, currently.

Taken together, curcumin may modulate the immune response to osteosarcoma by affecting various immune cells, cytokines, and molecular markers, which confers it to be a promising agent for immunotherapy in osteosarcoma.

Combination With Chemotherapy

Chemotherapy plays a great role in the treatments of tumors, particularly for extensive metastatic advanced ones that cannot be removed by surgical resection. To date, various chemotherapy regimens have been administrated clinically (e.g.. cisplatin, doxorubicin, 5-fluorouracil, methotrexate), and among them, cisplatin is the most used (86). These drugs perform anti-cancer activities through various mechanisms: damaging DNA, activating TP53, increasing the intracellular reactive oxygen species (ROS) level, etc. However, these chemotherapeutic agents are like a "double-edged" sword; they damage both cancer cells and normal somatic cells in the same way, terming as on-target toxicity (87). According to a great amount of previous research, cisplatin and doxorubicin have been confirmed to be toxic to many organs, especially the kidney and heart (88, 89), respectively. Another challenge for the current chemotherapy is multidrug resistance (MDR) impedes the efficacy of chemotherapeutic drugs regarding the activation of NF-κB, overproduced P-glycoprotein (P-gp), etc. (90–92). To overcome this issue, the strategy of escalating dose and group combination has been presented. Nevertheless, this means more toxicity to patients.

ROS plays a crucial role in on-target toxicity and MDR. For on-target toxicity, most chemotherapeutic agents will upregulate the intracellular ROS. Afterward, the accumulated ROS will damage DNA and proteins, and cell membranes, thereby inducing cell apoptosis. Normal cells will also be killed due to oxidative stress in this process. Cisplatin-induced kidney injury and doxorubicin-induced cardiotoxicity are reported to be relative to ROS (93, 94). On the other hand, upgraded ROS can active NF-κB following activation of some chemoresistance genes such as hypoxia-inducible factor 1 alpha and P-gp (95).

Regarding the role of ROS in chemotherapy, combining with antioxidants seems an appealing approach to protect normal cells and circumvent the chemoresistance simultaneously (96–98). Curcumin reverses chemotherapy resistance, which has also been reported. Ehherth et al. found curcumin sensitized CE/ADR5000 cell line from 883-fold doxorubicin-resistance to 0.9-fold (99). As mentioned above, curcumin is a safe natural antioxidant (maximum 12 g/day over 3 months) (100), with the application of it in chemotherapy, there may be an improved synergistic effect and can protect the normal tissues; it is implied that curcumin protects against doxorubicin toxicity (101); the protective effects can also be found in combination with cisplatin (102). Except for ROS, Ma reported that curcumin can increase the absorption of doxorubicin *in vivo* by inhibition of drug efflux, thereby enhancing the chemotherapy efficacy (103). This means

curcumin may play a versatile role in the combination with chemotherapeutic agents.

With the introduction of chemotherapy in osteosarcoma treatment, long-term survival rates have increased from less than 20 to 65–70%, and the first-line drugs are MAP: methotrexate, doxorubicin, and cisplatin (104). However, the survival of patients bearing osteosarcoma has not been improved since the last decades, although chemotherapy strategy for osteosarcoma has developed: neoadjuvant and adjuvant chemotherapy. To enhance the chemotherapy efficacy, numerous studies with the addition of some drugs to MAP have been conducted, however, data from these studies did not show any improvement. A trial started in 2005 conducted by the European and American Osteosarcoma Study Group showed the addition of interferon-alpha in neoadjuvant chemotherapy, and ifosfamide and etoposide in adjuvant chemotherapy did not show a statistical difference (105); and the French multicenter OS2006 added zoledronic to chemotherapy, there was no significant enhancement either (106). Other agents (topotecan, imatinib, oxaliplatin, ixabepilone, etc.) tested in selected phase II trials in osteosarcoma did not show any positive results (104). Although curcumin seems a drug with great synergistic effort in chemotherapy, there is little research about this strategy in the chemotherapy of osteosarcoma until now. Further investigations about this strategy are required in the future.

Combination With Bone Tissue Engineering Materials

To remove the primary tumor thoroughly, an extended resection area is the main approach currently. In osteosarcoma treatment, this may cause critical size bone defects, while insufficient resections are always responsible for the tumor recurrence. This seems to be in a dilemma. To repair the critical size bone defects (more than 2 cm, typically), autografts and allografts are prevailing strategies, and autografts are considered to be the "gold standard" (107). Nevertheless, the application of autografts and allografts will cause some side effects. Autografts may cause the morbidity of donor sites (pain, hematomas, nerve injuries, etc.); allografts may result in disease transmission. To overcome these issues, various biomaterials have been developed and applied clinically, among which the prevailing materials are polymers (natural or synthetic), bioceramic, and composite materials (108). These materials achieve great success in osteogeneration. However, most of these materials lack the anti-cancer property, which means they are ineffective for potential tumor recurrence. The combination of curcumin with these materials is a promising strategy to resolve this problem. As mentioned above, curcumin cannot suppress osteosarcoma development but induce osteogenesis. The addition of curcumin can promote bone repairment and protect against the potential remaining carcinoma. Naboneeta documented that curcumin loaded with hydroxyapatite-coated Titanium implant enhanced the cytotoxicity to MG-63 *in vitro* (109). In another study, he and his colleagues pointed out that curcumin loaded on 3D printed calcium phosphate scaffold presented selective toxicity to MG-63 cells and promoted normal osteoblast proliferation (110). Another benefit of this combination strategy is increasing the accumulation of curcumin in lesions. Due to extensive first-pass metabolism and poor curcumin bioavailability (111), traditional delivery methods are powerless to overcome these issues. Loading in/on these materials, curcumin can accumulate in the target area directly, therefore, its pharmacological efficacy boost.

To refine the stability and bioavailability of curcumin, some nanoparticles are used. In a review, encapsulating curcumin in liposomal nanoparticles, the most used way, improved its anticancer efficacy (112). Currently, some more sophisticated combination strategies have been proposed. The chemotherapeutic drug, photosensitizer, and immune checkpoint blockade were loaded in the same nanoscale polymers, by which the anticancer effect increased significantly (113). Based on this, curcumin, a versatile agent with all these properties, is a prospective candidate in a nano delivery system.

PROSPECT OF APPLICATION OF CURCUMIN IN PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is an emerging treatment modality. To date, it has been applied in many fields including dermatology, oncology, gynecology, and urology (114). It is thought to be a non-invasive remedy, as it kills pathogens or tumor cells depending on the phototoxicity resulting from the intracellular accumulation of ROS attributing to the "photodynamic effect" referred to in 1904 by Von Tappeiner (115). The production of exceeded ROS is based on the mutual interaction among the photosensitizers (PS), light with appropriated wavelength, and intracellular oxygen molecules. There are two types of reactions in PDT with the same initiation- exciting PS using appropriated light. Afterward, the excited PS may transport electrons to cellular substrates (Type I reaction) or molecular oxygen directly (Type II reaction) (116). In the former, free radicals and anion radicals (hydroxyl radical, and superoxide ion) were generated, and singlet oxygen was found in the latter, which is considered to be the most dangerous one among ROS as it can react with unsaturated lipids, proteins (117) with its potent oxidative property, thereby damaging the cell and nuclear membranes (118).

PDT was firstly approved in Canada in 1993 for the therapy of bladder cancer (119), and more than 200 clinical trials have been carried out. Photofrin, a first-generation and most used PS has been approved to treat cancers by FDA (120) and it is still used now. The anticancer effect of PDT is based on these mechanisms: direct killing cancers by ROS, inhibiting the angiogenesis (121), and activating the immune system (122) (**Figure 4**). ROS can cause the death of cancer cells and vascular endothelial cells. In this condition, oxygen and nutrition supplements for tumors will be dampened, causing cancer cell death. Afterward, some pro-inflammatory cytokines will be released to recruit and activate immune cells (123). The broken vascular walls also facilitate the recruitment of neutrophils and macrophages in the tumor microenvironment (124). Additionally, Castano et al. also found PDT can suppress the Treg (125) which always silences cytotoxicity T lymphocytes. Based on these, a combination of PDT with immune checkpoint inhibitors may enhance the anti-cancer effect. In a case report, a patient with advanced head and

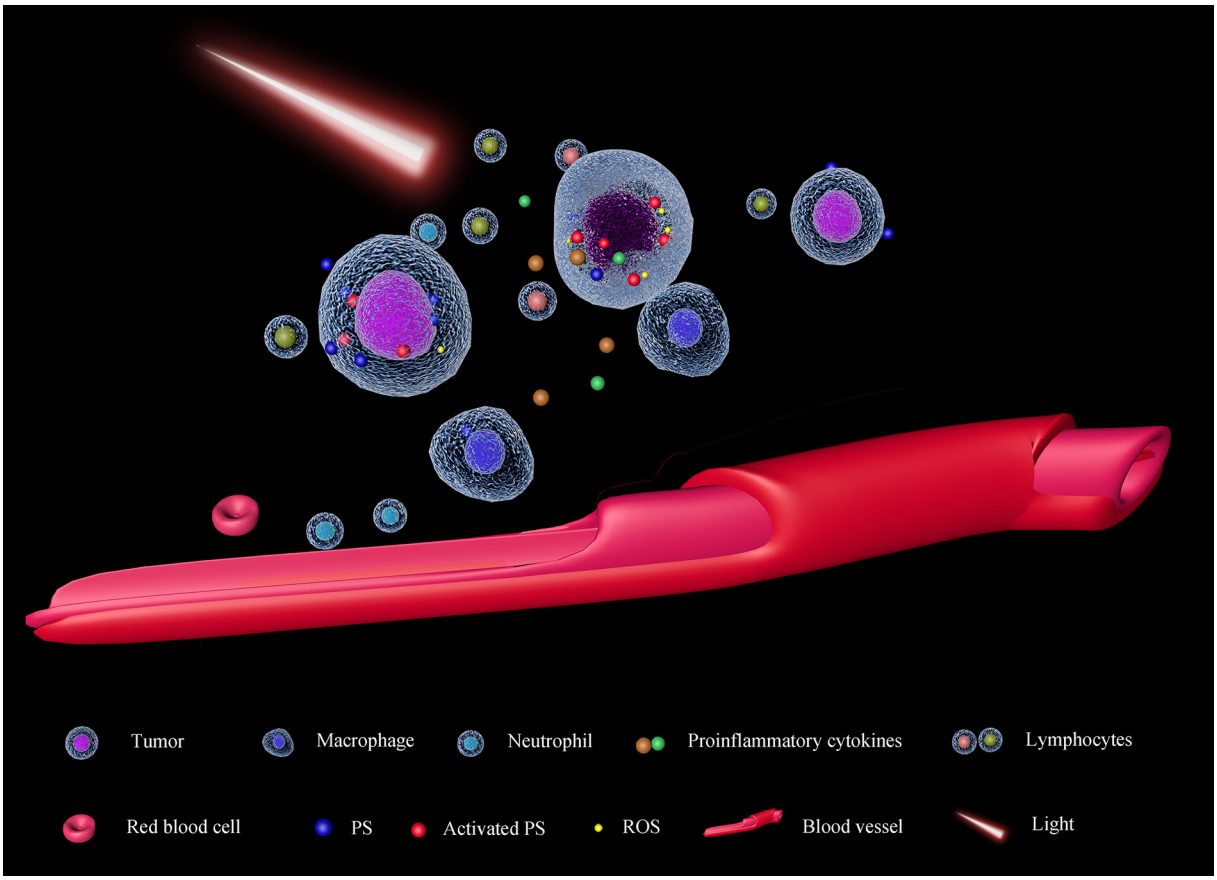

FIGURE 4 | Schematic diagram of PDT. The photosensitizer (PS) will be activated by light with an appropriate wavelength, causing the accumulation of ROS in the cells. Exceeded ROS damages tumor cells directly and epithelial cells of tumor-associated vessels. As the impairment of the tumor cell membrane, some pro-inflammatory cytokines will be released, facilitating the recruitment of immune cells in the tumor microenvironment (TME). On the other hand, as the tumor-associated vessels are also damaged, nutrients and oxygen supplements for the tumor will decrease; moreover, the neutrophils and macrophages can transport into the TME, suppressing tumor proliferation.

neck squamous cell cancer received radiotherapy, surgery, and chemotherapy, which did not control the development of cancer. Afterward, with PDT, the visible tumor vanished, and combining PD-1 blocker, the patient was with no signs of the disease two years later (126).

Although curcumin, to date, has not been applied in the treatment of osteosarcoma clinically, curcumin has been found that it can work as a PS in PDT with enhanced anti-cancer or anti-bacteria effect (127, 128). Curcumin although is a phytochemical agent, its chemical structure is declared clearly. It is available to obtain highly pure commercial production and meet the potential tremendous clinical need. Besides, regarding the non-toxicity of curcumin to normal tissues (100), curcumin used in PDT can reduce the potential damage to normal cells. Moreover, to reduce the damage to normal tissues, the selectivity of PS is also crucial. Ideally, the more PS distributed in tumors, the better efficacy, and fewer side effects can be induced. It has been proved that tumor takes up more curcumin than normal cells (129). All these suggest curcumin is a promising PS, while it also has a great challenge in clinical application. To excited PS, light with an appropriated wavelength is vital. The optimal wavelengths are between 600 and 850 nm, termed as "therapeutic windows", as the lower ones cannot penetrate deep tissues and higher ones without sufficient energy cannot excite PS to generate singlet oxygen (130). Unluckily, the Ex of curcumin is just around 425 nm (131), which is cannot penetrate skins to excite curcumin in osteosarcoma PDT. To overcome this problem, using a fiber optic device may be a practical approach. Another drawback of curcumin-hydrophobicity also dampens its efficacy in PDT. It is documented that PS can perform photoactive only in the monomeric form (132). Curcumin will aggregate in an aqueous environment, reducing its excitation. These disadvantages may be contributed to the limitation of its clinical trials. More advantages and modifications of curcumin are in high demand to adjust to the PDT.

CONCLUSION

Curcumin, a multifunctional phytochemical, has been identified to be a promising anticancer drug based on abundant *in vitro* and

in vivo studies. Nonetheless, due to its hydrophobicity, poor bioavailability, there are few clinical trials demonstrating comforting results, neither successful clinical applications. For osteosarcoma treatment, most of the current research about the effect of curcumin is carried out *in vitro*, which may weaken the comforting results from these studies. Established OS animal models using different OS cell lines have been reported, while few of them have been applied to test curcumin resulting from its inherent disadvantages that may affect the feasibility and impede the accurate assassination. To circumvent this limitation and provide more reliable conclusions from no matter cellular and animal research or pre/clinical trials, more measures have to be implemented. On one hand, chemical modification of curcumin or analogs has been carried out to enhance its solubility in water and bioavailability in physiological conditions. On the other hand, the combination of curcumin with other therapeutic strategies is also promising. Thanks to its versatile properties, curcumin can improve chemotherapy and immunotherapy efficiency. Moreover, curcumin can also work as a photosensitizer in PDT. Interestingly, these three approaches can work synergistically. In line with this, curcumin may combine a wide range of agents as a sophisticated systemic strategy to suppress oncogenesis. In osteosarcoma remedy, curcumin loaded in bone-engineering materials can inhibit osteosarcoma cells and promote osteogenesis simultaneously. This property makes curcumin stand out from a great variety of anticancer drugs. In this approach, bone-engineering materials not induce osteogenesis but work as a controlled delivery system of curcumin that enhances the local concentration of curcumin and prolongs its duration of action. Taken together, although curcumin has a great anticancer property, to widen its clinical application, more modifications and further studies are still required.

AUTHOR CONTRIBUTIONS

CX contributed to the conception of this work and drafted the manuscript. MW collected literature. WG and WS made important revisions and polished the language. YL edited and revised this manuscript and approved the publication. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 26 November 2021
doi: 10.3389/fphar.2021.781033

Alantolactone: A Natural Plant Extract as a Potential Therapeutic Agent for Cancer

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Alantolactone (ALT) is a natural compound extracted from Chinese traditional medicine *Inula helenium L.* with therapeutic potential in the treatment of various diseases. Recently, *in vitro* and *in vivo* studies have indicated cytotoxic effects of ALT on various cancers, including liver cancer, colorectal cancer, breast cancer, etc. The inhibitory effects of ALT depend on several cancer-associated signaling pathways and abnormal regulatory factors in cancer cells. Moreover, emerging studies have reported several promising strategies to enhance the oral bioavailability of ALT, such as combining ALT with other herbs and using ALT-entrapped nanostructured carriers. In this review, studies on the anti-tumor roles of ALT are mainly summarized, and the underlying molecular mechanisms of ALT exerting anticancer effects on cells investigated in animal-based studies are also discussed.

Keywords: Alantolactone, anticancer effects, cancer, signaling pathways, regulatory factors

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *22 September 2021*
Accepted: *03 November 2021*
Published: *26 November 2021*

Citation:
Cai Y, Gao K, Peng B, Xu Z, Peng J, Li J, Chen X, Zeng S, Hu K and Yan Y (2021) Alantolactone: A Natural Plant Extract as a Potential Therapeutic Agent for Cancer. Front. Pharmacol. 12:781033. doi: 10.3389/fphar.2021.781033

1 INTRODUCTION

Cancer is characterized by a very high incidence rate and fatality rate, and seriously affects human health (Fidler et al., 2017). Cancer maintains the malignancy by affecting the development of the embryo and destroying the repair mechanisms (Guan et al., 2020). It has been found that genomics-based assays can be used in clinical therapy, such as targeted treatment and antitumor vaccines (Berger and Mardis, 2018). Currently, surgical resection, radiotherapy, and chemotherapy are the main effective modalities for curing cancers. Chemotherapy uses anti-cancer compounds and medicine to attenuate cancer development (Seo et al., 2009). However, treatment failure and side effects are common in chemotherapy. Therefore, new drugs with better therapeutic effects and fewer adverse effects are needed for cancer treatment.

Nowadays, alantolactone (ALT), a natural herb compound derived from the traditional Chinese medicinal *Inula helenium L.*, has attracted extensive research attention because of the therapeutic potential in cancer treatment (Mi et al., 2014). It has been revealed that ALT can

Abbreviations: ALT, alantolactone; AKT, alpha serine/threonine-protein kinase; AP2M1, adaptor-related protein complex 2 subunit mu 1; EGFR, epidermal growth factor receptor; GSK, glycogen synthase kinase; HBV, hepatitis B virus; HCV, hepatitis C virus; HUVEC, human umbilical vein endothelial cells; ICD, immunogenic cell death; MAPK, Mitogen-activated protein kinases; Nrf2, nuclear factor E2-related factor 2; PINK1, putative kinase 1; PLCγ1, phospholipase C gamma 1; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TCM, traditional Chinese medicines; TrxR1, thioredoxin reductase 1; VEGFR2, vascular endothelial growth factor receptor 2.

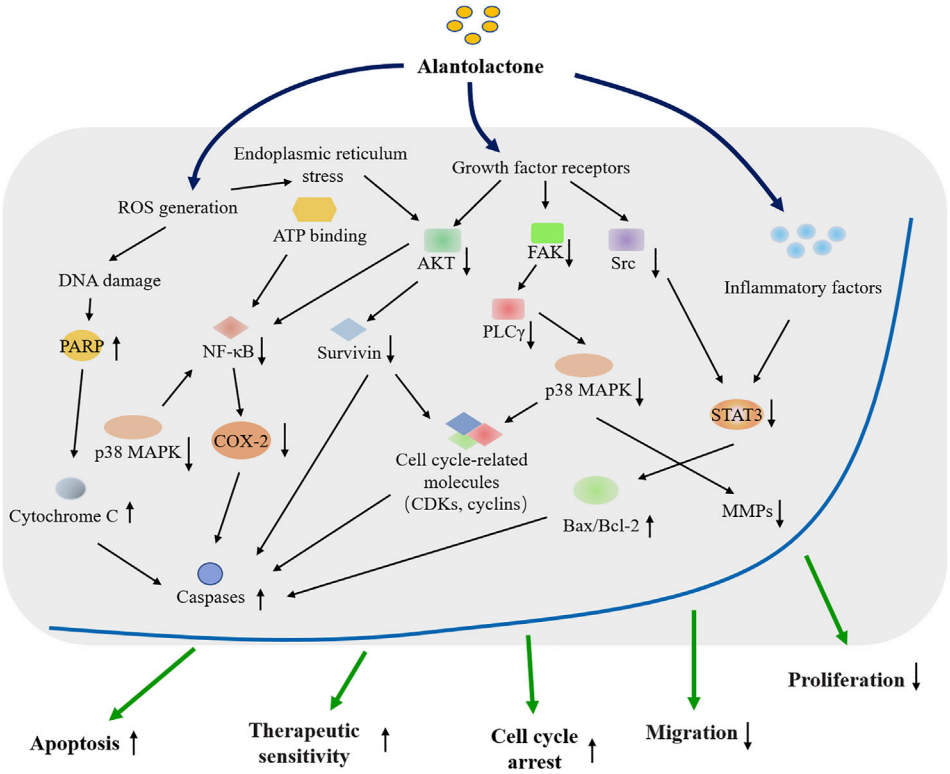

FIGURE 1 | Overview of the cytotoxic effects of the natural compound Alantolactone on cancer research and therapy.

exhibit anti-inflammatory and anti-tumor activities through modulating the abnormal signaling pathways in cancer cells (Gierlikowska et al., 2020; Babaei et al., 2021). For example, mitogen-activated protein kinases (p38 MAPK) and NF-κB signaling pathways are significantly attenuated by ALT, inhibiting cell viability and promoting cell apoptosis in lung cancer cell lines NCI-H1299 and Anip973 (Liu et al., 2019). And a recent study firstly reported that ALT could suppress the activation of YAP1/TAZ, leading to the inhibition of cancer cell growth (Nakatani et al., 2021). ALT could downregulate the serine/threonine kinase Aurora-A through directly binding to the interface pocket of Aurora-A-TPX2 complex, weakening several cancer-associated biological behaviors, including centrosome amplification, chromosomal instability and oncogenic transformations (Bhardwaj and Purohit, 2020; Nadda et al., 2020). Furthermore, with no obvious side effects, ALT could synergistically enhance the cytotoxic effects with other anti-cancer agents, such as oxaliplatin (Cao et al., 2019) and olaparib (Wang et al., 2020) *in vivo* and *in vitro*.

In this paper, the findings regarding the antagonistic effects of ALT in various cancers are summarized, and the underlying mechanism of ALT anticancer activity is explored (**Figure 1**, **Tables 1**, **2**). Besides, to explore the practical values of ALT in future clinical applications, the safety and efficacy of ALT are also discussed.

2 THE ACTION OF ALT AGAINST HUMAN CANCERS

2.1 Lung Cancer

Lung cancer is one of the most frequent human malignancies worldwide, causing about 1.6 million deaths annually. Risk factors of lung cancer include second-hand smoking, air pollution, genetic reason, etc. (Wu et al., 2020; Yang et al., 2020). In addition, non-small cell lung cancer, accounting for ~85% of lung cancer cases, is increasing in both incidence and mortality. Non-small cell lung cancer is divided into two histological subtypes, namely lung adenocarcinoma and lung squamous cell carcinoma (Chen et al., 2020; Tubio-Perez et al., 2020). Nowadays, the potential therapeutic effects of traditional medicine, like ALT on patients with both subtypes of non-small cell lung cancer have been studied. It has been found that ALT effectively induces cell apoptosis in both lung squamous carcinoma cells (SK-MES-1) and lung adenocarcinoma cells (NCI-H1299 and Anip973) and the cytotoxic influence of ALT is closely related to the improved treatment efficacy and prognosis of patients with lung cancer (Zhao et al., 2015; Liu et al., 2019). It has also been found that ALT could significantly enhance the anticancer effects of chemotherapy drug gemcitabine on lung adenocarcinoma cells A549 and lung squamous carcinoma cells NCI-H520 cells through inhibiting the activation of AKT/glycogen synthase kinase (GSK) 3β and

TABLE 1 | The anticancer activities and the underlying mechanisms of alantolactone *in vitro*.

Cancers	Cell lines	Modulated factors	Biological effects	References
Liver cancer	HepG2 cells	Bcl-2, caspase-3, STAT3	Inducing apoptosis, inhibiting cell proliferation, inducing G2/M phase arrest	Khan et al. (2013)
		Bcl-2, NF-κB, p53, Bax, caspase-3/8/9, t-Bid		Lei et al. (2012)
		p21, cyclin A1 cyclin B1, caspase-3, PARP		Kang et al. (2019)
Colorectal cancer	SW480 and SW1116 cells, non-cancer BEAS-2B and L-O2 cells	Bcl-2, Bax, caspase-3, p21	Inducing G1 cell cycle arrest, inducing apoptosis, inhibiting cell proliferation	Ding et al. (2016)
	Murine CT26-FL3 cells, Murine breast cancer 4T1 cells	Bcl-2, Bcl-xL		Zhang et al. (2019a)
	HCT116 and RKO cells	JNK, p38, MAPK, Ki-67		Cao et al. (2019)
	HCT-8, L02, HEK 293 T cells	Cripto-1, ActRIIA, activin, SMAD3, p21		Shi et al. (2011)
	RKO cells	MMP, Bcl-2, Bax, caspase-3/9, cytochrome c		Zhang et al. (2013)
Breast cancer	McF-7 cells	Bcl-2, Bcl-2-associated X protein, p53, p65, caspase-3, caspase-12, MMP-2, MMP-7, MMP-9, p38, MAPK, NF-κB, Nrf2	Inhibiting cell proliferation, inducing apoptosis, inhibiting motility, migration and tube formation, causing cell cycle arrest	Liu et al. (2018a)
	HUVECs, MDA-MB-231 cells	VEGFR2phosphorylation, PLCγ1, FAK, Src, Akt		Liu et al. (2018b)
	Triple-negative breast cancer (TNBC) cells	Bcl-2, Bax, caspase-3, CyclinB1, Cdc2, ATF4, CHOP, ki-67		Yin et al. (2019)
	MDA-MB-231, MCF-7 cells	Bax/Bcl-2, MMP, cytochrome c, caspase 9/3, PARP, MAPKs, p-NF-κB, p65, p-STAT3, NF-κB, AP-1, STAT3		Cui et al. (2018)
		STAT3, MAPKs, NF-κB, IL-6, EGFR, cyclin D1, c-Rel, p65, p50, JNK/AP-1		Chun et al. (2015)
Lung cancer	NCI-H1299 and Anip973 cells	Bcl-2, MMP-9, MMP-7, and MMP-2, β-actin, p38MAPK, NF-κB	Inducing cell apoptosis, suppressing migration, invasion, and colony formation, inhibiting cell proliferation	Liu et al. (2019)
	SK-MES-1 cells	Caspases-8, -9, -3, PARP, Bcl-2, Bax, CDK4, CDK6, cyclin D3, cyclin D1, p21, p27		Zhao et al. (2015)
	A549 cells and Ncl-H520 cells	Xiap, survivin, caspase-9, caspase-3, PARP, ATF4, eIF2α, CHOP, Bcl-2, Bax, STAT3, iNOS, COX-2, MMP-9		Maryam et al. (2017)
		PI3K/Akt, ER, p21, cyclin A2		Wang et al. (2019a)
Leukemia	HL-60 cells	Cytochrome c, Bax, caspase-3, PARP	Inducing apoptosis, inhibiting cell proliferation, inducing cell cycle arrest	Pal et al. (2010)
	THP-1 cells	STAT-3, survivin, Bcl-2, Bcl-xL, Bax, cl-caspase-3, cl-PARP, cytochrome c		Ahmad et al. (2021)
	K562 and K562r cells CML blast cells	NF-κB, p65 Bcr/Abl protein, caspase-3, PARP-1		Wei et al. (2013)
	BV173 and NALM6 cells	AP2M1, Beclin1, LC3-II/LC3-1, p62, Bax, cleaved caspase 3, cytochrome C, Bcl-2		Shi et al. (2020)
	B-ALL cell lines	PARP-1, capase-3, caspase-8, caspase-9, NF-κB, BCR-ABL, EGFR		Xu et al. (2019b)
Pancreatic cancer	MIA PaCa-2 and PANC-1 cells	TFEB, CTSB/CTSD	Inducing apoptosis, improving chemosensitivity, inhibiting proliferation, inhibiting migration	He et al. (2018)
	BxPC-3, AsPC-1, and PANC-1 cell lines	STAT3		Zheng et al. (2019)
	PANC-1 and SW1990 cells	Caspase 3/7, Bak, Bcl-2, Mcl-1, XIAP, STAT3		Yan et al. (2020)
Gastric cancer	SGC-7901 and BGC-823 cells	TrxR1, p38MAPK, p38, Ki-67, Bcl-2	Inhibiting proliferation, inducing apoptosis	He et al. (2019a)
		Bcl-2, Bax, cleaved PARP, cyclin D1, p21, p27, AKT, cyclin-dependent kinase inhibitor 1, cyclin-dependent kinase inhibitor 1B		Zhang and Zhang (2019)
		Bax, Bcl-2, p53, MMP-2, MMP-7, MMP-9, NF-κB, p38MAPK, p65		He et al. (2019b)
Cervical cancer	HeLa cells	Bcl-2, Bax	Inhibiting proliferation, inducing apoptosis	Jiang et al. (2016)
		Caspase-3, Bax, Bcl-2, NF-κB		Zhang et al. (2019b)
		TrxR, caspase 3		Zhang et al. (2019a)
Glioblastoma	U87 and U251 cells	IKKβ/NF-κB, p50, p65, p300, COX-2, cytochrome c, cyclin D1, CDK4, MMP-2, MMP-9, caspase-3/9, PARP, Bax, Bcl-2	Inhibiting cell growth, inducing apoptosis	Khan et al. (2012), Wang et al. (2017)
Osteosarcoma	U2OS and HOS cells	PI3K/AKT, cyclin D1, p27, Bcl-2, Bax, cleaved caspase-3/8, MMP-2, MMP-9	Inhibiting proliferation, promoting apoptosis	Zhang et al. (2019c)
Multiple myeloma	RPMI8226, NCI-H929, IM9, MM1R, MM1S, OPM2 and U266 cells	ERK1/2, IL-6, VEGF, caspase-3/8/9, Bcl-2, Bax, survivin, cyclin D, cyclin E, CDK 2, CDK 4, MAPK	Inhibiting proliferation, inducing G1 phase arrest, inducing apoptosis	Yao et al. (2015)

TABLE 2 | The anticancer activities and the underlying mechanisms of alantolactone *in vivo*.

Cancers	Animals	Modulated factors	Biological effects	References
Colorectal cancer	Six-week-old female Balb/c mice female sprague-dawley rats	HMGB1, CRT, MHCII, CD86, macrophages, MDSCs, TNF-α, IFN-γ	Promoting antitumor response, suppressing cell proliferation, inducing apoptosis	Zhang et al. (2019a)
	Five-week-old female athymic BALB/c mice	JNK, p38, MAPK, Ki-67		Cao et al. (2019)
Breast cancer	Chick embryo CAM BALB/c nude mice	VEGFR2phosphorylation, PLCγ1, FAK, Src, Akt	Inducing apoptosis, causing cell cycle arrest suppressing growth of xenograft tumors	Liu et al. (2018b)
	MDA-MB-231 xenografts in nude mice	Bcl-2, Bax, caspase-3, cyclinB1, Cdc2, ATF4, CHOP, ki-67		Yin et al. (2019)
	Female athymic BALB/c nude mice	STAT3, MAPKs, NF-κB, IL-6, EGFR, cyclin D1, c-Rel, p65, p50, JNK/AP-1		Chun et al. (2015)
Leukemia	BV173 xenograft nude mouse model	AP2M1, Beclin1, LC3-II/LC3-1, p62, Bax, cleaved caspase 3, cytochrome C, Bcl-2	Inhibiting cell proliferation, inducing apoptosis, inducing cell cycle arrest	Shi et al. (2020)
	B-ALL mice model (NOD-SCID mice)	PARP-1, capase-3, caspase-8, caspase-9, NF-κB, BCR-ABL, EGFR		Xu et al. (2019b)
Pancreatic cancer	Female nude BALB/c mice	TFEB, CTSB/CTSD	Inducing apoptosis, improving chemosensitivity	He et al. (2018)
	Female Wild-type BALB/c mice	STAT3		Zheng et al. (2019)
Gastric cancer	Athymic BALB/c nu/nu female mice	TrxR1, p38MAPK, p38, Ki-67, Bcl-2	Inhibiting proliferation, inducing apoptosis	He et al. (2019a)
Glioblastoma	BALB/c nu/nu male nude mice	IKKβ/NF-κB, p50, p65, p300, COX-2, cytochrome c, cyclin D1, CDK4, MMP-2, MMP-9, caspase-3/9, PARP, Bax, Bcl-2	Inhibiting cell growth, inducing apoptosis	Khan et al. (2012), Wang et al. (2017)

endoplasmic reticulum (ER) stress pathways (Wang J. et al., 2019). After treatment on A549 lung adenocarcinoma cells, ALT performs the biological functions to trigger oxidative stress mediated-cell apoptosis by abrogating the glutathionylation-dependent STAT3 activation (Maryam et al., 2017). The above studies show the molecular mechanism and biological significance of ALT in the treatment of lung cancer.

2.2 Liver Cancer

Liver cancer, with a high death rate and poor 5-years survival, is considered to be one of the most malignant cancers in the world (Feng et al., 2020). The factors leading to liver cancer are as follows: infection of hepatitis B virus (HBV), infection of hepatitis C virus (HCV), alcohol abuse, and alternations of genetic and epigenetic events (Zhang et al., 2020b). There are many strategies to treat liver cancer, such as chemotherapy, radiotherapy, molecular targeted therapy, surgical resection, and liver transplantation (Petrowsky et al., 2020). However, the prognosis is unsatisfactory because of the complex risks and pathological factors (Zhang et al., 2020a; Ruan et al., 2020). Therefore, a new treatment is needed. A recent study has explored the mechanism of ALT-mediated apoptosis in liver cancer cells HepG2 and found that through down-regulating reactive oxygen species (ROS)-mediated alpha serine/threonine-protein kinase (AKT) activation and weakening PTEN induced putative kinase 1 (PINK1)-mediated cell mitophagy, ALT treatment could induce apoptosis in HepG2 cells (Kang et al., 2019). It has also been shown that mitochondrial membrane in HepG2 cells loses the potential when being exposed to ALT and ALT induces apoptosis through modulating the levels of several apoptosis-associated proteins, including Bax, Bak, caspases, etc. (Lei et al., 2012). Another study has drawn a similar conclusion that ALT treatment could enhance Bax/Bcl-2 ratio, promote caspase-3 activation and elevate ROS generation, contributing to inducing apoptosis of HepG2 cells. The abnormally over-expressed and activated signal transducer and activator of transcription 3 (STAT3) signaling pathway have also been proved to be impaired by ALT in liver cancer cells (Khan et al., 2013). These studies indicate that ALT has the potential to be a leading chemotherapeutic candidate in the treatment of liver cancer.

2.3 Colorectal Cancer

At present, colorectal cancer ranks as the fourth most deadly cancer in the world. The incidence and mortality of colorectal cancer are much higher in developing countries than in developed countries because of the differences in medical service quality (Suliman et al., 2019; Almatroudi, 2020). It has been found that the incidence of colorectal cancer has a younger trend (The Lancet, 2017; The Lancet Gastroenterology, 2018). Colorectal cancer is a heterogeneous disease with many molecular subtypes, which is beneficial to the prognosis and immunotherapy of cancer (Becht et al., 2016; Wirth and Schneider, 2016). Nowadays, many traditional Chinese medicines (TCM) have been applied to the clinical therapy of cancers. Quercetin synergized with ALT could significantly induce immunogenic cell death (ICD) in colorectal cancer cells. This synergistic therapeutic effect is capable of reversing the immune-suppressive tumor microenvironment, thereby improving cell toxicity and antitumor immunity (Zhang J. et al., 2019). Ding et al. have explored the underlying molecular mechanism of ALT in human colorectal cancer cells SW480 and SW1116 and found that after ALT treatment, the accumulation of ROS causes oxidative DNA damage, contributing to the intrinsic apoptosis pathway of cancer cells (Ding et al., 2016). In addition to causing oxidative DNA damage,

ALT could strengthen the effects of oxaliplatin in HCT116 and RKO cells by inducing the activation of MAPK-JNK/c-Jun pathway, deactivation of the JNK pathway, inhibition of p38 MAPK pathway and decrease of intracellular ROS, as has been suggested by two independent studies. The two studies suggest that ALT could suppress cell proliferation and exhibit anticancer effects on colorectal cancer HCT-8 cells and HCT-116 cells (Shi et al., 2011; Babaei et al., 2021; Ren et al., 2021). Besides, ALT could exert the dose-dependently cytotoxic effects on RKO human colon cancer cells and induce cell apoptosis through modulating ROS-mediated mitochondria-dependent pathway (Zhang et al., 2013). The above studies show that ALT treatment could be clinically applied for patients with colorectal cancer in the future.

2.4 Breast Cancer

Breast cancer is a common cancer in women (Liu Y. et al., 2020; Wan et al., 2020). Although the diagnosis strategies like the mammogram, have been developed in recent years, the mortality rate of breast cancer is still high (Ranjkesh et al., 2020; Xu et al., 2020). As a result, innovative alternatives are needed to improve the therapeutic outcome of patients with breast cancer Studies have shown that ALT changes the cell morphology and decreases the cell viability of MDA-MB-231 and MCF-7 breast cancer cells (Liu J. et al., 2018; Cui et al., 2018). Administration of ALT can promote apoptosis and suppress migration of MCF-7 cells, which may be due to the decrease of p38 MAPK, NF-κB and nuclear factor E2-related factor 2 (Nrf2) signaling pathways (Liu J. et al., 2018). Liu et al. have revealed that ALT treatment is effective in inhibiting the motility, migration, and tube formation of human umbilical vein endothelial cells (HUVEC), which promote tumor angiogenesis. Besides, ALT impairs the angiogenesis and tumor growth by down-regulating vascular endothelial growth factor receptor 2 (VEGFR2) phosphorylation level and its downstream protein kinases, including phospholipase C gamma 1 (PLCγ1), protein tyrosine kinase 2 (FAK), SRC, and AKT (Liu Y. R. et al., 2018). Triple-negative breast cancer is one of the most challenging subtypes of breast cancers with a high probability of relapse, distant metastasis, and poor survival (Kim et al., 2018; Garrido-Castro et al., 2019). Therefore, analyzing the correlation of ALT and the anti-tumor potential in TNBC is potentially important. Yin et al. have shown that ALT promotes cell death and inhibits cell proliferation of triple-negative breast cancer cells by inducing ROS generation and subsequent ROS-dependent ER stress. Further analyses have shown that thioredoxin reductase 1 (TrxR1) expression and activity are weakened by ALT (Yin et al., 2019). Furthermore, other studies have demonstrated that ALT, serving as a STAT3 inhibitor, suppresses cell migration and the growth of triple-negative breast cancer cells both *in vitro* and *in vivo* (Chun et al., 2015; Kim et al., 2017), highlighting the therapeutic potential in breast cancer treatment.

2.5 Leukemia

Leukemia is a malignant progressive disease characterized by abnormal proliferation of haemopoietic stem cells (Abdellateif et al., 2020) and can be divided into four subtypes, namely acute myeloid leukemia, acute lymphoblastic leukemia, chronical myeloid leukemia, and chronical lymphoblastic leukemia. Chronical lymphoblastic leukemia is the most common one that occurs in adults (Hallek et al., 2018; Bosch and Dalla-Favera, 2019), whereas acute lymphoblastic leukemia is most commonly observed in children (Nordlund and Syvanen, 2018). Recently, the biological activities of ALT against THP-1 leukemia cells have been investigated and the results show that ALT plays an important role in inhibiting cell viability and inducing mitochondrial apoptosis in THP-1 cells by provoking ROS production and interfering in STAT3, survivin, c-Jun, and p38 MAPK signaling pathways (Ahmad et al., 2021). Shi et al. have also demonstrated that ALT could promote the expression level of adaptor-related protein complex 2 subunit mu 1 (AP2M1) and inhibit cell proliferation, colony formation, and autophagy of acute lymphoblastic leukemia cells in a dose-dependent manner through up-regulating AP2M1 signaling (Shi et al., 2020). Moreover, the n-hexane fraction extracted from *Inula racemosa Hook. f.*, a mixture of active ingredients mainly consisted of ALT, displays an inhibitory effect on leukemia HL-60 cells through enhancing the intrinsic and extrinsic apoptosis pathways without side effects to normal cells (Pal et al., 2010). ALT also induces cytotoxicity on B cell acute lymphoblastic leukemia *in vivo* and *in vitro* by prompting ROS overload and subsequently resulting in ROS-mediated DNA damage (Xu X. et al., 2019). After the evaluation about the potential activity of ALT in imatinib-sensitive and -resistant cells, Wei et al. have concluded that ALT treatment contributes to significant cell apoptosis in both imatinib-sensitive and -resistant leukemia cells, as indicated by the increase of caspases activation and poly (ADP-ribose) polymerase-1 (PARP-1) cleavage (Wei et al., 2013). These studies strongly support the application of ALT in leukemia treatment.

2.6 Pancreatic Cancer

Pancreatic cancer is the second leading cause of cancer death in Western countries, especially in the United States (Neoptolemos et al., 2018; Collisson et al., 2019). The treatment of pancreatic cancer is not easy as early diagnosis is hard (Moore and Donahue, 2019) and there are few effective clinical treatment approaches (Halbrook and Lyssiotis, 2017). It has been revealed that the bioactive mixture of ALT and the analogues (allo-ALT and iso-ALT) could exert significant anti-proliferation and anti-migration effects on PANC-1 and SW1990 pancreatic cancer cells (Yan et al., 2020). It has also been shown that the combination of ALT and other treatments could exert synergized cytotoxic effects on pancreatic cancer. For example, when combined with the chemotherapy drug oxaliplatin, ALT might play a crucial role in deducing tumor-killing effects on pancreatic cancer cells through blocking cathepsin B/cathepsin D activation (He et al., 2018). Similarly, Wang et al. have revealed that ALT triggers synergistic lethality with simultaneous PARP-1 inhibition in homologous recombination-proficient cancer cells (Wang et al., 2020), and promotes the therapeutic sensitivity of pancreatic cancer cells to the anti-cancer drugs, including oxaliplatin (He et al., 2018), PARP inhibitor (olaparib) (Wang et al., 2020), epidermal growth factor receptor (EGFR) inhibitors (erlotinib and afatinib) (Zheng et al., 2019), and so on. Therefore,

the combination of natural compound ALT and specific anti-cancer agents is a safe and effective strategy for pancreatic cancer treatment.

2.7 Other Tumors

Many studies have suggested that ALT could also exhibit cytotoxic effects on other types of cancers. It has been shown that ALT induces apoptosis and triggers cell-cycle arrest in gastric cancer cells through ROS generation and modulation of several ROS-dependent kinase signaling pathways, such as AKT, p38 MAPK, and NF-κB (He W. et al., 2019; He Y. et al., 2019; Zhang and Zhang, 2019). Furthermore, combined treatment of ALT and ferroptosis inducer erastin could exert a synergistic effect on inducing the death of gastric cancer cells (He W. et al., 2019). It has also been demonstrated that ALT exerts concentration-dependent effects on inhibiting proliferation and inducing apoptosis of cervical cancer cells through regulating the Bcl-2/Bax radio, NF-κB pathway, and thioredoxin reductase (TrxR) activation (Zhang J. et al., 2016; Jiang et al., 2016; Zhang Y. et al., 2019). Furthermore, a newly study have reported that ALT could inhibit the progression of HeLa cells via suppressing the expression of BMI1(Sun et al., 2021). Through down-regulating the NF-κB/COX-2-mediated signaling cascades or triggering the cofilin/G-actin signaling, ALT inhibits the growth and induces apoptosis of glioblastoma cells both *in vivo* and *in vitro* (Khan et al., 2012; Wang et al., 2017; Wang X. et al., 2021). The similar tumor-inhibition effects of ALT, accompanied by apoptosis promotion and growth depression, could also be observed in osteosarcoma (Zhang Y. et al., 2020), esophageal cancer (Wang Z. et al., 2021), multiple myeloma (Yao et al., 2015), etc. The above studies explore the underlying molecular mechanism of the biological activity of ALT, contributing to the application of ALT as a promising chemotherapeutic candidate for different kinds of cancers.

3 CLINICAL PERSPECTIVE OF ALT

As an important sesquiterpenoid extracted from a frequently utilized traditional herbal medicine, ALT has been confirmed to possess a broad spectrum of pharmacological properties, including anti-tumor, anti-fungal, and anti-inflammatory activities. Up to now, many studies have reported the anticancer effects of ALT *in vitro* and *in vivo*. However, the biological actions of ALT are easily influenced by some factors, like bioavailability.

Recently, a pharmacokinetics study has suggested that the oral bioavailability of ALT is quite low, which is one challenge in clinical trial design to explore the biological actions. Some defects of ALT, such as low water solubility, limit the absorption and bioavailability *in vivo* (Xu et al., 2015). Low oral bioavailability probably results from intestinal metabolism, poor permeability, and low aqueous solubility (Zhou et al., 2018). However, according to the compatibility principle in the Prescription Dictionary of Chinese Medicine, the combination of ALT and other herbs could effectively reduce the toxicity and enhance intestinal absorption, contributing to stronger bioavailability and therapeutic actions (Xu R. et al., 2019). It is well known that evaluation of intestinal bacteria is one challenge in clarifying the metabolism of oral drugs (Zimmermann et al., 2019). A biotransformation strategy based on the anaerobic culture of intestinal bacteria has been developed by Yao et al. for identifying ALT metabolites (Yao et al., 2016). In addition, ALT-entrapped nanostructured carriers have been developed to improve the bioavailability and potential cytotoxicity efficacy of ALT against cancers (Zhang J. et al., 2019). These studies are beneficial for the evaluations of ALT application in the future. Unfortunately, until now, there are no clinical trials to explore the bioavailability and anti-tumor effect of ALT in cancer patients. Therefore, to verify the pharmacological activities of ALT, more investigations, especially well-designed clinical trials, remain to be determined in the future.

4 IMPLICATION OF ALT FOR CANCER-ASSOCIATED SIGNALING PATHWAYS

As shown in previous studies, ALT has good clinical prospects as therapeutic agents for human cancers. It has been found that ALT exerts high cytotoxicity effects, such as anti-proliferation, anti-metastasis, and pro-apoptotic cascades on many human cancer cell lines through interfering with several molecular events (Zhang J. P. et al., 2016; Nadda et al., 2020).

Previous studies have illustrated the important roles of ROS in maintaining the stable microenvironment of tissues and affecting the genesis and development of malignant tumors (Ippolito et al., 2020; Shen et al., 2020). If the ROS production is not in balance, the extensive damage response in cells caused by oxidative stress would result in higher risks of diseases, like diabetes, cardiovascular disease, cancers, etc. (Tavares and Seca, 2019). Therefore, keeping the balance of ROS levels is beneficial for regulating cancer treatment efficacy (Jiang et al., 2019; Zhou et al., 2020). It has been found that ALT could increase the concentration of ROS and trigger the intrinsic apoptosis pathway of colorectal cancer cells (Ding et al., 2016). Kang et al. have reported that ALT could induce cell-cycle arrest and cell apoptosis in HepG2 cells by regulating intracellular ROS accumulation, which provides a new strategy to treat liver cancer (Kang et al., 2019).

In addition, as a transcription factor, NF-κB is related to the regulation of carcinogens, such as promoting cell proliferation, regulating apoptosis, facilitating angiogenesis, and stimulating metastasis (Liu Z. et al., 2020; Espinosa-Sanchez et al., 2020). NF-κB also modulates the immune and inflammatory responses, influencing cancer cell growth (Fusella et al., 2017; Taniguchi and Karin, 2018). Effective regulation of the activation of the NF-κB signaling pathway is significant in developing chemotherapies. It has been found that ALT-targeted NF-κB and the downstream signaling pathways inhibit the migration of breast cancer cells and trigger the apoptosis of chronical myeloid leukemia cells (Wei et al., 2013; Liu J. et al., 2018). It has also been demonstrated that ALT promotes cell apoptosis in acute lymphoblastic leukemia and gastric cancer through inhibiting NF-κB activation (He Y.

et al., 2019; Xu X. et al., 2019). Besides, ALT significantly delays the cell proliferation of HeLa cells in a dose-dependent manner through targeting NF-kB signaling pathways (Zhang Y. et al., 2019).

It is well-known that clarifying the underlying functions of VEGFR contributes to the understanding of the angiogenesis and therapeutic response of cancer cells (Haibe et al., 2020; Kratzsch et al., 2020). Furthermore, VEGF plays a crucial role in the development of molecular-targeted treatment or other novel anti-cancer drugs in clinical practice (Apte et al., 2019). Liu et al. have uncovered that ALT inhibits VEGFR2 phosphorylation, and impairs VEGF-VEGFR2 signaling in HUVECs (Liu Y. R. et al., 2018). ALT could also reduce VEGF secretion, thereby suppressing the adhesion of multiple myeloma cells (Yao et al., 2015). These findings suggest that ALT may be a promising agent to fight against angiogenesis and invasion in cancers through intervening in VEGF-VEGFR pathways.

The aberrant activation of the p38 MAPK signaling pathway is involved in various biological processes, facilitating the development and treatment of cancer (Wang K. et al., 2019; Reger de Moura et al., 2020). As an essential regulating factor, p38 MAPK participates in many cellular activities, making cancer cells perceive and adapt to environmental stress signals (Low and Zhang, 2016; Martinez-Limon et al., 2020). Studies have shown that deactivating the p38 MAPK pathway could facilitate the ALT-mediated cell apoptosis in colon cancer cells and breast cancer cells (Liu J. et al., 2018; Cao et al., 2019). Moreover, ALT exerts attractive pharmacological activities on lung cancer cells by blocking the p38 MAPK pathway (He W. et al., 2019; Liu et al., 2019). He et al. have further revealed that ALT modulates the ROS-mediated p38 MAPK pathway and induces cell apoptosis in gastric cancer. More importantly, ALT treatment markedly enhances the cell sensitivity to the ferroptosis inducer erastin (He W. et al., 2019).

In addition, there are a few studies concerning about the correlation between ALT administration and cell autophagy in cancer cells. ALT could play a significant role in promoting impaired autophagy, facilitating to allay osteoarthritis and strengthen pancreatic cancer cells' chemosensitivity (He et al., 2018; Pei et al., 2021). Another two studies have demonstrated that treatment with ALT could significantly downregulate the cell autophagy in ALL and liver cancer cells, implying that ALT have the potential to kill cancer cells through modulating autophagy (Kang et al., 2019; Shi et al., 2020).

Taken together, accumulating reports have showed that ALT exerts anticancer effects on various kinds of cancers, such as liver cancer, colorectal cancer, breast cancer, etc. And the potential molecular mechanisms involved in ALT's anticancer activities are inhibiting JNK and p38 MAPK pathways, PI3K/AKT/GSK3β pathways, NF-κB/COX-2 pathways and promoting cell apoptosis-associated signalings. These findings above-mentioned demonstrate that ALT may be a potent therapeutic candidate for cancer reseach and treatment. However, more comprehensive studies are still needed to further explore the detailed functions of ALT.

5 CONCLUSION

In summary, the exploration of agents from plants will help to develop new therapeutic strategies and drugs in future clinical treatment. ALT possesses superior anti-tumor properties besides anti-inflammatory and antimicrobial activities and can be a potential drug candidate for cancer therapy. From some experiments of ALT *in vivo* and *in vitro*, we can know that ALT can synergize with chemical drugs to enhance their anticancer effects, such as Quercetin and oxaliplatin. Additionally, it was reported that ALT could enhanced the therapeutic sensitivity on cancer treatment. Although there are some studies concerning the cytotoxic effects of ALT *in vivo* and *in vitro*, more profound investigations are still needed to clarify the underlying mechanisms of ALT in the treatment of human malignancies. Besides, accurate and reliable clinical research, for example, randomized controlled trials, are needed to prove the effectiveness of ALT as a therapeutic agent for cancers.

AUTHOR CONTRIBUTIONS

YC, JP and KG: Conceptualization, Data curation, Methodology, Writing-Original draft preparation. BP: Visualization, Investigation. JL: Supervision, Resources. XC and YY: Formal analysis, Funding acquisition. SZ and KH: Software, Validation. JP, ZX and YY: Writing- Reviewing and Editing.

FUNDING

This study is supported by grants from the China Postdoctoral Science Foundation (2021T140754, 2020M672521), the National Natural Science Foundation of China (81803035), the Natural Science Foundation of Hunan Province (2020JJ5934, 2019JJ50932), and the Postdoctoral Science Foundation of Central South University (248485). We thank the Language Editing Service of KetengEdit for assistance with the language editing.

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frontiers in Pharmacology

SYSTEMATIC REVIEW
published: 06 December 2021
doi: 10.3389/fphar.2021.770663

Platinum and Taxane Based Adjuvant and Neoadjuvant Chemotherapy in Early Triple-Negative Breast Cancer: A Narrative Review

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Platinum (Pt) derivatives such as cisplatin and carboplatin are the class of drugs with proven activity against triple-negative breast cancer (TNBC). This is due to the ability of Pt compounds to interfere with the DNA repair mechanisms of the neoplastic cells. Taxanes have been efficacious against estrogen receptor-negative tumors and act by disruption of microtubule function. Due to their distinct mechanisms of action and routes of metabolism, the combination of the Pt agents and taxanes results in reduced systemic toxicity, which is ideal for treating TNBC. Also, the sensitivity of *BRCA1*-mutated cells to taxanes remains unsolved as *in vitro* evidence indicates resistance against taxanes due to *BRCA1* mutations. Recent evidence suggests that the combination of carboplatin and paclitaxel resulted in better pathological complete response (pCR) in patients with TNBC, both in neoadjuvant and adjuvant settings. *In vitro* studies showed sequential dependency and optimal time scheduling of Pt- and taxane-based chemotherapy. Also, combining carboplatin with docetaxel in the NAC regimen yields an excellent pCR in patients with *BRCA*-associated and wild-type TNBC. TNBC is a therapeutic challenge that can be tackled by identifying new therapeutic sub-targets and specific cross-sections that can be benefitted from the addition of Pt- and taxane-based chemotherapy. This review summarizes the merits as well as the mechanism of Pt- and taxane-based adjuvant and neoadjuvant chemotherapies in early TNBC from the available and ongoing clinical studies.

Keywords: adjuvant chemotherapy, neoadjuvant therapy, platinum, taxane, triple negative breast cancer

OPEN ACCESS

Edited by:
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Hokkaido University, Japan

Reviewed by:
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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *04 September 2021*
Accepted: *11 November 2021*
Published: *06 December 2021*

Citation:
Tian H, Ma D, Tan X, Yan W, Wu X, He C, Zhong L, Zhang Y, Yu B, Zhang Y and Qi X (2021) Platinum and Taxane Based Adjuvant and Neoadjuvant Chemotherapy in Early Triple-Negative Breast Cancer: A Narrative Review. Front. Pharmacol. 12:770663. doi: 10.3389/fphar.2021.770663

INTRODUCTION

Triple-negative breast cancer (TNBC) is a subtype of breast cancer (BC) that collectively represents 15–20% of all the BC reported (Dent et al., 2007). It is usually associated with rapid disease progression, higher mortality rate, poorer prognosis, and distant recurrences when compared to other forms of breast cancer. Despite having larger tumors and a marked rate of node positivity, patients in the triple-negative category exhibit a weaker relationship between tumor size and node status (Dent et al., 2007).

In the TNBC subtype, negative expressions of progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor-2 (HER2) are observed (Jhan and

TABLE 1 | Summary of neoadjuvant studies with Pt derivatives and taxane combinations.

Study	Phase	Trial number	Molecular subtype of breast cancer	Intervention	Comparator	Outcomes
von Minckwitz et al. (2014)	II	NCT01426880 (Geparsixto)	TNBC	P + A Bev	P + A Bev with Cb	pCR: 43.7 vs. 36.9%, (OR = 1.33, 95% CI 0.96–1.85; p = 0.107) TNBC: pCR: 53.2 vs. 36.9% (p = 0.005) Grade 3 or 4 neutropenia (192 [65%] vs. 79 [27%]) Grade 3 or 4 anemia (45 [15%] vs. 1 [<1%]) Grade 3 or 4 thrombocytopenia (42 [14%] vs. 1 [<1%]) Grade 3 or 4 diarrhea (51 [17%] vs. 32 [11%]) Dose discontinuations (141 [48%] vs. 114 [39%]) (p = 0.031). Frequency of grade 3 or 4 hematological events decreased from 82% (n = 135) to 70% (n = 92) and grade 3 or 4 non-hematological events from 78% (n = 128) to 59% (n = 77) in the Cb arm when the dose of Cb was reduced from AUC 2.0 to 1.5.
Loibl et al. (2018)	III	NCT02032277 (BrighTNess)	TNBC	Veli + P + Cb → AC	P → AC and P + Cb → AC	pCR: Veli + P + Cb vs. P alone (168 [53%] vs. 49 [31%]), (p < 0.0001), pCR: Veli + P + Cb b vs. P + Cb (168 [53%] vs. 92 [58%]), p = 0.36).
Wu et al. (2018), Sharma et al. (2018)	II	ChiCTR-TRC-14005019	TNBC	TEL	TE	TpCR: TEL vs. TE (38.7% [24/62] vs. 12.7% [8/63]), (OR: 4.342, 95% CI 1.764–10.687; p = 0.001) ORR: TEL vs. TE (93.5% [58/62] vs. 73.0% [46/63]) Grade 3–4 anemia and thrombocytopenia: TEL vs. TE (52.5 vs. 10.0% and 34.4 vs. 1.7% respectively)
Gluz et al. (2018)	II	NCT01815242 (WSG-ADAPT-TN)	TNBC	Gem + nab-P	Cb + nab-P	pCR for 3-years EFS: 92 vs. 71%), p < 0.001) pCR for 3-years OS: 99.1 vs. 81.6%), p < 0.001) 3-years EFS: 77.6 vs. 80.8%), p = 0.48) 3-years OS: 84.7 vs. 92.2%), p = 0.08).
Sharma et al. (2021)	II	NCT02413320 (NeoSTOP)	TNBC	Cb + P → AC	Cb + T	pCR: 54%, RCB 0 + 1: 67% pCR in patients with BRCA TNBC: 59% pCR in patients with wild-type TNBC: 56% At least one grade 3: 21%, At least one grade 4: 7%
Zhang et al. (2020)	II	NCT03154749 (NeoCART)	TNBC	T + Cb	E + C → T	pCR: 27 (61.4%) vs. 17 (38.6%) (OR: 2.52, 95% CI 2.4–43.1; p = 0.033) stage II pCR: 73.3% (22/30) vs. 48.4% (15/31) (p = 0.046) stage III pCR: 35.7% (5/14) vs. 15.4% (2/13) (p = 0.384). Grade 3/4 AEs include anemia (4.5%), thrombocytopenia (2.3%), neutropenia (2.3%) and ALT/AST increased (2.3%) in the T + Cb group.
Sikov et al. (2015), Sikov et al. (2019)	II	NCT00861705 (CALGB 40603)	TNBC	P + Cb + Bev → AC	P + Bev → AC	pCR on addition of either: Cb (60 vs. 44%; p = 0.0018) or Bev (59 vs. 48%; p = 0.0089) pCR breast/axilla: Cb (54 vs. 41%; p = 0.0029)

A, doxorubicin; AUC, area under curve; Bev, bevacizumab; C, cyclophosphamide; CI, confidence-interval Cb, carboplatin; E, epirubicin; EFS, event-free survival; Gem, gemcitabine; nab-P, albumin paclitaxel; OR, odds Ratio; OS, overall survival, P, paclitaxel, PCR, pathological complete response; T, docetaxel; TNBC, triple negative breast cancer; Veli, veliparib; L, lobaplatin.

Andrechek, 2017). TNBC is a heterogeneous disease that is classified based on the specific histological characteristics of the tumor and the expression of single molecular markers (BCL2, p53, MDR-1, Ki67, etc.) and manifests into a range of clinical outcomes (Jhan and Andrechek, 2017; Diana et al., 2020).

The current treatment approach for TNBC consists of chemotherapy drugs such as anthracyclines, taxanes, platinum (Pt) derivatives, and targeted therapies such as angiogenesis inhibitors (bevacizumab), PARP1 inhibitors, EGFR inhibitors, tyrosine kinase and ERK inhibitors, and mTOR inhibitors (Mustacchi and De Laurentiis, 2015). Anthracyclines and taxanes have proven efficacy in both early-stage and metastatic ER-negative BC tumors and hence, both the classes are designated as first-line treatment of TNBC (Mustacchi and De Laurentiis, 2015).

BRCA1 and *BRCA2* genes synthesize proteins that aid the repair of damaged DNA. They are also called tumor suppressor genes as

TABLE 2 | Summary of adjuvant studies with Pt derivatives and taxane combinations.

Study	Phase	Trial number	Molecular subtype of breast cancer	Intervention	Comparator	Outcomes
Du et al. (2020), Burstein et al. (2019)	II	NCT01150513	TNBC	T/P + Cb	EC→T	5- year DFS: 84.4 vs. 85.8%, (*P*non-inferiority = 0.034) 5- year OS: 93.5 vs. 94.4%, (p = 0.770) Grade 3/4 adverse events: 48.7% (75/154) vs. 68.9% (106/154)
Yu et al. (2020), Korde et al. (2021)	III	NCT01216111 (PATTERN))	TNBC	P + Cb	CEF→T	5- year DFS: 86.5 vs. 80.3%, (HR = 0.65; 95% CI, 0.44–0.96; p = 0.03) RFS: 91.2 vs. 84.4%, (HR = 0.54; 95% CI, 0.34–0.88; p = 0.01) OS: 93.4 vs. 89.8%; (HR = 0.71; 95% CI, 0.42–1.22; p = 0.22) DDFS: 92.6 vs. 87.9%; (HR = 0.59; 95% CI, 0.35–0.999; p = 0.05)
Wang et al. (2019)	III	NCT01378533	TNBC	P + Cb with G-CSF	EC→P with G-CSF	DFS: (HR = 0.305, 95% CI = 0.134–0.693; p = 0.0046) 3-years DFS: 93.7 vs. 77.9% 3-years OS: 98.4 vs. 92.6%, p = 0.0268 Grade 3/4: 48.5 vs. 21.9%; p = 0.002
Nasr et al. (2015)	III		TNBC	FEC→ T	FEC→ T + Cb	mDFS:28 vs. 24 months, (p = 0.05) mOS: 37 vs. 29 months, (p = 0.04) distant metastasis recurrence rates: 26 vs. 37%
Frasci et al. (2009)	II		TNBC	Cis + E + P with G-CSF	NA	pCR: 46 women (62%; 95% confidence interval 50–73) in both breast and axilla. DFS: 41-months (range 3–119), 13 events (nine distant metastases) distant disease-free survival = 84% Five-year DFS in pCRs = 90% Five-year DFS in non-pCRs, = 56%. Severe neutropenia = 23 (31%) Severe anemia = 8 (10.8%) Severe non-hematological in <20% of patients

A, doxorubicin; C, cyclophosphamide; Cb, carboplatin; Cis, cisplatin; DDFS, Distant disease-free survival; DFS, Disease-free survival; E, epirubicin; F,5-fluorouracil; G-CSF, granulocyte stimulating factor; mDFS, median disease-free survival; mOS, median overall survival, P, paclitaxel, PCR, pathological complete response; RFS, Relapse-free survival; T, docetaxel; TNBC, Triple negative breast cancer.

they regulate cell division and are susceptible to the development of pathogenic mutations which can subsequently lead to carcinogenesis (Filippini and Vega, 2013; Diana et al., 2020). About 71% of germline *BRCA1* mutation carriers and 25% of germline *BRCA2* mutation carriers are affected by TNBC phenotype (Peshkin et al., 2010). Recently, Pt derivatives such as cisplatin and carboplatin have shown a revived interest in the treatment of TNBC. Preclinical data also suggest a favorable activity of Pt agents in TNBC and *BRCA1*-associated breast cancer (Rapoport et al., 2014).

The purpose of this narrative review is to objectively summarize the efficacy of Pt- and taxane-based neoadjuvant chemotherapy (NAC) and adjuvant chemotherapy in patients with early TNBC, as well as analyze its underlying pharmacological mechanism from a broader clinical perspective.

METHODOLOGY

A literature search was performed on PubMed for articles published in English from inception till May 2021, focusing on MeSH terms 'triple-negative breast cancer', 'taxanes', and 'platinum agents' in the context of 'adjuvant' and 'neoadjuvant' settings. The same search terms were used for the Embase and ClinicalTrials.gov registry of clinical trials. Abstracts from the annual meetings for the American Society of Clinical Oncology (ASCO) and European Society for Medical Oncology (ESMO) from 2015 to 2021 were also screened. We excluded publications if no clinical comparative information about the pCR or survival outcomes were reported (**Supplementary Figure S1**). A brief summary of the studies included in this review has been shown in **Tables 1** and **2**.

THE PHARMACOLOGICAL MECHANISM OF THE COMBINATION OF TAXANES AND PT DERIVATIVES

Although taxanes such as paclitaxel and docetaxel share a close resemblance in their molecular structure, they exhibit diverse pharmacology (Dorr, 1997). Both the taxanes bind to ß-subunit of tubulins in the neoplastic cell (**Figure 1**), influence microtubule polymerization, and repress the cell cycle at G2-M stage intersection. They both undergo metabolism in the liver. Furthermore, the mode of action of both the taxanes are quite similar; however, docetaxel shows a greater affinity

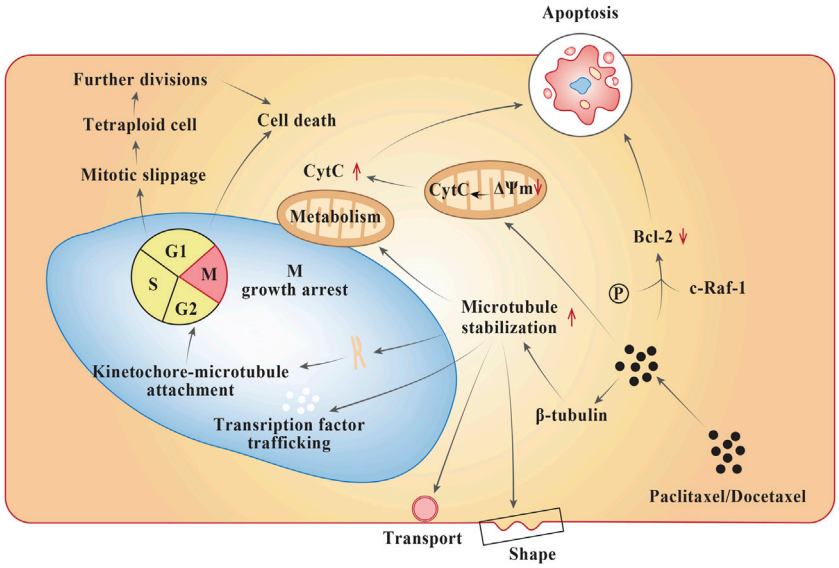

FIGURE 1 | Mechanism of action of taxanes in neoplastic cells.

for tubulin binding, a higher tendency towards microtubule depolymerization inhibition, stronger antitumor activity within *in vitro* and *in vivo* models, and more potent induction of BCL-2 phosphorylation leading to apoptosis (Pienta, 2001; Yiding and Zhongyi, 2021). The specific cytochrome P-450 (CYP) enzymes responsible for their hydroxylation are CYP2C8 and CYP3A4 for paclitaxel and docetaxel, respectively. The cytotoxic activity of the taxanes are discerned to increase with prolonging the duration of exposure (Dorr, 1997). At a mechanistic level, paclitaxel acts in a reversible manner to hyper stabilize the microtubules by binding to the N-terminal 31 amino acids of the ß-tubulin subunit thereby decreasing the threshold concentration of purified tubulin subunits. Also, paclitaxel has the ability to interact *in vitro* in microtubules formation at colder temperatures (4°C) and calcium concertrations (Kampan et al., 2015). As a result, the cancer cells treated with the drug are growth arrested in metaphase on bipolar spindles. Another mechanism of action of paclitaxel involves formation of tetraploid G1 cell due to improper chromosome segregation during mitosis. This results in cell death and arrest during growth phase (Weaver, 2014). Paclitaxel also activates multiple signal-transduction pathways such as toll-like receptor-4 (TLR-4) dependent pathway (either via MyD88 dependent or independent pathway), c-Jun N-terminal kinase (JNK), P38 Mitogen activated protein kinase (MAPK), nuclear factor kappa ß (NF-κß), Janus kinase-(JAK-) signal transducer and activator of transcription factor (STAT) pathway, which may be associated with proapoptotic signaling (Kampan et al., 2015). In case of docetaxel, the mode of anti-cancer activity is similar to that of paclitaxel, except that it differs structurally from the former at either the 3′ position on the side chain or the 10′ position on the baccatin ring (Montero et al., 2005).

Pt compounds have a central Pt particle, surrounded by chloride (Cl^-) molecules and ammonia groups. Pt compounds enter cells through an active carrier. Once inside the cell, the Cl^- particles separate, abandoning a reactive complex that interacts with the DNA (**Figure 2**). At a lower concentration of Cl^-, the dissociation for Cl^- ions are favored, while higher intercellular concentrations of Cl^- generally stabilizes the drug (Bardal et al., 2011). They act by alkylating DNA purine bases, which causes guanine-guanine (GG) synthesis that leads to inter- and intra-strand cross-linkage DNA adducts, which inhibits DNA synthesis and function (Bardal et al., 2011). This interferes with DNA repair mechanisms, intrinsic mitochondrial pathway, and forms a component of endoplasmic reticulum stress, ultimately leading to either necrosis or apoptosis. Cisplatin, a Pt-based compound binds to N7 reactive center on purine bases, forming 1,2-intrastrand [d (GpG) and d (ApG)] adducts of purines, eliciting DNA injury which can lead to cell apoptosis. The pathways activated during this process include p53, extracellular-signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) (Dasari and Tchounwou, 2014). Similarly, carboplatin, when penetrated into the cell membrane, is subjected to hydrolysis becoming positively charged. This compound follows the same process as cisplatin and interferes with G2/M growth arrest leading to cell apoptosis or necrosis (Sousa et al., 2014). They are primarily eliminated from the circulation via renal excretion (Bardal et al., 2011; Sousa et al., 2014).

Due to their distinct mechanisms of action, Pt and taxanes are often combined in cancer therapy contributing to their synergistic action. Different routes of metabolism of these two drugs lead to reduced systemic toxicity, making it an ideal candidate for chemotherapeutic treatment among patients with BC.

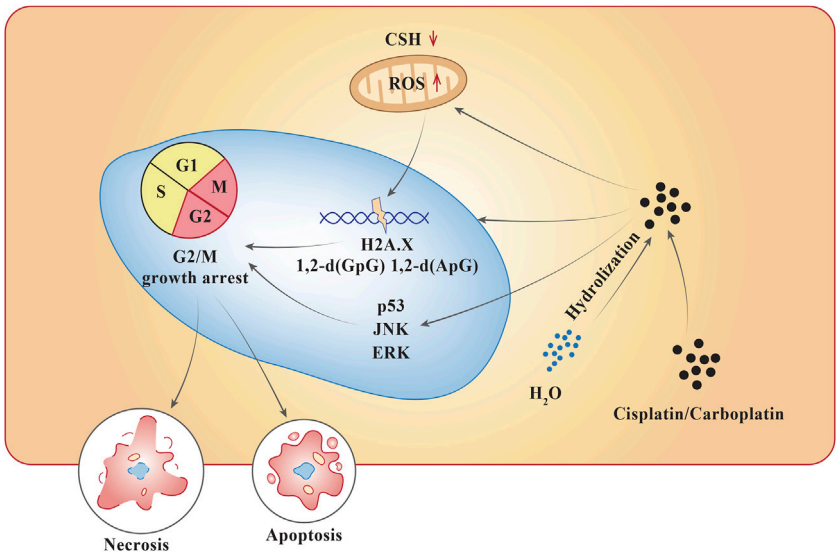

FIGURE 2 | Mechanism of action of platins in neoplastic cells.

The pharmacology of taxanes and Pt combination is illustrated by a well-designed study by Felici et al. utilizing a compartmental analysis for docetaxel and a non-compartmental investigation for cisplatin and 5-FU (Felici et al., 2006). They demonstrated that there was no pharmacokinetic interaction between the three drugs when given to patients with metastatic tumors while maintaining a manageable toxicity profile (Felici et al., 2006).

Multidrug resistance (MDR) is a phenomenon whereby tumor cells acquire resistance to a broad range of structurally and functionally diverse chemotherapeutic drugs, including alkylating agents, anthracyclines, vinca alkaloids, epipodophyllotoxins, and paclitaxel (Clynes, 1994). Multidrug resistance protein-1 (MRP-1) expression in primary BC is inversely correlated with both relapse-free survival (RFS) and overall survival (OS) (Nooter et al., 1997; Filipits et al., 1999), which could be one of the possible mechanisms of action for taxane-Pt-based chemotherapy. Previously, a study demonstrated that MRP-1 expression at diagnosis was associated with a worse prognosis in patients who received adjuvant systemic chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil (5-FU), in patients with small tumors (T1) and node-negative BCs (Vulsteke et al., 2013).

In vitro, cells resistant to Pt compounds were found to display increased levels of MRP-1 and MRP-4 (Beretta et al., 2010). Another research reported that overexpression of MRP-1 and MRP-3 was responsible for the decrease in drug sensitivity towards vincristine, etoposide, doxorubicin, and cisplatin in patients with lung cancer (Zhang et al., 2015). Shingo Maeda et al. evaluated the antitumor effects of cisplatin and docetaxel on gastric cancer cell lines MKN-74, MKN-45, and TMK-1. Strikingly, a sequence dependency was observed in gastric cancer cells *in vitro*, since docetaxel followed by cisplatin (DC) showed a stronger antitumor effect versus cisplatin followed by docetaxel (CD) in all cell lines (survival ratios for DC vs. CD: 0.462 vs. 0.666 for MKN-45 cells; 0.691 vs. 0.838 for MKN-74 cells; 0.570 vs. 0.766 for TMK-1 cells) (Maeda et al., 2004). Also, a higher Pt accumulation (twice the Pt accumulation than control cells 1.22 ± 0.26 vs. 0.64 ± 0.03 μg/10^7 for MKN-45 cells, 1.61 ± 0.34 vs. 0.77 ± 0.06 μg/10^7 for MKN-74 cells, respectively, $p < 0.05$) was noted in docetaxel followed by the cisplatin group in contrast to the cells treated with only cisplatin. Combining all the study results, we hypothesis that MRP-1 upregulation is a cause for drug resistance to platinum in cancer cells, while docetaxel could suppress the MRP-1 upregulation, thus performing a synergistic effect with platinum to increase the efficacy.

Another probable mechanistic pathway for the interaction between Pt- and taxane-based chemotherapy is via MicroRNAs (miRNAs). miRNAs are small regulatory non-coding RNAs that act through multiple cellular signaling pathways by controlling the degradation and translation of their target messenger RNAs (mRNAs). miRNAs base-pair with sequences within the 3′-untranslated region (UTR), 5′-UTR, and coding sequence regions of target mRNAs (He et al., 2005).

Paclitaxel elevates the level of miR-512-3p, which induces apoptosis in carcinoma cells (Chen et al., 2010). Another study revealed that high miR-9 expression downregulates *BRCA1* activity and improves paclitaxel/taxane chemotherapy response by increasing Pt sensitivity along with longer progression-free survival (PFS) (Sun et al., 2013). miRNA let-7 binding site genetic variants located in the HIF1AN and CLDN12 genes could predict pCR to taxane- and Pt-based NAC in locally advanced BC. Polymorphisms in microRNA let-7 binding sites of the HIF1AN and CLDN12 genes can predict pCR to taxane- and Pt-based NAC in BC. (Du et al., 2019) Similarly, another study reported low *BRCA1* expression and high expression of miRNA-9 was associated with Pt sensitivity and longer PFS (Strumidło et al., 2017).

TREATMENT SEQUENCE OF TAXANES AND PT-DERIVATIVES MIGHT LEAD TO DRUG-DRUG INTERACTIONS

Sequential treatment with taxanes and Pt derivatives might influence drug-drug interactions. Since paclitaxel undergo hepatic oxidation through the CYP system, pharmacokinetic interactions can be either sequential or schedule dependent (Scripture et al., 2005). Administration of paclitaxel following cisplatin causes an increase in myelosuppression, which is probably due to a decrease in paclitaxel clearance. In contrast, a favorable sequence-dependent pharmacodynamic interaction is seen when paclitaxel infusion is administered before carboplatin in terms of reducing platelet toxicity (Scripture et al., 2005). The systemic exposure to carboplatin alone (AUC for carboplatin = 34 μg × h/mL) showed a decrease in platelet count by 50% compared to carboplatin given after paclitaxel (AUC for carboplatin = 57 μg × h/mL), when the relationship between carboplatin and thrombocytopenia was analyzed (Scripture et al., 2005). The mechanism for this interaction remains obscure and warrants further probing. Nonetheless, the pharmacokinetic characteristics such as absorption, metabolism, distribution, and excretion of both paclitaxel and cisplatin do not vary based on their sequence of medication. A recent study suggested that sequential administration of paclitaxel 175 mg/m^2 followed by cisplatin 75 mg/m^2 should be preferred over a concurrent combination of both the drugs as the sequence of paclitaxel prior to cisplatin is associated with lesser toxicity (Elserafi et al., 2018). Although a similar response rate as well OS was observed in both sequential and concurrent regimens (Elserafi et al., 2018).

Some studies report that administration of paclitaxel followed by carboplatin led to a decreased formation of Pt dimers in patient's DNA, which is speculated to contribute towards the antitumor activity of carboplatin (Baker, 1997; Calvert, 1997; Kearns and Egorin, 1997). Patients treated by paclitaxel followed by carboplatin sequence had less hematopoietic toxicity than patients treated with carboplatin followed by paclitaxel. No significant difference in the pharmacokinetics of carboplatin or paclitaxel was observed with either of the administration schedule (Baker, 1997; Calvert, 1997; Kearns and Egorin, 1997). A similar finding was observed for the combination of cisplatin and docetaxel where a significantly lower Pt DNA dimers were detected in patients treated with docetaxel followed by cisplatin (Schellens et al., 1994). While the mechanism for this interaction has not been fully understood, it is assumed that taxanes show a reduced activity when administered before Pt compounds. Thus, in most phase II/III clinical studies, the sequence of Pt agent followed by a taxane is more commonly used.

PROGRESS OF NEOADJUVANT RESEARCH ON PT DERIVATIVES IN COMBINATION WITH TAXANE

Apart from increasing the breast conservation rate, NAC has demonstrated comparable benefits to adjuvant chemotherapy in terms of disease-free survival (DFS) and OS (Niu et al., 2014). Of note, complete response to NAC foretells a good prognosis (Liedtke et al., 2008). Neoadjuvant therapy might have greater clinical importance for patients with TNBC than the other types of BCs (Cortazar et al., 2014). Following NAC, a higher pCR (30–40%) is observed in TNBC than other BC subtypes (Cortazar et al., 2014). Achieving pCR had the strongest prognostic effect in patients with TNBC as it yielded better event-free survival (EFS: HR = 0.24, 95% CI 0.18–0.33) and OS (HR = 0.16, 0.11–0.25) in CTNeoBC pooled analysis compared to those without pCR. Conversely, those patients who failed to achieve pCR were at a higher risk of relapse (Kelland, 2007; Cortazar et al., 2014; Tutt et al., 2018; Dieci et al., 2019).

For many years, a taxane-anthracycline-based regimen has been incorporated as a standard for NAC regimen in treating BC. The earliest study, published in the year 2003 is the Trial B-27, drawing attention to the four cycles of sequential preoperative docetaxel to cyclophosphamide plus doxorubicin (AC) chemotherapy that provided a significantly superior outcome compared to the four cycles of AC alone (Bear et al., 2003). However, there were some flaws reported with this study design as the preoperative regimens were of different durations (4 vs. 8 cycles). Hence suggesting that the favorable results in the AC/docetaxel arm might be due to the delivery of additional cycles of chemotherapy rather than the addition of taxane. To clarify this ambiguity, 162 patients with locally advanced BC were treated with four cycles of neoadjuvant cyclophosphamide/vincristine/doxorubicin/prednisolone (CVAP). This was followed by randomization of those patients who attained complete or partial response to four additional cycles of docetaxel (100 mg/m^2) or CVAP. Patients who completed eight cycles of chemotherapy in totality, showed a higher clinical and pathological objective response rate (ORR) with docetaxel. Moreover, patients who did not respond to four cycles CVAP, docetaxel elicited a clinical response of 67% and a pathological response of 44% (including a pCR of 15%) (Smith et al., 2002).

The addition of neoadjuvant carboplatin to taxane-anthracycline-based chemotherapy has shown potential efficacy in several recent studies conducted in TNBC patients (**Table 1**). In BrighTNess (Loibl et al., 2018) Trial, a phase III randomized trial, patients were given 12 doses of paclitaxel weekly plus for four cycles of carboplatin every 3 weeks plus veliparib two times a day compared to those receiving paclitaxel plus carboplatin plus veliparib placebo. The pCR achieved was quite similar in both the arms (53% in veliparib containing arm vs. 58% in veliparib placebo arm, $p = 0.36$). However, the advantage of carboplatin and paclitaxel combination over paclitaxel alone was significantly highlighted (pCR: 58% in paclitaxel plus carboplatin arm vs. 31% in paclitaxel only arm, respectively, $p < 0.0001$) in the neoadjuvant therapy of TNBC. All patients received cyclophosphamide followed by doxorubicin as the standard part of the treatment (Loibl et al., 2018).

In GeparSixto (von Minckwitz et al., 2014) randomized phase II trial, patients with TNBC received four cycles of paclitaxel 80 mg/m^2 once a week and non-pegylated liposomal doxorubicin 20 mg/m^2 once a week simultaneously with bevacizumab 15 mg/kg intravenously every 3 weeks. Patients were further allocated (1:1) to receive either carboplatin once a week or no

carboplatin depending on their biological subtype and Ki-67 levels. This study showed that patients with additional carboplatin achieved a pCR of 53.2% which was higher than the pCR of 36.95% in the arm without carboplatin ($p = 0.005$). (von Minckwitz et al., 2014).

CALGB 40603 (Alliance), a 2 × 2 factorial, randomized phase II trial assessed the addition of carboplatin and/or bevacizumab to neoadjuvant paclitaxel once-a-week ensuing dose-dense doxorubicin and cyclophosphamide on pCR rates in stage II-III TNBC patients (Sikov et al., 2015). Earlier, CALGB 40603 showed that the addition of carboplatin to NAC significantly improved pCR (54 vs. 41%; $p = 0.0029$) in the breast/axilla compared to bevacizumab (Sikov et al., 2015; Sikov et al., 2019). However, long-term results showed no improvement in 5-years EFS with either carboplatin (HR = 0.99, 0.70–1.40) or bevacizumab (HR = 0.91, 0.64–1.29). The administration of ≥11 doses of weekly paclitaxel was linked to a better EFS (HR = 1.92, 1.33–2.77) in the exploratory analysis which was more pronounced in carboplatin-treated arms (Sikov et al., 2019).

Adding platinum to the taxane-anthracycline-based regimen can increase the AE incidence rate (Zheng et al., 2015), thus many current studies are excluding anthracyclines from NAC regimen. In a combined analysis of two cohorts by Sharma et al.,[49]carboplatin with docetaxel in NAC regimen yielded pCR in patients with *BRCA*-associated and wild-type TNBC as high as 59% (95% CI: 40–78%) and 56% (95% CI: 48–64%) respectively ($p = 0.83$) (Sharma et al., 2017). They also conducted a survival study (Sharma et al., 2018) presenting RFS and OS according to the degree of pathological response. It was seen that a pCR of 55% (100/183; 95% CI, 48–62) was obtained which is analogous to the pCR achieved when carboplatin is added to anthracycline-taxane chemotherapy. Patients with pCR had a 3-years RFS of 90% compared to the 66% in those who failed to attain pCR (HR = 0.30; 95% CI: 0.14–0.62, $p = 0.0001$). Also, the 3-years OS of 94% was noted in those with pCR while 79% in patients without pCR (HR = 0.25; 95% CI: 0.10–0.63, $p = 0.001$) (Sharma et al., 2018). Noteworthily, the pCR results observed in the aforementioned studies are remarkably higher than the traditional neoadjuvant anthracycline-taxane combinations, where at most 28–40% of TNBC patients achieve pCR (Loibl et al., 2018; von Minckwitz et al., 2014; Sharma et al., 2017; Sharma et al., 2018; Arun et al., 2011).

NeoSTOP (Neoadjuvant Study of Two Platinum Regimens in Stage I-III Triple Negative Breast Cancer) trial was aimed at assessing the efficacy of anthracycline-free and anthracycline-containing neoadjuvant carboplatin regimens in two different centers. This randomized phase II trial showed that the pCR rates (54%) were similar in both the arms, however, grade 3/4 AEs were more common in paclitaxel plus carboplatin followed by doxorubicin plus cyclophosphamide arm when compared to the carboplatin plus docetaxel only arm (73 vs. 21%, $p < 0.0001$) (Sharma et al., 2021). The NeoCART trial was a phase II, randomized, multicenter study devised to evaluate the efficacy of neoadjuvant docetaxel plus carboplatin versus epirubicin plus cyclophosphamide followed by docetaxel in early-stage TNBC patients (Zhang et al., 2020). This study reported a higher pCR in docetaxel plus carboplatin group than the standard NAC group (61.4% vs. 38·6%, OR = 2.52, 95% CI 2.4–43.1; $p = 0.033$). Noteworthily, more significantly higher pCR rates were observed in earlier disease stages and negative lymph node patients (Zhang et al., 2020). A phase II randomized study by Wu et al. also showed a pCR rate of 38.7% in the arm containing lobaplatin with epirubicin and docetaxel combination as NAC regimen compared to the pCR rate of 12.7% (odds ratio (OR) = 4.342, 95% CI 1.764–10.687, $p = 0.001$) in patients who were not given lobaplatin (Wu et al., 2018).

Several studies have explored the association between platinum and *BRCA* mutant subtype prognosis. According to the WSG-ADAPT TN randomized phase II trial, neoadjuvant nab-paclitaxel plus carboplatin indicated an excellent pCR rate of 64% in *BRCA1/2*-mutated cases versus 34.5% in all others mutations (OR = 3.41, 95% CI: 1.11–10.50; $p = 0.03$) supporting the de-escalation strategy in *BRCA1/2* mutations in early TNBC patients (Richters et al., 2021). A study conducted in a neoadjuvant setting with small sample size, involving 12 *BRCA1* mutation carriers, four cycles of chemotherapy with single-agent cisplatin at 75 mg/m^2 every 21 days yielded a pCR rate of 80% (Byrski et al., 2010). When the same regimen was studied as NAC in 28 patients with TNBC (including 12 *BRCA1* mutation carriers), a pCR rate of 22% was reported (Silver et al., 2010). These two small phase II clinical trials suggest that triple-negative, particularly *BRCA1*-mutant tumors, are more susceptible to DNA-damaging agents such as cisplatin. A retrospective evaluation of 12 patients with BC and *BRCA1* gene mutations also revealed that Pt-based NAC was highly effective in BC patients with *BRCA1* gene mutations (Sæther et al., 2018). Additionally, NeoSTOP trial showed a higher pCR (76 vs. 49%, OR = 3.35, 95% CI: 0.99–11.37; $p = 0.052$) in patients with *BRCA1/2* mutation than the non-*BRCA1/2* mutated patients (Sharma et al., 2021). However, in the secondary analysis of the GeparSixto randomized clinical trial, it was found that patients without *BRCA1/2* mutation showed a higher pCR with the carboplatin (55 vs. 37%, OR = 2.14; 95% CI, 1.28–3.58; $p = 0.004$) compared to the non-carboplatin arm, whereas those with *BRCA1/2* mutation did not significantly improve the pCR with the addition of carboplatin (Hahnen et al., 2017). The secondary analysis of the randomized phase III BrighTNess trial, concurred the benefit of carboplatin across all molecular subtypes of TNBC (Filho et al., 2021). Thus, neoadjuvant therapy improves the pCR rates in patients with TNBC, consequently enhancing the survival benefits and the quality of life (Cortazar et al., 2014).

Clinical Guidelines and consensus conferences provide differing viewpoint regarding the use of Pt- and taxane-based regimens for TNBC. The National Comprehensive Cancer Network (NCCN) Breast Cancer Guidelines (National Cancer Comprehen, 2021), version 5.2021 does not recommend the routine usage of Pt agents as part of NAC in TNBC for a majority of the patients (including *BRCA* mutation carriers). while the adjuvant treatment with Pt agents is discouraged. Also, it is suggested that there is a paucity of data regarding the optimum combination of taxanes and/or ideal chemotherapy regimen in situations where a Pt agent has to be included in an anthracycline-based regimen. However, the guideline suggests the utility of Pt agents in NAC setting only in specific scenarios where local control is imperative.

The European Society of Medical Oncology (ESMO) 2019 clinical practice guidelines for early breast cancer recommend a sequential anthracycline/taxane-based regimen as the standard for the majority of patients. In selected lower-risk patients, four cycles of anthracycline- or taxane-based chemotherapy or cyclophosphamide/methotrexate/5-fluorouracil (CMF) may be used. The addition of a Pt compound may be considered in triple-negative tumors and/or in patients with deleterious *BRCA1/2* mutations (Cardoso et al., 2019).

The panelists at St. Gallen International Consensus Conference for the primary therapy of early breast cancer 2019 recommended against the routine inclusion of Pt-based chemotherapy in women already slated to receive alkylator-, taxane-, and anthracycline-based regimens. However, they favored the inclusion of Pt-based chemotherapy among women with known, deleterious germline *BRCA1/2* mutations, though the evidence supporting this is inadequate. Further, they endorsed dose-dense (accelerated schedules of anthracycline- and alkylator-based therapy, followed sequentially by dose-dense or weekly taxane) treatment as a preferred approach for anthracycline- and taxane-based NAC and adjuvant chemotherapy regimens (Burstein et al., 2019).

The latest guidelines from the American Society of Clinical Oncology (ASCO) suggests that TNBC patients with node-positive and/or at least T1c disease should be given an anthracycline- and taxane-containing regimen while those with either cT1a or cT1bN0 TNBC must avoid NAC. Carboplatin can be added to the NAC regimen for treating TNBC patients to increase the likelihood of pCR. In premenopausal women with hormone-receptor-positive/HER2-negative early-stage BC, endocrine therapy in neoadjuvant setting should be avoided with exception to clinical trials (Korde et al., 2021).

PROGRESS OF ADJUVANT RESEARCH ON PT DERIVATIVES IN COMBINATION WITH TAXANES

TNBC is associated with a higher risk of recurrence within 3 years, increased risk of distant metastases and brain metastases with rapid progression from distant recurrence to death, as well as absence of known therapeutic targets. It is therefore crucial to optimize the early-stage chemotherapy for such patients (Dent et al., 2007). In daily practice, usual chemotherapy for adjuvant treatment of TNBC includes anthracycline, and taxane-containing regimens, whereas the dose-dense chemotherapy approach is still debated (Joensuu and Gligorov, 2012). The efficacy of Pt added to taxane in the adjuvant setting is still being explored, nevertheless, few recent studies in this therapeutic area are summarized in **Table 2**.

In a single-arm study, a high pCR rate (65%) was seen in patients with TNBC (n = 74) treated with cisplatin 30 mg/m^2, epirubicin 50 mg/m^2, and paclitaxel 120 mg/m^2 weekly for 8 weeks with granulocyte colony-stimulating factor (G-CSF) on days 3–5. Patients who attained a pCR had a 3-years DFS rate of 97% and a 5-year DFS rate 90% (Frasci et al., 2009).

In a phase II trial, a subset of randomly assigned chemotherapy-naïve patients with TNBC after surgery received six cycles of taxane and platinum (TP) regimen (docetaxel: 75 mg/m^2 or paclitaxel 175 mg/m^2; carboplatin AUC = 5, day 1) or epirubicin, cyclophosphamide, and taxane (EC-T) regimen (4 cycles of epirubicin: 90 mg/m^2; cyclophosphamide: 600 mg/m^2, day 1 accompanied with four cycles of docetaxel 75 mg/m^2 or paclitaxel 175 mg/m^2, day 1) (Du et al., 2020). Both regimens were repeated every 3 weeks. The above study indicated non-inferiority of carboplatin plus taxanes to epirubicin plus cyclophosphamide followed by taxanes (TP vs. EC-T, 5-year DFS = 84.4 vs. 85.8%; absolute difference: 1.4%, 95% CI −5.3–8.1%; $p = 0.034$) as adjuvant chemotherapy for early TNBC (Du et al., 2020).

In 2020, PATTERN (Yu et al., 2020) study reported that when six cycles of paclitaxel with carboplatin were compared with a standard-dose regimen of three cycles of cyclophosphamide, epirubicin, and fluorouracil followed by three cycles of docetaxel (CEF-T), the DFS after a follow-up of 62 months was found to be higher (5-year DFS, 86.5 vs. 80.3%, hazard ratio [HR] = 0.65; 95% CI, 0.44–0.96; $p = 0.03$) in the paclitaxel with carboplatin group versus CEF-T group.

FUTURE PERSPECTIVES AND IMPLICATIONS

In light of the current advancement towards treating TNBC, the combination of Pt to neoadjuvant taxane-based chemotherapy results in favorable outcomes, with a majority of the studies pointing toward a higher pCR. Evidence favor carboplatin with docetaxel in the NAC regimen, which yields an excellent pCR in patients with *BRCA*-associated and wild-type TNBC (Sharma et al., 2017).

Historically, *BRCA1/2* mutations were regarded as an accessible biomarker for predicting longer PFS and clinical outcomes with carboplatin in comparison with docetaxel under adjuvant settings (Tutt et al., 2018). However, results from the randomized, phase III, TNT trial revealed that one-third of the TNBC patients with *BRCA1/2* mutation were non-responders to the Pt therapy. This may arise due to the homologous recombinant repair (*HRR*) gene defect that is retained in *BRCA1/2* mutation carriers that forms a hard epigenetic *BRCA*ness (Tutt et al., 2018).

GeparSixto (von Minckwitz et al., 2014) and BrighTNess (Loibl et al., 2018) trials reported similar pCR benefits in *BRCA* mutated cohorts in patients receiving Pt-based chemotherapy compared to the non Pt containing arms. The secondary analysis of the BrighTNess phase III randomized clinical trial also showed that the addition of carboplatin to standard NAC can yield pCR benefits across all the molecular subtypes (Filho et al., 2021). Compared to the other BC subtypes, 11–31% of women with TNBC are found to have germline *BRCA* mutations (Cocco et al., 2020). Although these studies show that the Pt agents under neoadjuvant setting may significantly improve the pCR in TNBC patients regardless of the gBRCA1/2 mutation status, their effectiveness remains debatable to date. This critique can be attributed to the fact that the supporting evidence was derived from a post hoc exploratory analysis with a small number of *BRCA*-mutated patients. Moreover, no clear recommendations are provided by

TABLE 3 | Summary of ongoing and recently reported clinical trials with Pt derivatives and taxane combinations in triple-negative breast cancer.

phase	NCT	Study population	Setting	Stage	Experimental arm	Control arm	Primary endpoint
II	NCT01042379	TNBC	Neoadjuvant	II–III	Veli + Cb → standard NACT	Standard NACT	pCR
III	NCT02032277 (BrighTNess)	TNBC	Neoadjuvant	II–III	Veli + Cb + P → AC	Placebo + Cb + P → AC	pCR
II/III	NCT03150576 (PARTNER)	TNBC and/or gBRCA mutated BC	Neoadjuvant	II–III	Ola + Cb + P → AC/EC	P + Cb → AC/EC	Safety, pCR
II	NCT03639948 (NeoPACT)	TNBC	Neoadjuvant	I–III	Cb + T + pembro	NA	pCR
III	NCT02620280 (NeoTRIPaPDL1)	High-risk TNBC	Neoadjuvant	II–III	Cb + nab-P + atezo → AC/EC/FEC	Cb + nab-P → AC/EC/FEC	EFS
III	NCT03036488 (Keynote-522)	TNBC	Neoadjuvant	II–III	Cb + P + pembro → AC/EC + pembro	Cb + P + placebo → AC/EC + placebo	pCR, EFS
III	NCT03281954 (NSABP B-59)	TNBC	Neoadjuvant	II–III	P + Cb + atezo → atezo + AC/EC	P + Cb + placebo → placebo + AC/EC	pCR, EFS
II	NCT03872505 (PANDoRA)	TNBC	Neoadjuvant	II–III	Durva + Cb + P + radiation	Durva + Cb + P	pCR
II	NCT03650738	TNBC	Neoadjuvant	II–III	Apatinib + nab-P + Cb	NA	pCR, safety
II	NCT03193853	TNBC	Neoadjuvant	IV	Tak-228 + Tak-117 → cis + nab-P	NA	ORR
II/III	NCT02221999	TNBC and Hormone-receptor-positive	Neoadjuvant	III-IV	P + cis + leuprolide/goserelin versus P + cis + letrozole	P + cis	pCR
II	NCT04537286	TNBC	Neoadjuvant	IV	Nab-P + cis + carilizumab	NA	PFS, safety
II	NCT04159142	TNBC	Neoadjuvant	III	Nab-P + Cb	Nab-P + capecitabine	PFS
II	NCT03121352	TNBC	Neoadjuvant	IV	Cb + nab-P + pembro	NA	ORR
II	NCT04083963 (BRE-01)	TNBC	Neoadjuvant	I-IV	P + Cb → AC/EC	NA	pCR
II	NCT02876107	TNBC	Neoadjuvant	I-III	P + Cb + panitumumab	P + Cb	pCR
II	NCT02124902	TNBC	Neoadjuvant	II–III	T + Cb	T + Cb	pCR
IV	NCT04136782	TNBC	Neoadjuvant	II-III	Nab-P + Cb	E + T	pCR
II	NCT02547987 (CADENCE)	TNBC	Neoadjuvant	II-III	T + Cb	NA	pCR
II	NCT01525966	TNBC	Adjuvant	II–III	Cb and nab-P	NA	pCR
III	NCT02455141 (TCTN)	TNBC	Adjuvant		EC → P or T	EC → P or T + Cb	DFS
III	NCT03876886	TNBC	Adjuvant	II-III	AC + P	P + Cb	DFS
III	NCT02441933 (PEARLY Trial)	TNBC	Adjuvant/ Neoadjuvant	II-III	AC → P or T + Cb	AC→ P or T	DFS

A, doxorubicin; Atezo, atezolizumab; C, cyclophosphamide; Cb, carboplatin; Cis, cisplatin; DFS, Disease-free survival; Durva, durvalumab; EFS, event-free survival; E, epirubicin; F,5-fluorouracil; NA, not available' nab-P, albumin paclitaxel (weekly cycle if not specially noted); ORR, objective response rate; Ola, olaparib, P, paclitaxel (weekly cycle if not specially noted), PCR, pathological complete response, Pembro, pembrolizumab; RFS, Relapse-free survival; T, docetaxel; TNBC, triple negative breast cancer; Veli, veliparib.

current guidelines on the usage of Pt in neoadjuvant settings for TNBC.

The poly ADP-ribose polymerase inhibitors have also shown huge potential with promising clinical efficacy and lower toxicity profiles when given in monotherapy in TNBC with *BRCA1/2* mutations (Guney Eskiler et al., 2018). These effects are due to HRR deficiency that results in faulty DNA repair mechanisms. In phase II BROCADE (Han et al., 2018) trial, there was a statistically significant increase in ORR from 61.3 to 77.8% when veliparib was added to carboplatin with paclitaxel regimen versus carboplatin with paclitaxel alone. An increase in median PFS (14.1 vs. 12.3 months, HR = 0.789; 95% CI 0.536–1.162; $p = 0.227$) and OS (28.3 and 25.9 months, HR = 0.750; 95% CI 0.503–1.117; $p = 0.156$) was also observed when veliparib was added to the Pt-based taxane chemotherapy in metastatic BC. (Han et al., 2018)

On the other hand, biomarkers such as tumor-infiltrating lymphocytes (TILs) in TNBC are linked to a higher mutation rate and pCR with NAC and an improved survival outcome with adjuvant therapy (de Boo et al., 2020). Another common biomarker that is expressed in 20% of TNBC is PD-L1 (Cocco et al., 2020). Tumor mutational burden (TMB) being a good marker of tumor antigenicity has a high prevalence in TNBC. Moreover, PI3K, AKT, and mammalian target of rapamycin (mTOR) pathway alterations also occur in approximately 35% of TNBC (Cocco et al., 2020).

Although conducting studies in the adjuvant setting with Pt-based treatment is fairly feasible, the complexity in obtaining enriched tumor samples for research purposes is an enormous challenge (Agrawal and Mayer, 2014). Pt resistance due to prior exposure to Pt agents in preoperative settings can lead to increased toxicity during adjuvant therapy and is possibly the cause of inadequate and sparse studies reinforcing Pt-based adjuvant therapy (Agrawal and Mayer, 2014). In addition, a relatively higher survival benefit that is offered by the conventional adjuvant therapies might be associated with the modicum of studies evaluating Pt- and taxane-based combination therapy in the adjuvant setting. Also, the utility of Pt-based adjuvant therapy is highly controversial as there are insufficient trials assessing the DFS and the OS in such regimens. A meta-analysis revealed higher DFS

(HR = 0.73, 95% CI 0.59–0.91, $p = 0.005$) as well as OS improvement (HR = 0.69, 95% CI 0.56–0.85) from adjuvant addition of capecitabine to four cycles of epirubicin and cyclophosphamide. in patients with early TNBC, treated with neoadjuvant carboplatin and docetaxel chemotherapy and without pCR data (Li et al., 2020). The accumulating toxicities of Pt agents can present barriers for the long-term use of these agents. Previous *in vivo* studies have also suggested that cells resistant to platinum often become sensitive to taxanes and vice-versa. As a result, combination of these two drugs is feasible for treatment of early TNBC. A phase II safety and efficacy study in preoperative weekly cisplatin-epirubicin-paclitaxel support in operable TNBC showed high 3- and 5-year DFS rates of 97 and 90% respectively, in contrast to the DFS rates of 61 and 56%, respectively, in those with residual disease after NAC. The numbers of patients with T2 and T3 tumors were same, however the pCR rate was significantly higher in the T2 tumors (74 versus 51%) (Frasci et al., 2009). Thus, the findings from the majority of the available studies suggest that the regimens containing both taxane plus Pt agents might be an effective alternative in adjuvant settings for patients with operable TNBC (Foulkes et al., 2010; Liedtke and Rody, 2017; Yu et al., 2020).

Many studies are underway that are directed towards evaluating the advantages of adding Pt to various adjuvant and NAC regimen for treating TNBC (**Table 3**). CALGB40603 (Sikov et al., 2015) is one such ongoing trial that is set to assess the long-term benefits of adding weekly paclitaxel to carboplatin in neoadjuvant setting. So far, the results are promising with a high pCR, but the survival outcomes such as RFS and OS are awaited (Sikov et al., 2015).

CONCLUSION

The use of Pt- and taxane-based chemotherapy in the neoadjuvant and adjuvant setting has tremendous potential to improve survival of patients with early TNBC by achieving a high pCR. TNBC in general provides a therapeutic challenge that can be tackled by identifying new therapeutic sub-targets and a specific subgroup that can benefit from a Pt- and taxane-based chemotherapy. Results from ongoing trials are expected to further validate the clinical benefits of this combination, especially in patients with early-stage or operable TNBC.

AUTHOR CONTRIBUTIONS

HT, YaZ, and DM performed the literature search and wrote the paper. XT, WY, and XW contributed to table and figure preparation. CH, BY, and LZ contributed to data extraction from all the studies. XQ and Yi Z contributed to the conception and design of the study and the revision of the manuscript. HT and DM contributed equally to this work.

FUNDING

This work was supported by the Project of National Key Clinical Specialty Construction (413F1Z113), Chongqing Basic Research and Frontier Exploration Project (No. cstc2018jcyjA0137) and Military Medical Staff Innovation Plan of Southwest Hospital (No. SWH2018BJLC-04) as well as Army Medical University (No. XZ-2019-505-042), and Sanofi China funding.

ACKNOWLEDGMENTS

The authors are very grateful to Mohammed Osama Akhtar and Anwesha Mandal of Indegene Lifesystems Pvt. Ltd. for providing assistance with the writing and reviewing of this original manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.770663/full#supplementary-material

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Conflict of Interest: YZ and BY are employed by Sanofi.

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GLOSSARY

AUC area under curve
BC breast cancer
CI confidence-interval
CVAP cyclophosphamide/vincristine/doxorubicin/prednisolone
CYP cytochrome P-450
DDFS distant disease-free survival
DFS disease-free survival
EFS event-free survival
ER estrogen receptor
G-CSF granulocyte colony-stimulating factor
HER2 human epidermal growth factor receptor-2
HRR homologous recombinant repair
mTOR mammalian target of rapamycin
mDFS median disease-free survival
mOS median overall survival
miRNAs MicroRNAs
mRNAs messenger RNAs
MRP-1 multidrug resistance protein-1
MDR multidrug resistance
NAC neoadjuvant chemotherapy
OR odds ratio
ORR objective response rates
OS overall survival
PFS progression-free survival
Pt platinum
pCR pathological complete response
PR progesterone receptor
RFS relapse-free survival
5-FU 5-fluorouracil
TNBC triple-negative breast cancer
TILs tumor-infiltrating lymphocytes
TMB tumor mutational burden

frontiers in Oncology

SYSTEMATIC REVIEW
published: 24 January 2022
doi: 10.3389/fonc.2021.818839

Efficacy and Safety of Thalidomide As a Pre-Medication of Chemotherapy-Induced Nausea and Vomiting (CINV) Following Highly Emetogenic Chemotherapy (HEC): A Systematic Review and Meta-Analysis

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Background: In China, thalidomide (THD) has been used to prevent chemotherapy-induced nausea and vomiting (CINV) following highly emetogenic chemotherapy (HEC); however, there is limited evidence on the efficacy and safety of THD in this setting. The aim of this study was to evaluate the efficacy, safety, and impact on quality of life (QoL) of THD on CINV following HEC.

Methods: Electronic databases were systematically searched for all randomized controlled trials (RCTs) in HEC using THD. The primary outcomes were complete response (CR) and no nausea, Secondary outcomes were the incidence of adverse events and QoL related indicators. We calculated risk ratios (RRs) and 95% confidence intervals (CIs) using a fixed-effects model. In the case of heterogeneity ($I^2 \geq 50\%$), a random-effects model was performed.

Results: A total of 3168 patients were included from 34 RCTs. In terms of CR rate, THD plus 5-HT_3 receptor antagonist (5-HT_3RA) with or without dexamethasone (DEX) was significantly higher than 5-HT3RA with or without DEX in the acute phase (74.4% vs 67.4%; RR 1.10), delayed phase (70.6% vs 50.4%; RR 1.53), and overall phase (68.4% vs 53.4%; RR 1.28). In terms of no nausea rate, the THD group was also significantly higher than the control group in the acute phase (61.7% vs 55.5%; RR 1.12), delayed phase (50.5% vs 30.0%; RR 1.69), and overall phase (44.6% vs 29.9%; RR 1.50). There was no statistical difference in the incidence of fatigue, headache, diarrhea, rash, hepatorenal

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: *20 November 2021*
Accepted: *29 December 2021*
Published: *24 January 2022*

Citation:
Xie J, Zhang C, Li S, Dai R, Sullivan MA, Deng B, Xu Q, Wang J, Shi C and Zhang Y (2022) Efficacy and Safety of Thalidomide As a Pre-Medication of Chemotherapy-Induced Nausea and Vomiting (CINV) Following Highly Emetogenic Chemotherapy (HEC): A Systematic Review and Meta-Analysis. Front. Oncol. 11:818839. doi: 10.3389/fonc.2021.818839

damage, and myelosuppression between those with and without THD. The incidence of increase in KPS scores, weight gain, appetite improvement, and sleep quality improvement were significantly higher with the addition of THD.

Conclusions: THD may be effective and safe for the prevention of CINV patients treated with HEC and may improve QoL.

Keywords: chemotherapy-induced nausea and vomiting, thalidomide, safety, efficacy, highly emetogenic chemotherapy

1 INTRODUCTION

Chemotherapy-induced nausea and vomiting (CINV) is one of the most common disturbing adverse effects of anticancer chemotherapy, which can significantly impair the patient's quality of life (QoL), adherence with future therapy, and nutritional status. American Society of Clinical Oncology (ASCO) guideline (2020) (1) classify chemotherapeutic agents according to their emetogenic potential (high, medium, low and minimal) and make recommendations based on their level of risk. For patients receiving highly emetogenic chemotherapy (HEC; CINV risk>90%), such as cisplatin- and anthracycline/cyclophosphamide (AC)-based regimens, National Comprehensive Cancer Network (NCCN) antiemesis guideline recommend a four-drug combination of a 5-HT_3 receptor antagonist (5-HT_3RA), a neurokinin-1 (NK1) RA, dexamethasone (DEX), and olanzapine (2). Even if CINV prevention is now dramatically improved, there is still a need to find more effective, safer and more economical drug regimens for better prevention because CINV remains a frequent and feared adverse effect.

The unintended teratogenic effect of thalidomide (THD), prescribed to treat morning sickness in pregnant women, is a historic tragedy, however with the approval of this drug for indications such as multiple myeloma. A randomized controlled double-blind phase III clinical study (3) in the Chinese population suggested that THD combined with palonosetron and DEX is efficacious and well-tolerated for the prevention of delayed CINV in anticancer chemotherapy-naive patients who undergo HEC. Rates of complete response and no nausea in the delayed phase were higher and adverse effects were mild to moderate in the THD group. Since pregnancy and childbirth are nearly impossible during anticancer chemotherapy in patients with malignant tumors, and THD prices are relatively low in China, there is some potential for THD to be useful in the management of CINV.

In China, there have been many controlled clinical trials using THD, in addition with antiemetic regimens, with results showing that THD can be used as a complementary and alternative medicine to prevent CINV following HEC. However, there is no systematic review or meta-analysis of its efficacy in the prevention of CINV, the incidence of adverse effects, and the improvement of QoL under HEC. Therefore, all controlled clinical trials using THD under HEC were systematically evaluated for efficacy in the prevention of CINV through multiple studies and large sample size.

2 METHODS

The meta-analysis was pre-registered at PROSPERO (CRD42020158732).

2.1 Literature Search

This systematic review and meta-analysis was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (4). Relevant publications were searched in the Chinese National Knowledge Infrastructure (CNKI), the VIP Information Database, Wanfang Database, PubMed, EMBASE, and the Cochrane Library. The systematic review was performed in December 2019 and updated in August 2020.

The keywords for searching included: "chemotherapy-induced nausea and vomiting", "CINV", "vomit", "emesis", "thalidomide", "highly emetogenic chemotherapy", "CDDP", "cisplatin", or "anthracycline and cyclophosphamide". References of the selected articles were also checked to identify further eligible trials.

2.2 Study Selection Criteria

Selecting studies that met the inclusion and exclusion criteria was independently performed by two authors(JX, CZ). Any disagreement between reviewers was resolved through public discussions until a consensus was reached.

Inclusion criteria: (a) randomized controlled trials (RCTs) in patients who received HEC (such as cisplatin-based treatment or AC regimen); (b) studies that reported either THD as an add-on treatment (5-HT_2RA, with or without DEX) or THD monotherapy compared to standard treatment.

Exclusion criteria: (a) review articles or studies involving non-human subjects; (b) duplicate published articles; (c) studies where anticancer chemotherapy regimens and basic antiemetic regimens were inconsistent between experimental and control groups; (d) studies with a high risk of bias.

2.3 Outcomes

The primary outcomes: Complete response (CR) and no nausea. CR is defined as having no emetic episode and requiring no use of rescue medication. Nausea was categorized by using a 4-point Likert scale (0, no symptoms; 3, severe). CR and no nausea were measured in the acute phase (0-24 h), the delayed phase (24-120 h), and the overall phase (0-120 h). Secondary outcomes included the adverse events which was graded according to the common terminology criteria for adverse events (CTCAE) (5) and indicators

related to QoL: Karnofsky performance scale (KPS) scores, weight, appetite, and sleep quality.

2.4 Quality Assessment

The quality of the included studies was assessed independently by two authors(SL, RD) based on the Cochrane Handbook for Systematic Review of Interventions (6). The Cochrane Collaboration's tool for assessing the risk of bias for RCTs includes the following seven items: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessments (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), and other sources of bias. Each item was described as high risk of bias, low risk of bias, or unclear risk of bias. Disagreements were discussed and resolved by consensus between both reviewers or *via* consultation with a third reviewer (JX).

2.5 Statistical Analysis

Results were quantitatively synthesized by means of meta-analysis using the Review Manager (version 5.3; Cochrane Collaboration, Oxford, England). The Mantel-Haenszel method was used to estimate the pooled risk ratio (RR) for each dichotomous variable. I^2 was used to evaluate heterogeneity across studies. When heterogeneity ($I^2 \geq 50\%$) was detected, random-effects meta-analyses were performed. $I^2 < 50\%$, a fixed effect statistical model was used. Results obtained from the analyses were displayed by generating a forest plot. A p-value of < 0.05 was considered statistically significant.

3 RESULTS

3.1 Study Selection and Trial Characteristics

There were 898 records identified *via* database searching. 537 of the records were searched in PubMed, EMBASE, and the Cochrane library, 361 of the records were searched in the CNKI, VIP Information Database, and Wanfang Database (**Figure 1**).

After removing the duplicates, there were 462 results. The titles and abstracts of 462 studies were screened, and the full text of 61 articles was reviewed. 27 studies were excluded for the following reasons: not a RCT study (n = 10), not HEC (n = 11), a different outcome (n = 5), and same data source (n = 1). Finally, 34 studies were assessed for eligibility and included in the quantitative synthesis. A total of 3168 patients were included. The characteristics of the included studies are shown in **Table 1**. All studies were RCTs. Patients' tumor types include breast, gastric, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), cervical, and others. All patients in these studies received HEC.

The included studies contained a total of 8 outcomes: CR (acute phase) (3, 9, 10, 14–17); CR (delayed phase) (3, 7, 9–12, 14, 16, 17); CR (overall phase) (3, 8, 9, 13); no nausea (acute phase) (3, 9, 10, 14, 16–18); no nausea (delayed phase) (3, 9, 10, 14, 16–18); no nausea (overall phase) (3, 9, 18); adverse events (3, 7, 9–19, 21–26, 28–33, 37–39); QoL (12, 13, 17, 20–23, 26, 27, 34–36).

3.2 Risk of Bias and Quality Assessment

All of the included studies had a low risk of attrition bias and reporting bias. Only one study (25) had a high risk of performance bias and detection bias due to its single-blind method. Two of the included studies (28) and (39) had a high risk of other bias due to a possible conflict of interest or small sample size (**Figure 2**).

3.3 Primary Outcomes

3.3.1 CR in the Acute Phase

Data of CR in the acute phase were available in 7 studies, including 1071 patients: 531 patients in the experimental group were treated with THD added to the 5-HT_3RA-based conventional antiemetic regimen, and 540 patients in the control group were treated with the 5-HT_3RA-based conventional antiemetic regimen. The CR rate was significantly higher with the addition of THD in the acute phase: 74.4% vs 67.4% (RR 1.10, 95%CI 1.03-1.18, p=0.008), without significant heterogeneity among studies (I^2=19%) (**Figure 3**).

3.3.2 CR in the Delayed Phase

Data of CR in the delayed phase were available in 9 studies, including 1270 patients: 633 patients in the experimental group and 637 patients in the control group. The CR rate was significantly higher with the addition of THD in the delayed phase: 70.6% vs 50.4% (RR 1.53, 95%CI 1.28-1.82, p<0.00001), with significant heterogeneity among studies (I^2=54%). Due to significant heterogeneity among the studies, a random-effects model was chosen for analysis (**Figure 4**).

3.3.3 CR in the Overall Phase

Data of CR in the overall phase were available in 4 studies, including 870 patients: 434 patients in the experimental group and 436 patients in the control group. The CR rate was significantly higher with the addition of THD in the overall phase: 68.4% vs 53.4% (RR 1.28, 95%CI 1.15-1.43, p<0.00001), without significant heterogeneity among studies (I^2=9%) (**Figure 5**).

3.3.4 No Nausea in the Acute Phase

Data of no nausea in the acute phase were available in 7 studies, including 1291 patients: 648 patients in the experimental group and 643 patients in the control group. The no nausea rate was significantly higher with the addition of THD in the acute phase: 61.7% vs 55.5% (RR 1.12, 95%CI 1.02-1.22, p=0.02), without significant heterogeneity among studies (I^2=0%) (**Figure 6**).

3.3.5 No Nausea in the Delayed Phase

Data of no nausea in the delayed phase were available in 7 studies, including 1291 patients: 648 patients in the experimental group and 643 patients in the control group. The no nausea rate was significantly higher with the addition of THD in the delayed phase: 50.5% vs 30.0% (RR 1.69, 95%CI

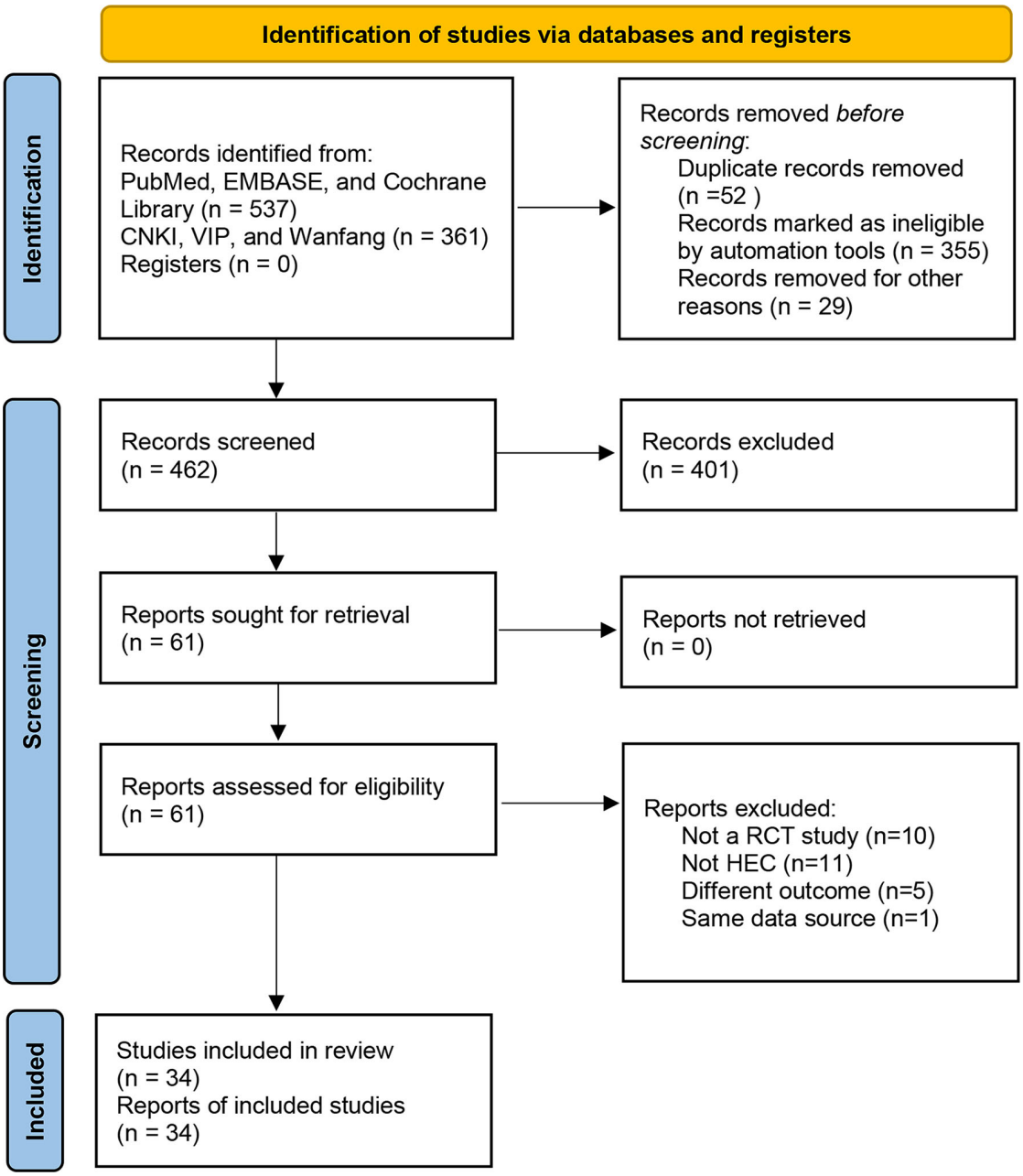

FIGURE 1 | Flow diagram.

1.47-1.94, p<0.00001), without significant heterogeneity among studies (I^2=42%) (**Figure 7**).

3.3.6 No Nausea in the Overall Phase

Data of no nausea in the overall phase were available in 3 studies, including 987 patients: 495 patients in the experimental group and 492 patients in the control group. The no nausea rate was significantly higher with the addition of THD in the overall phase: 44.6% vs 29.9% (RR 1.50, 95%CI 1.27-1.77, p<0.00001), without significant heterogeneity among studies (I^2=3%) (**Figure 8**).

3.4 Secondary Outcomes

3.4.1 Adverse Events

Data from 28 out of the 34 included articles involved safety studies of THD and 11 adverse events were included: fatigue (12 studies), constipation (26 studies), mucositis (7 studies), headache (5 studies), diarrhea (7 studies), rash (11 studies), peripheral neuropathy (9 studies), hepatorenal damage (13 studies), myelosuppression (7 studies), somnolence (13 studies), and anorexia (3 studies). There was no significant

TABLE 1 | Characteristics of studies included.

Study	T/C(n)	Cancer Types	Chemotherapy Regimens	Interventions	Control	Outcomes
Cheng et al. (7)	45/45	Cervical	CDDP	THD(D0-4:50mg qn)+TRO+DEX	TRO+DEX	(2)(7)
Wang (8)	40/40	Lung	CDDP-containing	THD(100mg qn)+PAL+DEX	PAL+DEX	(3)
Song et al. (9)	40/43	Gastric/Lung/Cervical/ Other	CDDP	THD(D1-5:100mg qd)+OND+MET+DEX	OND+MET+DEX	(1)(2)(3)(4)(5)(6)(7)
Zhang et al. (3)	317/321	Lung/Breast/Other	CDDP-containing/ AC	THD(D2-4:100mg bid)+PAL+DEX	PAL+DEX	(1)(2)(3)(4)(5)(6)(7)
Li et al. (10)	30/30	Lung	CDDP-containing	THD(D1-5:100mg qn)+OND+DEX	OND+DEX	(1)(2)(4)(5)(7)
Zhao et al. (11)	39/39	Unknown	CDDP-containing/ AC	THD(25mg bid)+TRO+DEX	TRO+DEX	(2)(7)
Han et al. (12)	40/38	Gastric/Lung/Ovarian	CDDP-containing	THD(D0:100mg qn,50mg was added per night up to 200 mg)+AZA	AZA	(2)(7)(8)
Han et al. (13)	38/32	Gastric/Lung/Ovarian	CDDP-containing	THD(D0:100mg qn,50mg was added per night up to 200 mg)+TRO	TRO	(3)(7)(8)
Zuo (14)	41/40	Breast	GP	THD(D1-8:25mg bid)+TRO	TRO	(1)(2)(4)(5)(7)
Cui et al. (15)	21/25	Breast	AC	THD(D1:25mg bid)+TRO	TRO	(1)(7)
Yu et al. (16)	30/31	NSCLC	GP	THD(D1-5:50mg bid)+RAM+MET	RAM+MET	(1)(2)(4)(5)(7)
Zhang et al. (17)	52/50	SCLC	CDDP-containing	THD(D1-7:100mg qn)+PAL+MP	TRO+MP	(1)(2)(4)(5)(7)(8)
Jiang (18)	138/128	Lung/Breast	CDDP-containing/ AC	THD(D1-5:100mg bid)+PAL+DEX	PAL+DEX	(4)(5)(6)(7)
Xing et al. (19)	38/38	Gastric	DP	THD(D1-7:100mg qd)	Placebo	(7)
Luo (20)	26/28	NSCLC	GP	THD(D1-7:100mg qd D8-42: 200mg qd)	Placebo	(8)
Niu et al. (21)	32/28	Gastric	TP	THD(D1-42: 100mg qn)	Placebo	(7)(8)
Peng et al. (22)	51/53	NSCLC	TP	THD(D1-7:100mg qn D8-90:200mg qn)+5-HT_2RA	5-HT_2RA	(7)(8)
Peng et al. (23)	30/31	NSCLC	TP	THD(D1-7:100mg qn D8-90:200mg qn)+5-HT_2RA	5-HT_2RA	(7)(8)
He et al. (24)	19/20	NSCLC	NP	THD(D1-7:100mg qn D8-14:150mg qn D15-90: 200mg qn)+GRA	GRA	(7)
Zhang (25)	48/48	NSCLC	TP	THD(D1-7:100mg qd D8-84:200mg qd)+5-HT_2RA	5-HT_2RA	(7)
Gu et al. (26)	33/33	NSCLC	NP	THD(200mg qd)	Placebo	(7)(8)
Huang (27)	36/30	NSCLC	GP	THD(D1-30:200mg qn)+5-HT_2RA	5-HT_2RA	(8)
Pujol et al. (28)	49/43	SCLC	PCDE	THD(D1-112: 400mg qd)	Placebo	(7)
Sun and Xu (29)	30/30	NSCLC	DP	THD(D1-7:100mg qd D8-90:300mg qd)+GRA+DEX	GRA+DEX	(7)
Liang (30)	35/31	NSCLC	CDDP-containing	THD[a]	Placebo	(7)
Wang et al. (31)	60/60	NSCLC	GP	THD(D1-180:200mg qn)+5-HT_2RA	5-HT_2RA	(7)
Zuo (32)	37/37	SCLC	EP	THD(D6-21: 100mg/m^3 21d for 1 cycle, total 6 cycles of treatment)	Placebo	(7)
Xie et al. (33)	29/29	Breast	GP	THD(200mg qn)	Placebo	(7)
Dong (34)	30/30	NSCLC	TP	THD[b]+5-HT_2RA	5-HT_2RA	(8)
Huang and Wu (35)	30/30	NSCLC	TP	THD(D1-7:100mg qd D8-84:200mg qd)	Placebo	(8)
Liu et al. (36)	40/40	NSCLC	TP	THD(D1-7:100mg qd D8-90:200mg qd)+5-HT_2RA	5-HT_2RA	(8)
Jiang et al. (37)	31/30	NSCLC	GP	THD(D1-60:200mg qn)+AZA	AZA	(7)
Sun et al. (38)	36/21	NSCLC	NP	THD(D1-21:100mg bid)	Placebo	(7)
Shen et al. (39)	15/10	NSCLC	NP	THD[c]	Placebo	(7)

NSCLC, Non-small cell lung cancer; SCLC, Small cell lung cancer; CDDP, Cisplatin; AC, Anthracycline + Cyclophosphamide; GP, Gemcitabine+Cisplatin; DP, Docetaxel+Cisplatin; TP, Paclitaxel+Cisplatin; NP, Vinorelbine+Cisplatin; PCDE, Etoposide+Cisplatin+Cyclophosphamide+4-epidoxorubicin; EP, Etoposide+Cisplatin; THD, Thalidomide; TRO, Tropisetron; DEX, Dexamethasone; PAL, Palonosetron; OND, Ondansetron; MET, Metoclopramide; AZA, Azasetron; RAM, Ramosetron; MP, Methylprednisolone; GRA: Granisetron; 5-HT_2RA: 5-HT_2 receptor antagonist; (1): Complete response (acute phase); (2): Complete response (delayed phase); (3): Complete response (overall phase); (4): No nausea (acute phase); (5): No nausea (delayed phase); (6): No nausea (overall phase); (7): Adverse events; (8): Quality of Life.

a: 100mg qd (D1-7) and then weekly increase of 100 mg until reaching the tolerated dose.

b: 100mg qn (D1) and increase to 200 mg/d within one week, and then the maintenance dose lasts for 3 months.

c: 100mg qn (D1-7) and weekly increase of 50 mg until reaching the tolerated dose (400 mg/d is the maximum dose), treatment lasts for at least 3 months.

heterogeneity among all studies (I^2<50%) and all analyses were performed using a fixed-effects model.

The incidence of mucositis and anorexia was significantly lower with the addition of THD: namely, 14.6% vs 23.3% (RR 0.64, 95%CI 0.46-0.88, p=0.006) of mucositis; and 19.6% vs 37.4% (RR 0.52, 95%CI 0.34–0.81, p=0.003) of anorexia.

The incidence of constipation, peripheral neuropathy, and somnolence was significantly higher with the addition of THD: namely, 39.5% vs 26.9% (RR 1.45, 95%CI 1.30-1.61, p<0.00001) of constipation; 27.4% vs 16.2% (RR 1.61, 95%CI 1.25-2.08, p=0.0002) of peripheral neuropathy; and 25.9% vs 10.2% (RR 2.41, 95%CI 1.78-3.28, p<0.00001) of somnolence.

There was no statistical difference in the incidence of fatigue, headache, diarrhea, rash, hepatorenal damage, and myelosuppression between those with and without THD (p>0.05) (**Table 2**).

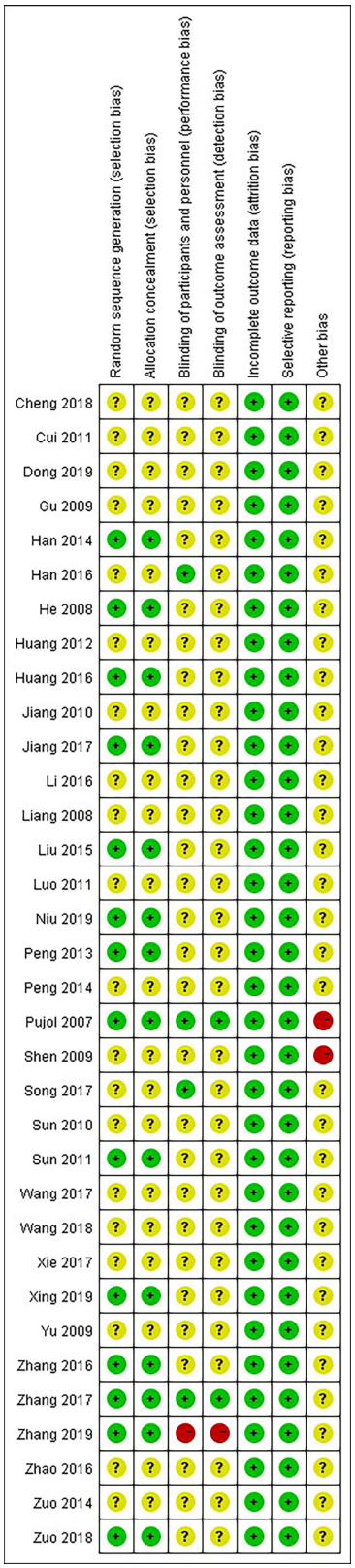

FIGURE 2 | Assessment of risk of bias.

3.4.2 QoL

Data from 12 out of the 34 included articles examined the impact of THD on QoL and included 4 items: increase in the KPS scores (11 studies), weight gain (7 studies), appetite improvement (6 studies), and sleep quality improvement (4 studies). There was no significant heterogeneity among all studies (I^2<50%) and all analyses were performed using a fixed-effects model.

The incidence of an increase in KPS scores, weight gain, appetite improvement, and sleep quality improvement was significantly higher with the addition of THD: namely, 55.9% vs 34.7% (RR 1.61, 95%CI 1.38-1.88, p<0.00001) of an increase in KPS; 49.4% vs 25.6% (RR 1.95, 95%CI 1.55-2.45, p<0.00001) of weight gain; 59.7% vs 41.0% (RR 1.47, 95%CI 1.23-1.74, p<0.00001) of appetite improvement; and 69.4% vs 25.9% (RR 2.66, 95%CI 1.92-3.69, p<0.00001) of sleep quality improvement (**Table 3**).

4 DISCUSSION

There is evidence that THD should be considered as an effective additional antiemetic medication (40). This meta-analysis suggests that the addition of THD to 5-HT_3RA treatment (with or without DEX) is beneficial. Our findings showed that the addition of THD prevents CINV following HEC during the acute, delayed, and overall phase. Among these phases, the THD group had the most significant improvement in CINV during the delayed phase (70.6% vs 50.4% and 50.5% vs 30.0% in CR and no nausea, respectively).

This meta-analysis also suggests a high safety profile for the use of THD in patients with tumors undergoing HEC. Although the THD group increased the incidence of constipation, peripheral neuropathy, and somnolence, the incidence was significantly lower in mucositis and anorexia. The addition of THD did not increase the incidence of many adverse events (fatigue, headache, diarrhea, rash, hepatorenal damage, and myelosuppression). Researchers speculate that THD protects the oral mucosa by inhibiting NF-κB and supporting epithelial repopulation (41). Chemotherapy-induced intestinal mucositis and delayed diarrhea are associated with AIM2 (absent in melanoma 2) inflammasome activation, while THD eliminates AIM2 signaling and significantly reduces the incidence of drug-induced diarrhea (42). This study shows that there is no statistical difference in the incidence of diarrhea between the THD group and the control group, which may require more rigorous clinical trials and a wider population.

As a complementary drug, THD has been shown to improve QoL in cancer patients in this meta-analysis.

THD significantly improves KPS scores, weight, sleep quality, and appetite in cancer patients receiving HEC (55.9% vs 34.7%, 49.4% vs 25.6%, 59.7% vs 41.0%, and 69.4% vs 25.9%, respectively). A Cochrane meta-analysis shows that there is insufficient evidence to refute or support the use of THD for the treatment of cachexia in patients with advanced cancer (43). THD combined with megestrol acetate was shown to be effective in terms of appetite, body weight, and QoL (44).

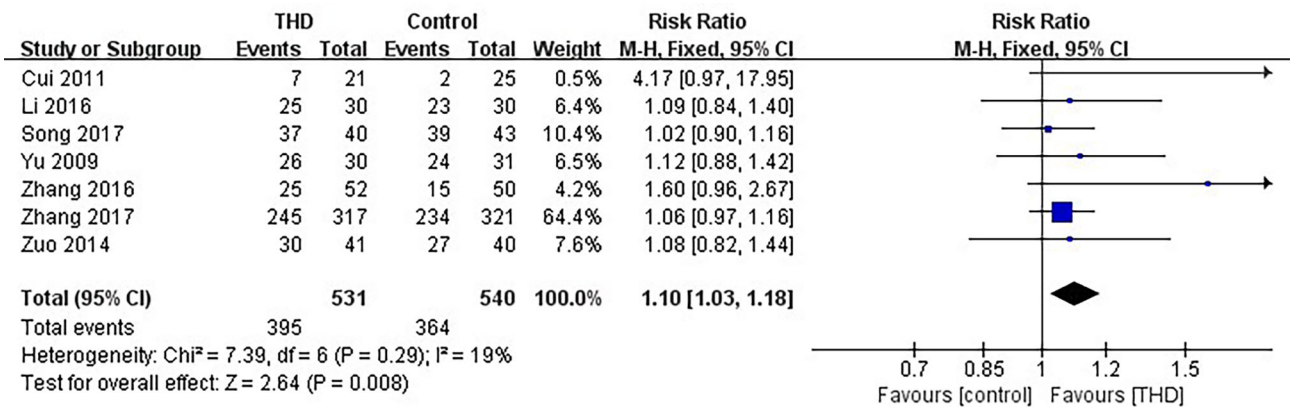

FIGURE 3 | Meta-analysis on CR (acute phase).

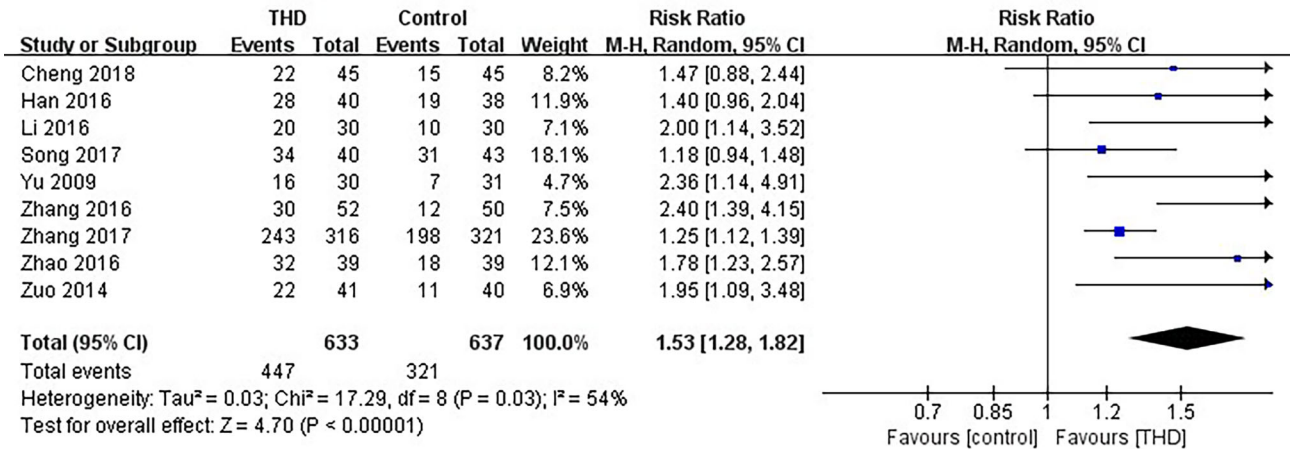

FIGURE 4 | Meta-analysis on CR (delayed phase).

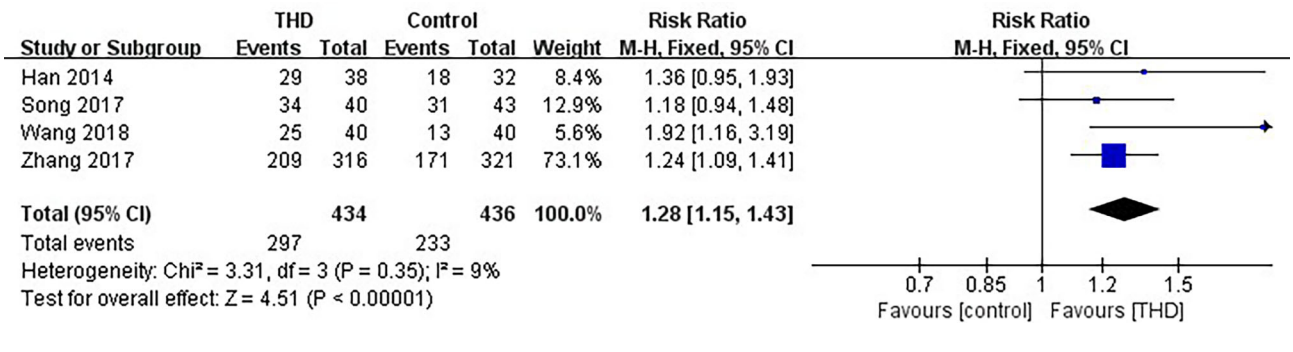

FIGURE 5 | Meta-analysis on CR (overall phase).

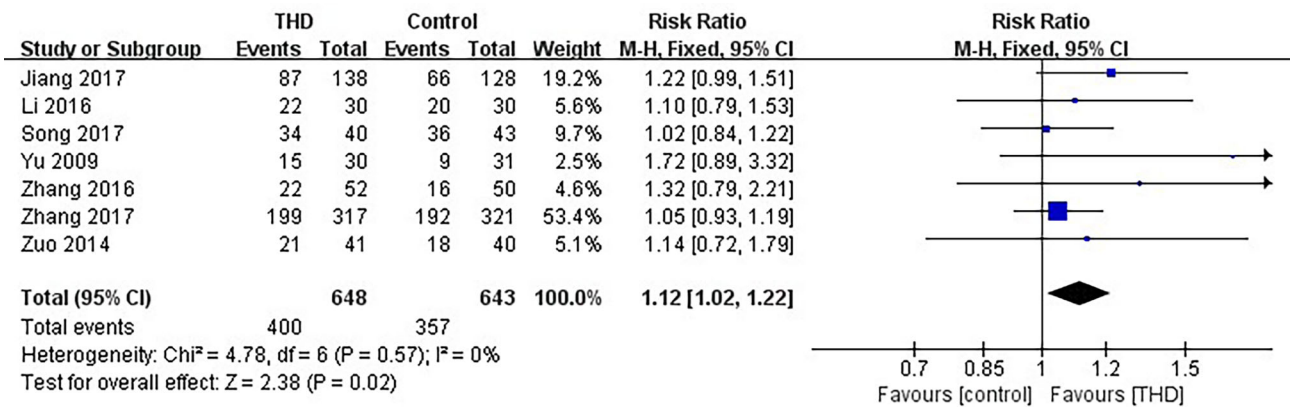

FIGURE 6 | Meta-analysis on no nausea (acute phase).

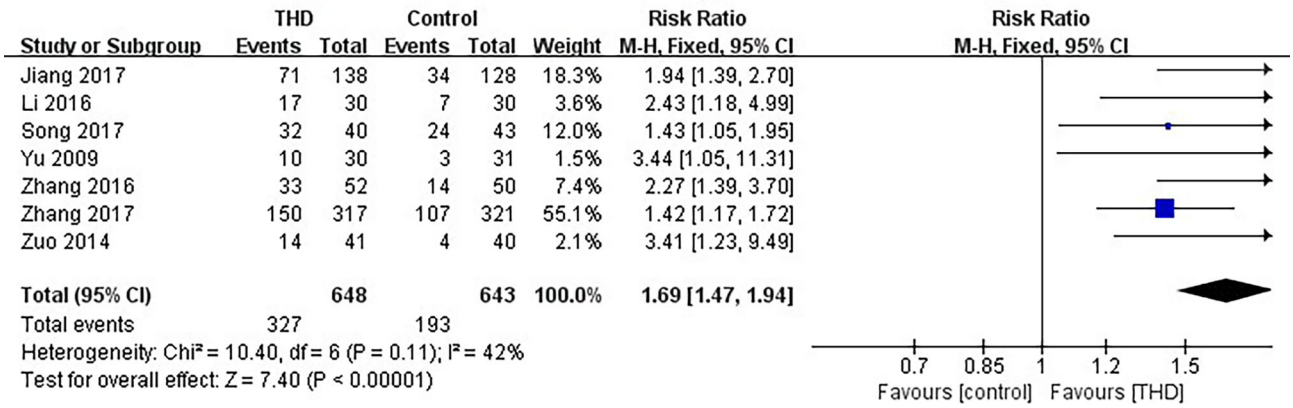

FIGURE 7 | Meta-analysis on no nausea (delayed phase).

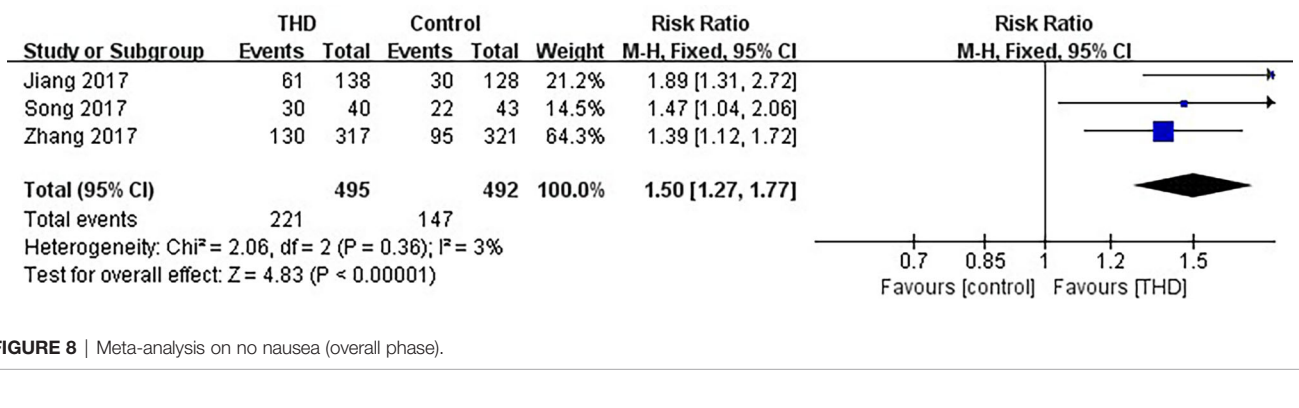

FIGURE 8 | Meta-analysis on no nausea (overall phase).

TABLE 2 | Meta-analysis on adverse events.

Adverse Effects	Numberof trials	THD			Control			Heterogeneity analysis			Statistical analysis model	Statistical analysis	
		Events	Total	Incidence	Events	Total	Incidence	Chi^2	P	I^2		RR (95%CI)	P
Fatigue	12	333	837	39.8%	303	817	37.1%	19.53	0.05	44%	Fixed effect	1.06(0.95, 1.18)	0.3
Constipation	26	526	1333	39.5%	346	1285	26.9%	41.59	0.02	40%	Fixed effect	1.45(1.30, 1.61)	<0.00001
Mucositis	7	49	336	14.6%	72	309	23.3%	4.91	0.56	0%	Fixed effect	0.64(0.46, 0.88)	0.006
Headache	5	48	456	10.5%	52	454	11.5%	1	0.91	0%	Fixed effect	0.91(0.63, 1.31)	0.6
Diarrhea	7	54	640	8.4%	42	621	6.8%	5.47	0.49	0%	Fixed effect	1.22(0.84, 1.78)	0.3
Rash	11	55	484	11.4%	48	466	10.3%	12.24	0.27	18%	Fixed effect	1.09(0.76, 1.56)	0.64
Peripheral neuropathy	9	117	427	27.4%	63	388	16.2%	7.83	0.45	0%	Fixed effect	1.61(1.25, 2.08)	0.0002
Hepatorenal damage	13	59	474	12.4%	51	451	11.3%	4.94	0.96	0%	Fixed effect	1.06(0.76, 1.48)	0.72
Myelosuppression	7	86	260	33.1%	99	259	38.2%	7.24	0.3	17%	Fixed effect	0.88(0.71, 1.09)	0.25
Somnolence	13	121	468	25.9%	46	453	10.2%	23.72	0.02	49%	Fixed effect	2.41(1.78, 3.28)	<0.00001
Anorexia	3	22	112	19.6%	43	115	37.4%	3.84	0.15	48%	Fixed effect	0.52(0.34, 0.81)	0.003

TABLE 3 | Meta-analysis on QoL.

Quality of Life	Numberof trials	THD			Control			Heterogeneity analysis			Statistical analysis model	Statistical analysis	
		Events	Total	Incidence	Events	Total	Incidence	Chi^2	P	I^2		RR(95%CI)	P
Increase in KPS scores	11	227	406	55.9%	137	395	34.7%	6.42	0.78	0%	Fixed effect	1.61(1.38, 1.88)	<0.00001
Weight gain	7	131	265	49.4%	67	262	25.6%	5.35	0.5	0%	Fixed effect	1.95(1.55, 2.45)	<0.00001
Appetite improvement	6	139	233	59.7%	96	234	41.0%	1.04	0.96	0%	Fixed effect	1.47(1.23, 1.74)	<0.0001
Sleep quality improvement	4	86	124	69.4%	30	116	25.9%	4.53	0.21	34%	Fixed effect	2.66(1.92, 3.69)	<0.00001

This study has several strengths. Firstly, we included 34 RCTs and 3168 cases, expanding the scope and number of THD studies and greatly improving sample size and test efficacy. Secondly, we compared the differences in the incidence of 11 adverse events between the THD and control groups to provide a reference for the safety study of THD use in cancer patients. Finally, we also analyzed the effect of THD in increasing KPS scores, increasing weight, improving sleep quality, and increasing appetite from the perspective of QoL of cancer patients.

This meta-analysis also has some limitations. First, although the search for this study was extensive and included both English and Chinese databases, the final population of the literature included in the study was Chinese, which is not representative of other regional populations and ethnicities. Second, many of the studies we included scored poorly on quality assessment, which to some extent affects the final results of the meta-analysis. Finally, the number of studies containing the same outcome was no more than 10, so a funnel plot was not used to test for publication bias.

5 CONCLUSION

According to this systematic review and meta-analysis, we conclude that THD is effective and safe for the prevention of CINV in patients being treated with HEC, and has a significant tendency to improve QoL. More high-quality RCTs with more participants are warranted to support our findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

JX and CZ contributed to study design, literature search, data collection, data analysis, and manuscript drafting. SL and RD contributed to quality assessment and data collection. MS, BD, and QX contributed to critical revision. JW, CS, and YZ contributed to conception, design, supervision, and manuscript drafting. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China [82073402] and Key research and development program of Hubei Province [2020BCA060].

ACKNOWLEDGMENTS

The authors thank all the staff member for their invaluable efforts to this study.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 09 February 2022
doi: 10.3389/fphar.2022.826785

Whether Patients With Stage II/III Colorectal Cancer Benefit From Adjuvant Chemotherapy: A Modeling Analysis of Literature Aggregate Data

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OPEN ACCESS

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *01 December 2021*
Accepted: *20 January 2022*
Published: *09 February 2022*

Citation:
Zha S, Li T, Zheng Q and Li L (2022) Whether Patients With Stage II/III Colorectal Cancer Benefit From Adjuvant Chemotherapy: A Modeling Analysis of Literature Aggregate Data. Front. Pharmacol. 13:826785. doi: 10.3389/fphar.2022.826785

Objective: This study used model analysis to clarify the benefits and risks of postoperative adjuvant chemotherapy compared with surgery alone in patients with stage II/III colorectal cancer.

Methods: Clinical trials involving patients with stage II/III colorectal cancer who underwent surgery alone or those who received post-surgical adjuvant chemotherapy were searched in the PubMed and embase databases. By establishing a survival model, the overall survival (OS) and disease-free survival (DFS) of patients who underwent surgery alone or postoperative adjuvant chemotherapy were quantitatively analyzed to compare the differences between the two. In addition, the incidence of grade 3/4 adverse reactions in the adjuvant chemotherapy group was analyzed using the random effects model in the single-arm meta-analysis.

Results: A total of 34 studies containing 33,069 patients were included in the analysis. This study found that postoperative adjuvant chemotherapy can effectively improve the OS and DFS of patients with colorectal cancer. The median OS of the adjuvant chemotherapy group and the surgery-only group was 118.8 months (95% CI: 96.6, 146.6) and 74.6 months (95% CI: 57.8, 96.1) respectively; and median DFS was 86.3 months (95% CI: 67.6, 110.6) and 40.8 months (95% CI: 23.7, 69.6) in the adjuvant chemotherapy and surgery-only groups, respectively. Common grade 3/4 adverse reactions in the adjuvant chemotherapy group include diarrhea, stomatitis, leukopenia, and nausea or vomiting, with an incidence of approximately 3%–6%.

Conclusion: Patients with mid-stage colorectal cancer can benefit significantly from postoperative adjuvant chemotherapy. This study provides the necessary quantitative information for decision-making regarding the benefits and risks of receiving adjuvant chemotherapy after resection in patients with colorectal cancer.

Keywords: colorectal cancer, adjuvant chemotherapy, survival, modeling, quantitative evaluation, MBMA

INTRODUCTION

Colorectal cancer is one of the most common gastrointestinal malignancies, with the fourth highest incidence of malignancies and the second highest cancer-related mortality worldwide (Center et al., 2009; Salehiniya et al., 2017). Approximately 70%–80% of patients with colorectal cancer are amenable to radical resection; however, postoperative recurrence is the main cause of treatment failure for colorectal cancer, and approximately 19%–28% of patients develop metastases after resection (Jemal et al., 2009; Van Cutsem et al., 2014; Kim et al., 2016; Pinson et al., 2018). The purpose of adjuvant chemotherapy is to eliminate small metastases or micro residual foci that have not been detected during surgery, such that one can improve their prognosis and prolong the survival of patients to obtain more clinical benefits. Adjuvant chemotherapy for colorectal cancer is defined as any 5-FU-based chemotherapy after radical resection of colorectal cancer, including portal vein infusion chemotherapy (Benson et al., 2004).

According to the different depths of invasion, lymph node metastasis, and organ metastasis status of this disease, the current NCCN international guidelines recommend that patients with stage I colorectal cancer should be treated mainly by surgical resection without adjuvant therapy. Patients with stage II colorectal cancer along with other high-risk factors and stage III progressive colorectal cancer should be treated with radical surgery following postoperative adjuvant chemotherapy, while those with advanced or recurrent colorectal cancer are recommended to undergo multidisciplinary evaluation to determine whether there is a chance of resection or radiotherapy, which should be followed by adjuvant chemotherapy (Benson et al., 2020; Benson et al., 2021). However, whether patients with stage II/III disease benefit from adjuvant chemotherapy remains controversial, there is currently no such study for quantitatively evaluating the efficacy of adjuvant chemotherapy regimens after radical surgery for colorectal cancer patients, as recommended in the NCCN guidelines (Carvalho and Glynne-Jones, 2017).

In addition, a series of adverse reactions are also important factors affecting whether a chemotherapy should be performed on patients. For example, the combination of oxaliplatin and fluoropyrimidine will increase the incidence of neutropenia, thrombocytopenia, diarrhea, nausea and depression. There will also be a chronic irreversible peripheral neuropathy (Kuebler et al., 2007; Iveson et al., 2019). Although the survival improves, the incidence of adverse events and the decline in quality of life were also significant for patients. Therefore, quantified comparisons of survival benefit and incidence of adverse events are necessary for physician and patient during a therapy.

Model-based meta-analysis (MBMA) is a quantitative method for evaluating the efficacy or safety of drugs based on traditional meta-analysis using models. Through this method, we can simultaneously correct for multiple influencing factors, deduce the influence of inter-study heterogeneity on the results, and make predictions of drug efficacy at different time points and at different covariate levels (Mandema et al., 2011; Demin et al., 2012). This study aimed to clarify the difference in survival benefit between surgery alone and adjuvant chemotherapy after surgery for patients with stage II/III colorectal cancer using MBMA, as well as evaluate the effect of multiple factors on survival time, aiming to provide reliable quantitative information on the clinical efficacy of adjuvant chemotherapy for colorectal cancer.

METHODS

Search Strategy

Relevant literature was searched in the PubMed and embase databases, with a search deadline of 29 December 2021, for keywords such as colorectal cancer and adjuvant chemotherapy, with "Or" connecting terms in the same category and "And" connecting terms in different categories. The type of literature included clinical trials, and the language was restricted to English. The specific search strategy is detailed in the Supplements.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: 1) the patient had a stage II/III colorectal cancer; 2) the patient had undergone surgical resection; 3) the adjuvant chemotherapy used was the NCCN guideline recommended regimen; and 4) the patient had not received any other treatment prior to enrollment.

The literature exclusion criteria included the following: 1) combination of cancers other than colorectal cancer, 2) patients who received non-adjuvant chemotherapy regimens, and 3) no survival data extracted (**Supplementary Figure S1** in Supplements).

Data Extraction and Quality Evaluation

The following data were extracted using Microsoft Excel (Microsoft Office package, 2019 version): 1) literature characteristics (author, year of publication, clinical trial registration number, etc.); 2) trial characteristics (sample size, dosing method, dosing regimen, etc.); 3) subject characteristics (age, sex, initial status, tumor *in situ*, cancer grade, etc.); and 4) outcome indicators (overall survival [OS], disease-free survival [DFS], and the incidence of grade 3/4 adverse reactions).

The above information was extracted from the data by two investigators independently, with inconsistencies adjudicated by a third investigator. When graphically presenting the data in the literature, the GetData Graph Digitizer software was used to extract the data in the graph. If the error during the extraction between the two researchers was greater than 2%, the data had to be extracted again, and the average value was taken as the final analysis data.

The quality of the literature was evaluated using the Cochrane risk of bias table which includes the evaluation in random sequence generation (Deeks et al., 2019), allocation concealment, performer and participant blinding, outcome assessment blinding, incomplete outcome data, selective publication, and other biases. Among them, we defined other bias as the trial being sponsored by a drug company and the trial being incomparable across subject groups at baseline. Each entry

was categorized as low risk, high risk, or unclear risk. The quality of the literature was scored by two researchers independently, and inconsistencies were adjudicated by a third researcher.

Model Building

Parametric survival models were used to analyze survival data, such as OS and DFS, of patients treated with surgery alone and adjuvant chemotherapy after radical surgery. Visual inspection of the data shows that the survival data was related to the hazard function h(t), which can be interpreted as the instantaneous risk of death at moment t. The hazard function can be described by the equations below.

$$h(t) = \frac{\left(\sigma t\sqrt{2\pi}\right)^{-1} e^{\left(-\frac{1}{2}z^2\right)}}{1 - \varnothing(Z)}, \quad Z = \frac{\ln(t) - \mu}{\sigma} \tag{1}$$

h(t) in **Eq. 1** conforms to the log-normal distribution, where μ and σ are the median and standard deviation of the log-normal distribution, respectively (Ding et al., 2020).

Once the base model was constructed, factors that have a potential impact on the model parameters were examined, including subjects' age, sex, location of carcinoma *in situ*, Dukes' classification, and the treatment regimen (with or without fluorouracil, with or without fluorouracil combined with calcium folinic acid regimen). Forward inclusion and backward elimination methods were used to screen the covariates using NONMEM software (Mandema et al., 1992; Wahlby et al., 2001). The bound of OFV decreasing in the forward method was set at 3.84 ($p < 0.05$), while in the backward method, the bound was set at 6.63 ($p < 0.01$). The detailed description of the construction of the model is available in Supplements, page five to six.

Model Evaluation

Several approaches were used to evaluate the model's performance after the final model was established. First, the goodness-of-fit of the proposed model was evaluated using model diagnostic plots. The model diagnostic plots included scatter plots of observation (OBS) versus population prediction (PRED) and individual predictions (IPRED), conditional weighted residuals (CWRES) versus time and PRED scatter plots, respectively. Second, the visual predictive check (VPC) was used to compare the model predictions with the observed values and evaluate the predictive performance of the model. Finally, the bootstrap method was used to assess the robustness of the model, that is, 1,000 new datasets were taken from the original dataset to obtain the median of the model parameter distribution and its 95% confidence interval (CI), and compared with the estimated values of the model parameters obtained from the original dataset; if they were closer, it indicated that the model was robust and less influenced by individual studies.

Safety Analysis

The incidence of common grade 3/4 adverse reactions, such as leukopenia, diarrhea, nausea or vomiting, and stomatitis, in the adjuvant chemotherapy group was pooled using a random-effects model in a single-arm meta-analysis to assess the safety of postoperative adjuvant chemotherapy for patients.

Software

The modeling and simulation processes were performed using NONMEM 7.3 (Level 1.0, ICON Development Solutions, New York, United States), and the model parameters were estimated using first-order conditional estimation. Meta-analysis and graphical visualization were performed using R software (version 4.0.3, The R Foundation of Statistical Computing, Vienna, Austria). The literature quality assessment was performed using RevMan (version 5.4, Nordic Cochrane Center, Copenhagen, Denmark).

RESULTS

Characteristics of the Included Studies

The study ultimately included 34 publications that enrolled 33,069 patients, which consisted of 21 publications (23 treatment arms, sample size of 7,020) in the surgery-only group and 31 publications (48 treatment arms, sample size of 26,049) in the postoperative adjuvant chemotherapy group (**Table 1**).

From the publications, we extracted data on OS, the gold standard for assessing clinical benefit in oncology, and DFS, the most common endpoint for evaluating adjuvant therapy after radical surgery. A total of 32 publications reported data on OS at different time points, comprising 67 trial arms (23 in the surgery-only group and 44 in the adjuvant chemotherapy group); 20 publications reported data on DFS, comprising 42 trial arms (11 in the surgery-only group and 31 in the adjuvant chemotherapy group).

The mean age of patients in the 34 studies was 54–68 years (median age, 62 years), with a median male prevalence of 56.0% (14.2%–73.3%). The proportion of primary tumors located in the colon was 12.7%–100% (median, 61.1%), and the proportion of primary tumors located in the rectum was 0%–65.8% (median, 36.7%). Among the included studies, 9 (26.5%) were of high quality, 25 (73.5%) were of medium quality, and 0 were of low quality (**Supplementary Table S1** & **Supplementary Figure S2** in Supplements).

Model Building and Evaluation

The results showed that the log-normal model had lower OFV values and a smaller relative standard error (RSE) % of the model parameters. Therefore, the log-normal model was selected to fit the OS and DFS data. In covariate screening, we did not find any factors that had a significant impact on the parameters in the OS and DFS models. The estimated values of the final model parameters are listed in **Table 2**.

The RSE of the model parameters in both the OS and DFS models was small, indicating that the model parameter estimates were relatively stable. The bootstrap method with 1,000 iterations converged successfully 993 times and 996 times, respectively, with the 95% CIs very close to the parameter estimates of the final OS and DFS models, suggesting that the final models were robust and influenced by the data of individual studies was relatively small.

The model diagnostic plots (**Supplementary Figure S3, S4** in Supplements) showed that the model-predicted values for OS and DFS fit well with the observed data without significant bias. The

TABLE 1 | Brief characteristics of included studies.

	Control	ACT	Overall
Number of trials (arms)	21 (23)	31 (48)	34 (71)
Total sample size	7,020	26,049	33,069
Age, yr, median (min-max)	62 (15–86)	62 (15–95)	62 (15–95)
Male, %, median (min-max)	53.5 (14.2–70.5)	56.0 (42.7–73.3)	56.0 (14.2–73.3)
Primary tumor, %, median (min-max)			
Colon	55.5 (12.7–71.0)	67.8 (30.6–100)	61.1 (12.7–100)
Rectum	39.0 (11.7–71.0)	32.2 (0–65.8)	36.7 (0–65.8)
Dukes' stage, %, median (min-max)			
Dukes' B	43 (0–92)	41 (0–91)	41 (0–92)
Dukes' C	38 (8–100)	47 (8–100)	42 (8–100)

ACT, indicates adjuvant chemotherapy; Control, indicates surgery alone.

TABLE 2 | Parameter estimations of model.

	Overall survival model				Disease-free survival model			
	Final model		Bootstrap (993/1,000)		Final model		Bootstrap (996/1,000)	
Parameters	Value	RSE%	Median	95%CI	Value	RSE%	Median	95%CI
SIGM1 (ACT)	1.44	3.8	1.44	1.32–1.55	2.08	2.4	2.08	1.98–2.17
SIGM2(CONTROL)	1.34	5.9	1.35	1.20–1.53	1.90	8.1	1.89	1.52–2.26
MU1(ACT)	4.87	2.1	4.86	4.69–5.08	4.90	2.5	4.90	4.65–5.13
MU2(CONTROL)	4.35	2.8	4.36	4.12–4.62	4.05	6.4	4.04	3.49–4.67
Variability parameters								
η(SIGM),%	23.6	13.9	22.9	16.9–44.2	13.2	32.8	12.4	3.99–27.7
η(MU),%	12.9	8.9	12.7	10.4–15.2	15.3	11.7	14.7	11.1–18.8
ε	1.03	9.8	1.03	0.85–1.23	1.63	9.6	1.62	1.31–1.93

ACT, indicates adjuvant chemotherapy; CONTROL, indicates surgery alone; η is the inter-study variability of pharmacodynamic parameter; ε is the residual error. CI, confidence interval; RSE, relative standard error.

visual predictive check (VPC) plots showed that the 95% CIs predicted by the OS and DFS models included most of the measured values, suggesting that the models have good predictive ability (**Figure 1**).

Model Simulation

Based on the final model, the typical OS and DFS values and their 95% CIs were simulated for the surgery-only and adjuvant chemotherapy groups, showing a median OS of 118.8 months (95% CI: 96.6, 146.6) and 74.6 months (95% CI: 57.8, 96.1) for the adjuvant chemotherapy and surgery-only groups, respectively, with the former being 1.6 times higher than the latter; 5-years survival rates of 71.6% (95% CI: 65.1, 77.7) and 57.8% (95% CI: 48.7, 67.2), respectively; and 10-years survival rates of 49.7% (95% CI: 42.9, 56.8) and 33.1% (95% CI: 25.6, 41.8), respectively.

The median DFS rates were 86.3 months (95% CI: 67.6, 110.6) and 40.8 months (95% CI: 23.7, 69.6) months in the adjuvant chemotherapy group and the surgery-only group, respectively; 5-years survival rates of 58.5% (95% CI: 52.9, 63.9) and 40.1% (95% CI: 28.1, 53.8), respectively; and 10-years survival rates of 42.3% (95% CI: 36.7, 48.0) and 24.1% (95% CI: 14.8, 35.7), respectively (**Figure 2**; **Table 3**).

Safety Analysis

A total of 18 publications reported the incidence of grade 3/4 adverse reactions, and the results showed that the incidence of grade 3/4 diarrhea, leukopenia, stomatitis, and nausea or vomiting in the postoperative adjuvant chemotherapy group was 6% (95% CI: 4, 9), 3% (95% CI: 1, 6), 4% (95% CI: 3, 8), and 4% (95% CI: 3, 6), respectively (**Supplementary Figure S5** in Supplements).

DISCUSSION

In recent years, despite a variety of emerging therapies for patients with colorectal cancer, such as radiation therapy, targeted therapy, and preoperative neoadjuvant therapy, adjuvant chemotherapy remains the basic therapy for this disease because of its wide application in various clinical situations. Disputes still exists on whether patients with middle-stage colorectal cancer can benefit from postoperative adjuvant chemotherapy, while what has already been agreed upon is that those with early-stage colorectal cancer are not advised with an adjuvant chemotherapy, and those with advanced ones should be treated with palliative care (Carvalho and Glynne-Jones, 2017; Dekker et al., 2019; Benson et al., 2020; Benson et al., 2021). Therefore, it is essential to conduct a quantitative comparison between adjuvant chemotherapy and surgical treatment alone in patients with middle-stage colorectal cancer.

Previous studies have focused on whether patients with other specific types of colorectal cancer benefit from adjuvant chemotherapy, for example, whether those with lung

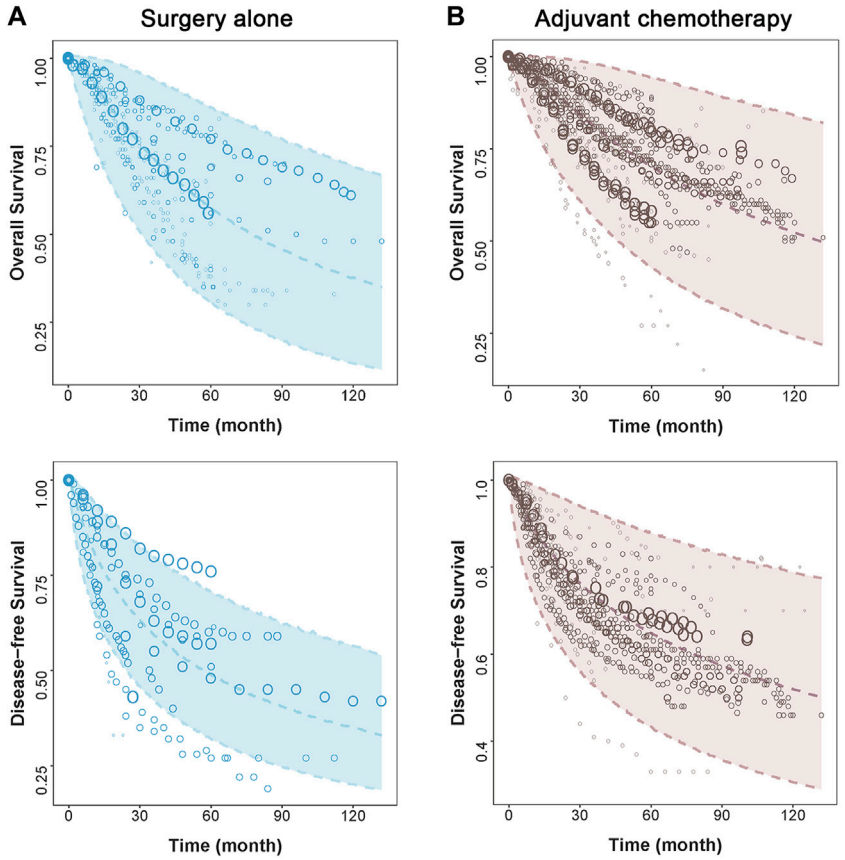

FIGURE 1 | Visual predictive check of the final model. The points represent observed survival data, and symbol size is proportional to sample size. The shade area is the model predicted 95% CI of the curve. The dashed lines are the model-predicted 2.5th, 50th, and 97.5th percentiles of survival. The two figures on the left side represent **(A)** surgery alone group, and two figures on the right side represent **(B)** adjuvant chemotherapy group.

metastases benefit from adjuvant chemotherapy, whether those with peritoneal metastases benefit from intraperitoneal hyperthermia chemotherapy, and whether there is a difference between long-term prognosis from three drugs and two drugs in palliative first-line treatment (Gill et al., 2004; Group et al., 2007; E. and; Mitry, 2008; Zhang et al., 2019)_ENREF_20. However, few studies have been done to quantitatively describe the survival benefits for middle-stage colorectal cancer patients from a adjuvant chemotherapy. A Meta analysis (Böckelman et al., 2015) found that only patients with stage III colorectal cancer could benefit from adjuvant chemotherapy, while the 5-years DFS of patients with stage II colorectal cancer with adjuvant chemotherapy was even lower than that without adjuvant chemotherapy. However, this study only included the literature published in 2005–2013 for analysis, and did not analyze OS, the conclusions of the study may be biased.

In this study, we established a survival model with a hazard function to reflect the difference among middle-stage colorectal cancer patients being treated with drugs recommended by the NCCN guideline and explored whether they benefit from adjuvant chemotherapy. We found a significant difference in OS and DFS between patients who received surgical treatment only and those who received adjuvant chemotherapy after surgery. We also found that the difference in DFS was more significant than that in OS, in which the adjuvant chemotherapy group had a 2.1 times larger DFS than those in the surgery group. Compared with previous studies, this study not only confirmed that patients with middle-stage colorectal cancer can benefit from adjuvant chemotherapy, but also can predict the survival time at any arbitrary time point, not limited to the median survival time and 1-year survival rate, so as to show the benefits of adjuvant chemotherapy in the whole time period.

There is a clinical debate on whether it deserves to use the adjuvant chemotherapy on patients compared to its safety issues (Yothers et al., 2011; Carvalho and Glynne-Jones, 2017; Zhang et al., 2019; La Regina et al., 2020). Although one may live a little longer after adjuvant chemotherapy, it depends on various adverse events, and whether one can live with a higher quality life matters more. In this study, we could not find any record of adverse events in the surgery-only group; thus, a single-arm meta-analysis was applied to the data of adverse events in the adjuvant chemotherapy group. The results showed that the incidence of regular grade 3/4 adverse events was no more than 6% in the adjuvant chemotherapy group, which included diarrhea and stomatitis (4%), leukopenia (3%), and nausea or vomiting (4%). The results can be useful for decision makers in

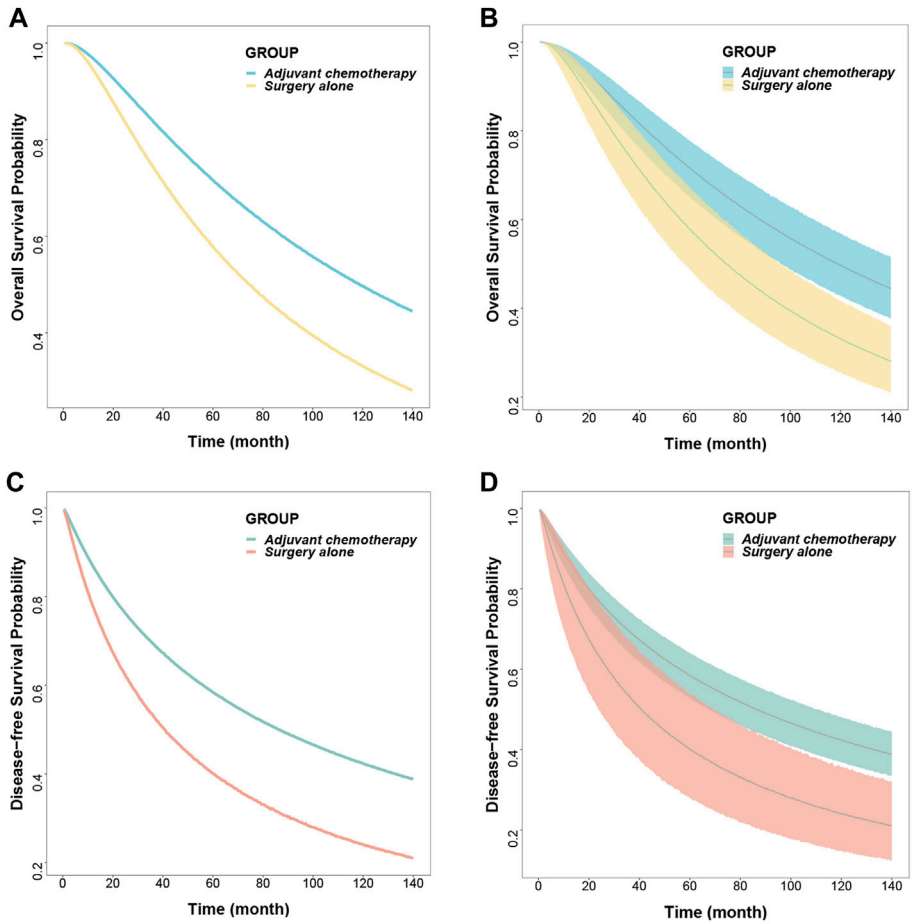

FIGURE 2 | Predicted typical time course **(A) (C)** and 95% confidence interval **(B) (D)** of overall survival and disease-free survival.

TABLE 3 | The predicted typical time course with 95% confidence interval of OS and DFS model.

	Median Overall Survival (month)	Five-year Overall Survival (%)	Ten-year Overall Survival (%)
Surgery	74.6 (57.8, 96.1)	57.8 (48.7, 67.2)	33.1 (25.6, 41.8)
Surgery + ACT	118.8 (96.6, 146.6)	71.6 (65.1, 77.7)	49.7 (42.9, 56.8)
	Median Disease-free Survival (month)	**Five-year Disease-free Survival (%)**	**Ten-year Disease-free Survival (%)**
Surgery	40.8 (23.7, 69.6)	40.1 (28.1, 53.8)	24.1 (14.8, 35.7)
Surgery + ACT	86.3 (67.6, 110.6)	58.5 (52.9, 63.9)	42.3 (36.7, 48.0)

ACT, indicates adjuvant chemotherapy.

determining whether adjuvant chemotherapy should be administered after surgery.

Reports have indicated that factors such as age and the location of carcinoma *in situ* can affect the survival of patients with colorectal cancer (Holch et al., 2017; Nikolic et al., 2021). Researchers have also reported that those under the age of 65 years can survive better than those older than 65 years. Moreover, some reported that patients with rectal cancer had a higher OS than those who experienced colon cancer in a 5-years range (Gill et al., 2004; Schmoll et al., 2014). However, this study did not find any covariate that had a significant impact on survival rate, including age, sex, initial status, tumor *in situ*, and cancer grade. The reason may be that our research is based on literature aggregate data, which to some extent masks individual differences and reduces the chances of finding covariates. Besides that, because the missing rate of factors is more than 30%, such as MSI status, perineural invasion, histologic grade, and serum CEA level, the covariates can not be investigated. This is one of the limitations of this study. Beside that, due to multifarious medication regimens and chemotherapy cycles, it is hard for us to make a more detailed category for our enrolled literatures. Finally, only studies

published in English were included, therefore, the risk of publication bias could be present.

CONCLUSION

This study quantified the survival benefit of adjuvant chemotherapy for colorectal cancer and found that postoperative adjuvant chemotherapy significantly prolonged patients' OS and DFS compared with surgery alone, providing quantitative evidence that patients with intermediate colorectal cancer benefit from adjuvant chemotherapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

SZ wrote the manuscript; LL and QZ participated in conception and design of the work and revised the paper critically for important intellectual content. All the information was independently extracted by SZ and TL.

FUNDING

This study was financially supported by the National Natural Science Funds (82174229).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.826785/full#supplementary-material

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frontiers in Oncology

SYSTEMATIC REVIEW
published: 09 February 2022
doi: 10.3389/fonc.2022.823705

Effects of Concomitant Antibiotics Use on Immune Checkpoint Inhibitor Efficacy in Cancer Patients

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OPEN ACCESS

Edited by:
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Reviewed by:
Muhammad Bilal Abid, Medical College of Wisconsin, United States
Simone Scagnoli, Sapienza University of Rome, Italy

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: 28 November 2021
Accepted: 14 January 2022
Published: 09 February 2022

Citation:
Jiang S, Geng S, Chen Q, Zhang C, Cheng M, Yu Y, Zhang S, Shi N and Dong M (2022) Effects of Concomitant Antibiotics Use on Immune Checkpoint Inhibitor Efficacy in Cancer Patients. Front. Oncol. 12:823705. doi: 10.3389/fonc.2022.823705

Objective: Immune checkpoint inhibitors (ICIs) have changed the outcomes of a variety of cancers in an unprecedented manner. Gut microbiome plays a crucial regulatory role in the antineoplastic therapy of ICIs, which can be influenced by antibiotic (ABX) administration. In this efficacy evaluation, we aimed to clarify the correlations of ABX administration with the survival of cancer patients receiving ICIs treatment.

Method: The eligible literatures were searched using PubMed, Cochrane Library, Web of Science, and Clinical trials.gov databases before Nov 2021. The correlations of ABX administration with progression-free survival (PFS) and overall survival (OS) were determined using Hazard ratios (HRs) coupled with 95% confidence intervals (CIs).

Results: A total of 12 studies enrolling 6010 cancer patients receiving ICIs treatment were included in this efficacy evaluation. ABX administration was significantly correlated worse PFS (HR=1.60, 95%CI=1.33-1.92, P<0.00001) and OS (HR=1.46, 95%CI=1.32-1.61, P<0.00001). Similar results were found in the subgroup analysis of non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and melanoma.

Conclusions: ABX use during ICIs treatment of cancer may significantly shorten PFS and OS. ABX should be used cautiously in cancer patients receiving ICIs. However, further validations are still essential due to existing publication bias.

Keywords: cancer, immune checkpoint inhibitors (ICIs), antibiotics, progression-free survival (PFS), overall survival (OS)

INTRODUCTION

In recent years, as new antitumor drugs, Immune checkpoint inhibitors (ICIs) have significantly improved the prognosis of patients with various types of tumor which brings a "Immune Era" with representative drugs included programmed cell death 1(PD-1)/programmed cell death ligand 1(PD-L1) inhibitors and cytotoxic T lymphocyte-associated antigen 4(CTLA4) antibodies (1). Gut microbes play an important role in regulating the efficacy and toxicity of cancer immunotherapy (2, 3). Phase I clinical trials in animal models suggested that gut microbes may be key modulators of ICIs efficacy and toxicity. Routy et al. (4) confirmed that transplanting intestinal microorganisms from patients into sterile mice

could enhance the anti-tumor efficacy of PD-1 inhibitors. Therefore, it is suggested that the response of cancer patients to ICIs may be influenced by conditions of altering the composition of gut microbes, including dysbiosis due to antibiotic use (ABX).

The relationship between ABX use and cancer therapy has been extensively studied, especially in the prevention of perioperative infection and immunosuppressive associated infection induced by chemoradiotherapy (5). There are few reports on the role of ABX in the treatment of ICIs in tumor patients, but the conclusions varied greatly which were influenced by the type and duration of administration. Several studies have compared the effects of ABX on clinical outcomes before/during/after the use of ABX with those without, and some patients have negative effects on treatment response and survival, such as Huang (6), Lurienne (7), etc. Other studies (8, 9) have shown no significant correlation between ABX administration during or before ICIs treatment and remission rates and PFS in cancer patients. Therefore, the prognostic effect of ABX in the treatment of ICIs is still unclear, and the comprehensive and objective evaluation is urgently needed. In the present study, we evaluated the efficacy of 12 studies in 6010 patients treated with ICIs and analyzed the association between ABX use and survival, with the expectation that the results would contribute to the individualized clinical management of cancer immunotherapy and the improvement of patient survival, we evaluated 12 studies of 6010 patients treated with ICIs and analyzed the association between ABX use and survival, with the aim of improving individual clinical management and patient survival during cancer immunotherapy.

METHODS AND MATERIALS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to report our meta-analysis. We systematically searched domestic and foreign literatures on antibiotic application versus non-antibiotic application before, during or after ICIs treatment in cancer, and systematically evaluated the impact of antibiotics in cancer treatment on the efficacy of ICIs.

Search Strategy

We use a variety of retrieval tools to conduct a comprehensive literature search. (1) Computer literature database search: ①Chinese search terms included "immune checkpoint inhibitors", "cancer", "immunotherapy", "programmed cell death protein 1", "programmed cell death protein ligand 1", "cytotoxic T lymphocyte antigen 4", etc. ②English keywords included "ICIs", "cancer", "immunotherapy", "PD-1", "PD-L1", "CTLA-4", etc. ③Different combinations of PubMed, Cochrane Library, Embase and EBSCO evidence-based medicine databases were searched, including title, abstract and keywords, and the search period was from self-establishment to November 2021.

Study Selection

As immunotherapy becomes more widely used in many cancer patients, some studies showed that both PFS and OS were significantly reduced in patients treated with ICIs and antibiotics. Therefore, it is important to determine whether antibiotics affect the prognosis of patients treated with ICIs. At present, systematic evaluation in this field mainly focuses on multi-factor analysis, while antibiotic single-factor analysis is rare. In order to further systematically evaluate the single factor effect of antibiotic and ICIs combination, the following inclusion criteria were used: (1) Included population: solid tumor patients treated with ICIs; (2) Literature type: prospective or retrospective study; (3) Interventions: antibiotic use before, during, or after ICIs treatment versus no antibiotic use; (4) Outcome measures: PFS and/or OS-related hazard ratios (HRs) with 95% confidence intervals (95%CI). Meanwhile, the following exclusion criteria were used: (1) No control group was established; (2) Repeatability study; (3) Non-Chinese and English literature; (4) HRs literature for PFS and/or OS is not provided

Data Extraction and Quality Assessment

Data were extracted from the eligible studies included according to the PRISMA statement: author's name, year of publication, type of publication (such as publication poster and abstract), country patient sample size, HRs and 95%CI of antibiotic treatment window, PFS was defined as spanning from randomization to either recurrence or death, and OS was defined as spanning from randomization to death. The Newcastle-Ottawa scale (NOS) was used to evaluate the quality of the literature (10), and the quality of the included studies was evaluated according to the following 8 criteria: (1) the representativeness of the exposure cohort; (2) the non-exposure cohort Selection; (3) Determination of exposure method; (4) No subject had an outcome event before the start of the study; (5) Comparability of exposure cohort and non-exposure cohort; (6) Evaluation of outcome events; (7) Whether the follow-up time is long enough; (8) Whether the follow-up is complete. Documents rated 7-9 points are considered "high" quality, 4-6 points are "fair", and 3 points or lower are considered "low". The quality evaluation is carried out independently by two researchers and cross-checked. If there is a disagreement, the third researcher is requested to assist in the resolution.

Statistical Analysis

Meta-analysis was performed using RevMan 5.2 software provided by the Cochrane Collaboration. All the HRs included in the study were pooled together to provide an overall effect size. Cochrane χ^2 test was used to analyze the heterogeneity between studies, and I^2 was used to evaluate the heterogeneity. When $P > 0.1$ and $I^2 < 50\%$, there was no statistical heterogeneity for RCTs, and the fixed-effect model was used. On the contrary, the random effect model was adopted on the premise of excluding clinical heterogeneity. An inverted funnel plot was used to analyze publication bias, and sensitivity analysis was conducted for each included literature. The experimental bias of included literature was also discussed.

RESULTS

Search Results and Patient Characteristics

Through database retrieval, 81 relevant literatures were obtained, including 8 Chinese literatures, 73 English literatures, 23

conference papers and abstracts, and 67 duplicated literatures, case reports, reviews and irrelevant contents were excluded. 37 literatures were screened strictly in accordance with the above screening process, and finally 12 (11–22) studies were included in the quantitative analysis. A total of 6010 cancer patients were involved, of whom 1414 were treated with ABX in the treatment window of ICIs, as shown in **Figure 1**.

A total of 6010 cancer patients meeting the requirements were included in the 12 literatures, including 1414 patients who received antibiotics during ICIs treatment and 4596 patients who did not receive antibiotics. All 12 literatures were of high quality, as shown in **Table 1**.

Meta-Analysis Results

Effect of Concomitant ABX Use on PFS of ICIs

PFS data could be obtained from 12 studies for heterogeneity analysis, I^2 = 68%,P=0.0001. There was statistical heterogeneity among studies, and random effect model was used for analysis. As shown in **Figure 2**, HR=1.60 (95%CI=1.33-1.92, P<0.00001) these results suggest that the use of antibiotics in the cancer immunotherapy window can significantly shorten PFS. In view of the heterogeneity, it was analyzed that the cause might be caused by different cancer diseases. Furthermore, subgroup analysis of PFS based on different cancers (NSCLC, RCC and Melanoma) showed that there was no heterogeneity among studies in the NSCLC group (I^2 = 47%, P=0.13) and small heterogeneity among studies in the RCC group (I^2 = 84%, P=0.0003). There was no heterogeneity among studies in the Melanoma group (I^2 = 71%, P=0.06), as shown in **Figure 3**.

Effect of Concomitant ABX Use on OS of ICIs

We obtained OS data from 11 studies and conducted heterogeneity analysis(I^2 = 37%, P=0.08). There was no statistical heterogeneity between studies and fixed effect model was used for analysis. The results showed that HR=1.46 (95% CI=1.32-1.61, P < 0.00001), suggesting that the application of antibiotics in the immunotherapy window of cancer patients can significantly shorten OS, as shown in **Figure 4**.

Sensitivity Analysis

The pooled HRs for PFS were not significantly different after excluding one study at a time in the sensitivity analysis, ranging from 1.52 [95% CI=1.29-1.80, after excluding KOSUKE's study (14)] to 1.67 (95%CI=1.37-2.02, after excluding Laura M.Chambers's study (20)). Moreover, the pooled HRs for OS also did not significantly change in the sensitivity analysis.

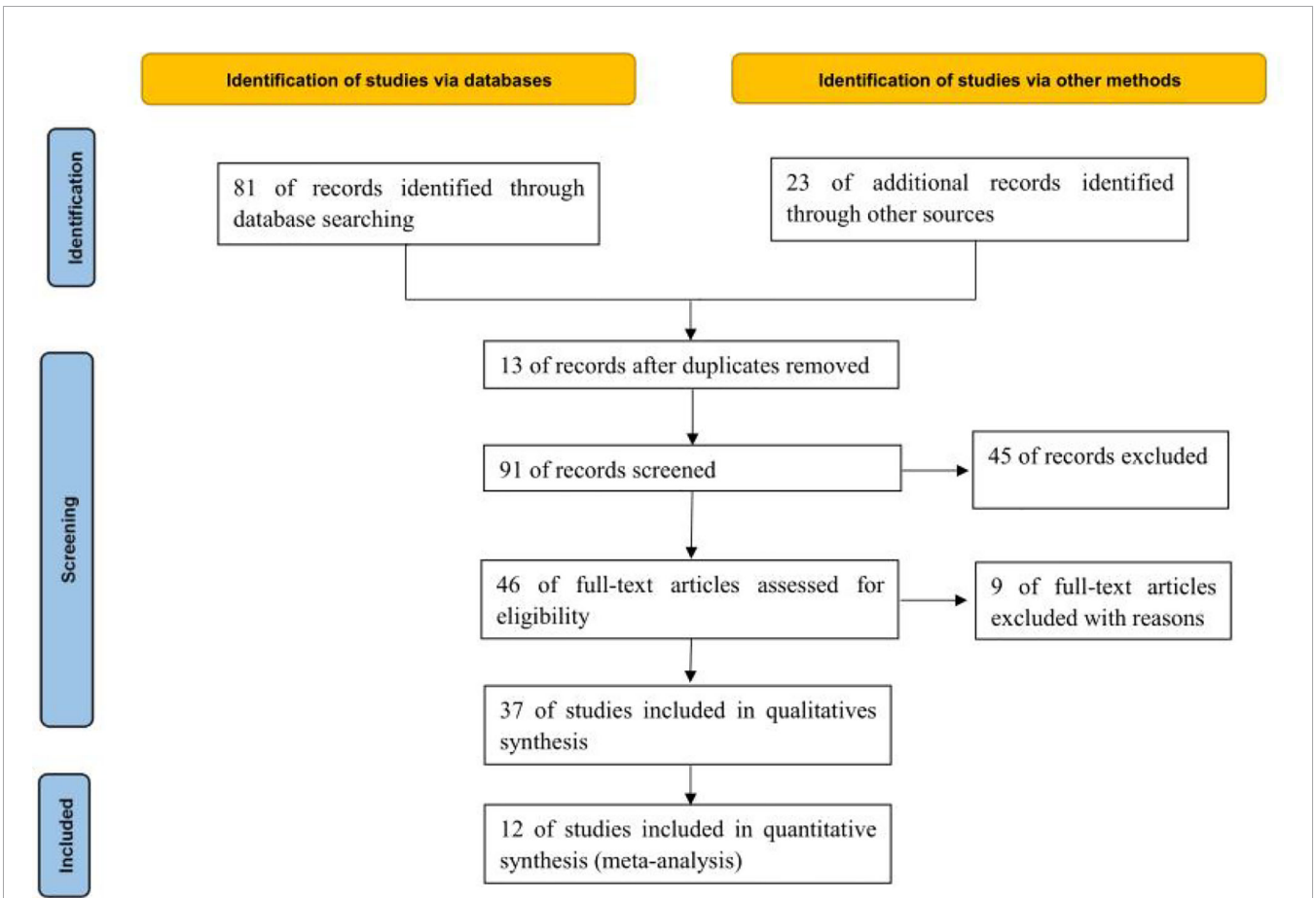

FIGURE 1 | PRISMA Flow chart of article selection.

TABLE 1 | Basic characteristics of included studies.

First Author	Year	Journal	Country	Type of Study	Type of Cancer	Patients (ATB+/ATB-)	mPFS,ABX+ vs ABX- (months)	mOS,ABX+ vs ABX-(months)	HR for PFS [95% CI]	p-Value for PFS	HR for OS [95% CI]	p-Value for OS	Quality
Umang Swami (11)	2020	Antibiotics	USA	Retrospective	Melanoma	30/169	NA	NA	1.28 [0.80,2.04]	0.30	1.73 [1.00,2.99]	0.05	7
Cortellini (12)	2021	Annals of oncololgy	UK	Retrospective	NSCLC	47/302	5.6 vs 6.3	11.2 vs 16.6	1.25 [0.84,1.84]	0.26	1.63 [0.99,2.68]	0.05	7
KAZUYUKI HAMADA (13)	2021	Anticancer Research	Japan	Retrospective	NSCLC	18/69	6.4 vs 19.9	20.6 vs 72.8	3.16 [1.55,6.25]	0.002	1.99 [0.91,4.09]	0.082	7
KOSUKE UEDA (14)	2019	Anticancer Research	Japan	Retrospective	RCC	5/31	2.8 vs 18.4	NA	6.52 [1.86,21.42]	0.0004	NA	NA	7
Anne Schett (15)	2019	Cancer Chemotherapy and Pharmacology	Switzerland	Retrospective	NSCLC	33/218	1.4 vs 5.5	1.8 vs 15.4	1.27 [0.94,1.71]	0.12	1.74 [1.24,2.44]	0.001	7
Lalani-1 (16)	2019	European Urology Oncology	Canada	Retrospective	RCC	31/146	NA	NA	1.96 [1.20,3.20]	0.007	1.44 [0.75,2.77]	0.27	7
Lalani-2 (16)	2019	European Urology Oncology	Canada	Retrospective	RCC	709/3435	NA	NA	1.16 [1.04,1.30]	0.008	1.25 [1.10,1.41]	0.001	
Chirayu Mohindroo (17)	2020	Cancer Medicine	USA	Retrospective	PDAC	209/580	4.4 vs 2.0	13.3 vs 9.0	2.08 [1.44,3.01]	0.0001	2.08 [1.44,3.01]	0.0001	7
Arielle Elkrief (18)	2019	OncoImmunology	Canada	Retrospective	Melanoma	10/74	2.4 vs 7.3	10.7 vs 18.3	3.57 [1.36,9.40]	0.01	1.92 [0.76,4.87]	0.17	7
L. Derosa-1 (19)	2018	Annals of oncololgy	France	Retrospective	NSCLC	48/239	1.9 vs 3.8	7.9 vs 24.6	1.5 [1.0,2.2]	0.03	4.4 [2.6,7.7]	0.01	7
L. Derosa-2 (19)	2018	Annals of oncololgy	France	Retrospective	RCC	16/121	1.9 vs 7.4	17.3 vs 30.6	3.1 [1.4,6.9]	0.01	3.5 [1.1,10.8]	0.03	
Laura M. Chambers (20)	2021	Gynecologic Oncology	USA	Retrospective	GC	58/101	7.3 vs 6.8	11.6 vs 19.5	0.96 [0.59,1.54]	0.85	1.20 [0.70,2.09]	0.51	7
Nadina Tinsley (21)	2020	The Oncologist	UK	Retrospective	NSCLC, others	92/291	3.1 vs 6.3	10.4 vs 21.7	1.401 [1.028,1.920]	0.033	1.4723 [1.038,2.107]	0.033	7
Hyunho Kim (22)	2019	BMC Cancer	Korea	Retrospective	NSCLC, others	108/234	2.0 vs 4.0	5.0 vs 17.0	1.715 [1.264,2.326]	0.001	1.785 [1.265,2.519]	0.001	7

NSCLC, Non-small cell lung cancer; RCC, Renal cell carcinoma; PDAC, Pancreatic ductal adenocarcinoma; GC, Gynecological cancer; PFS, Progression free survival; OS,Overall survival; ABX, Antibiotics; ABX+, Antibiotics exposure; ABX-, No antibiotics exposure; HR, Hazard ratio; NA, Not available.

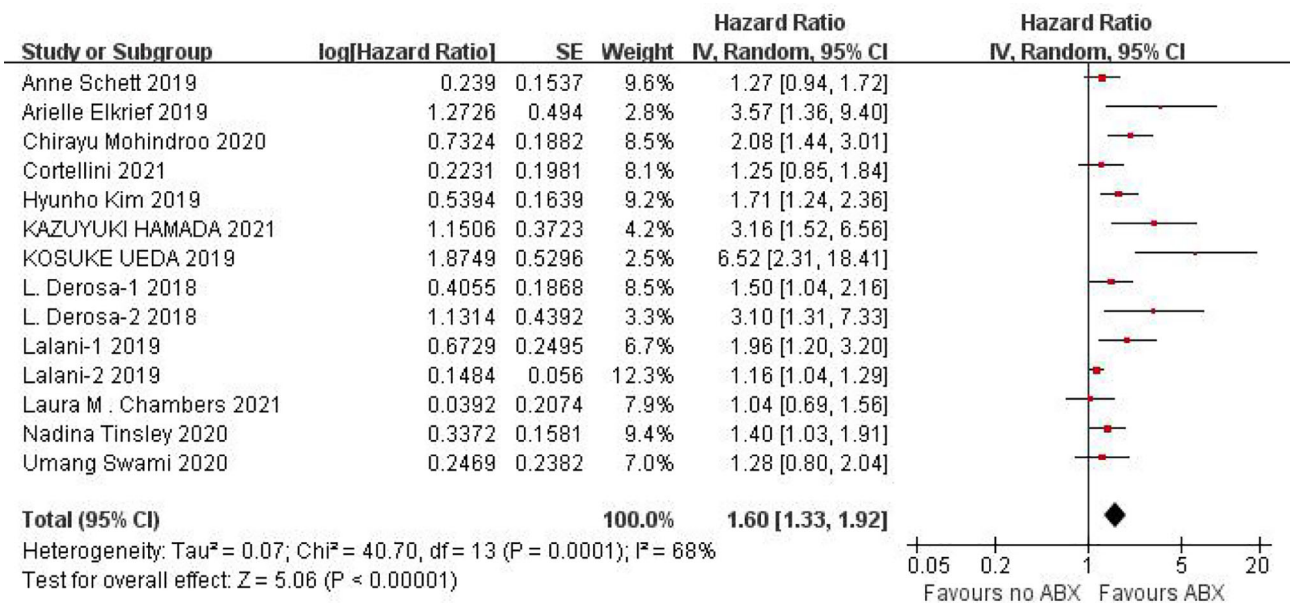

FIGURE 2 | Meta-analysis results of PFS between antibiotics exposed group and non-exposed group.

The overall HRs ranged from 1.42 [95%CI=1.29-1.57, after omitting Chirayu Mohindroo's study (17)] to 1.47 [95% CI=1.33-1.62, omitting Laura M. Chambers's study (20)].

Publication Bias

While performing Meta-analysis and comparison of PFS and OS data indicators, an inverted funnel plot was drawn for the included studies. The results showed that PFS has publication bias. Analysis of the reasons may be caused by different types of cancer. Therefore, it is necessary to conduct subgroup analysis and discussion. The OS funnel plot was symmetrical and mainly concentrated in the middle and upper part. Only a few studies may be less rigorous in design, poor research methods and other factors lead to the outside of the inverted funnel chart, suggesting a small bias, as shown in **Figures 5** and **6**.

DISCUSSION

Immunotherapy has now become one of the important and effective treatment methods for various cancers. In the first-line anti-tumor treatment, KEYNOTE024 (23) and KEYNOTE042 (24) clinical studies have shown that pembrolizumab single-agent contrast chemotherapy can significantly prolong the PFS and OS of PD-L1 (TPS≥50%) NSCLC patients; Keynote-021 (25), Keynote-189 (26), Keynote-407 (27) found that pembrolizumab combined with chemotherapy compared with chemotherapy can significantly prolong the PFS and OS of patients. With the advent of different types of immune checkpoint inhibitors and their gradual introduction into health insurance coverage, the total cost of immunotherapy for cancer patients has gradually decreased, thus enabling an increasing number of cancer patients to benefit from immunotherapy (28, 29). In the era of precision treatment, it is necessary to continue finding ways to further improve the clinical efficacy of immune checkpoint inhibitors.

In recent years, researchers have gradually realized that gut microbes may be a key factor in improving the prognosis of cancer patients (30–32). A lot of evidence shows that the application of ABX is related to the clinical efficacy of cancer immunotherapy. Gajewski et al. (33) found that bifidobacteria enhanced the anti-tumor effect of PD-L1 inhibitors in experimental mice models. In 2018, the team analyzed the composition of the fecal flora of 42 patients with metastatic melanoma, further revealing that the composition of the intestinal flora is significantly related to the effectiveness of PD-1 inhibitor immunotherapy (34). The influence of gut microbes on the efficacy of ICIs has become a research hotspot. However, in patients treated with ICIs, the predictive role of ABX exposure remains unclear. In this study, we evaluated the impact of ABX on the survival of cancer patients treated with ICIs based on multiple tumor types (including NSCLC, melanoma, RCC, etc.) and different dimensions. The results showed that the combined use of ABX is associated with the shortened PFS and OS, and ABX may be a negative prognostic factor for malignant tumors treated with ICIs.

The influence mechanism of ABX on ICIs response is as follows: First of all, the inherent anti-inflammatory effects of ABX, such as quinolone drugs can reduce the levels of pro-inflammatory cytokines (such as interleukin-1, tumor necrosis factor-α) and macrolide drugs, reduce T cell responses, and thereby ICIs have a potential antagonistic effect (35). Secondly, the modification of the intestinal microbiota by ABX will lead to the selection of bacterial species, which will have a negative impact on the response of ICIs. In animals, the transplantation of certain "favorable" bacteria can

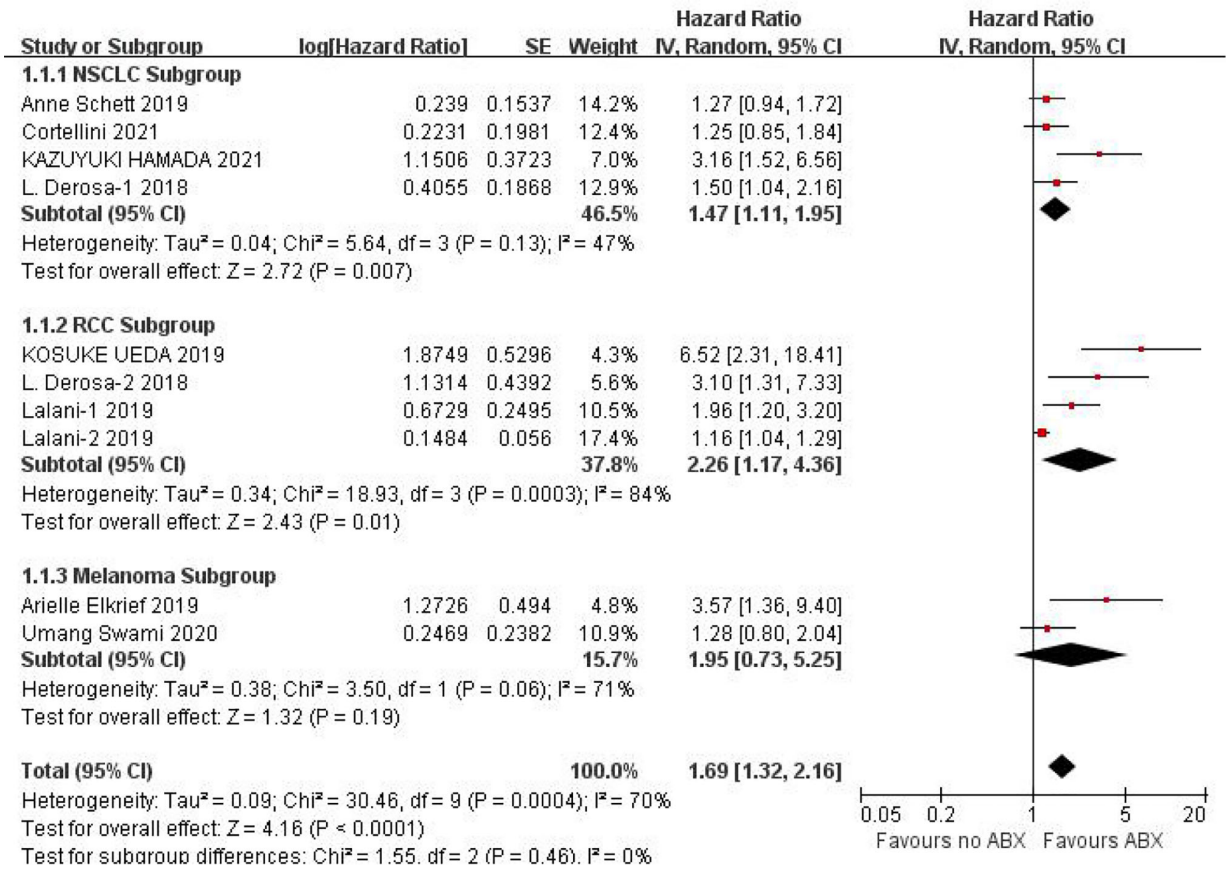

FIGURE 3 | Meta-analysis results of PFS subgroups between antibiotics exposed group and non-exposed group.

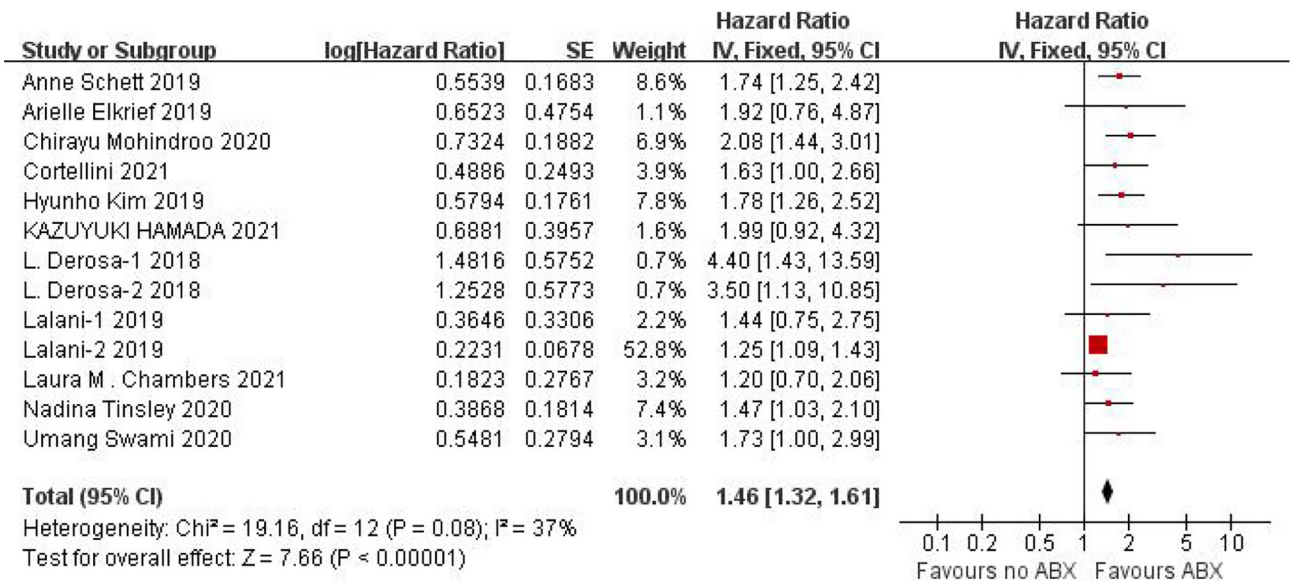

FIGURE 4 | Meta-analysis results of OS between antibiotics exposed group and non-exposed group.

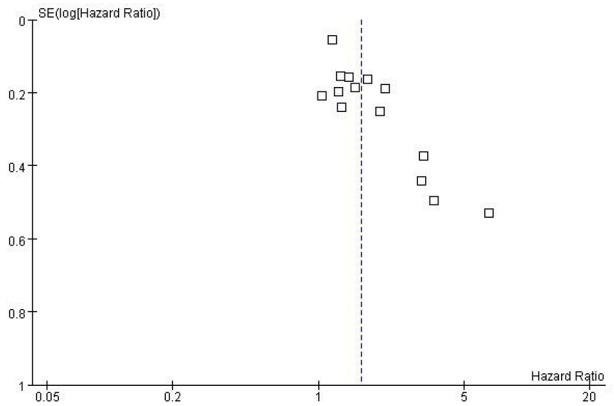

FIGURE 5 | Funnel Plot of PFS.

restore the response to ICIs after broad-spectrum antibiotic treatment (23). Third, the use of ABX affects the diversity of intestinal microbes, which is related to the negative reaction of anti-PD-1 immunotherapy (36). Finally, some ABX independent of ICIs may also have an inherent negative effect on the clinical course of malignant tumors by promoting canceration and metastasis (37).

Due to the poor physical condition and low immunity of cancer patients, the incidence of infection is relatively high, and the probability of using antibiotics is relatively high. Due to the poor physical condition and low immunity of cancer patients, the incidence of infection and the use of antibiotics are relatively higher. This study shows that the application of antibiotics during ICIs treatment of cancer can shorten the PFS (HR=1.60, 95% CI=1.33-1.92, P<0.00001) and OS (HR=1.46, 95%CI=1.32-1.61, P<0.00001) of cancer patients, the results are significantly different. In view of the small heterogeneity of PFS, we analyzed that its source may be related to different cancer types, so we conducted subgroup analysis according to cancer types. The results of subgroup analysis showed that NSCLC (HR=1.47, 95%CI=1.11-1.95, P=0.007), RCC (HR=2.26, 95%CI=1.17-4.36, P=0.01), melanoma (HR=1.95, 95% CI=0.73-5.25, P=0.19). There is no heterogeneity among the studies in the NSCLC group (I^2 = 47%, P=0.13), there is little heterogeneity among the studies in the RCC group (I^2 = 84%, P=0.0003), and there is no heterogeneity among the studies in the Melanoma group Heterogeneity (I^2 = 71%, P=0.06).

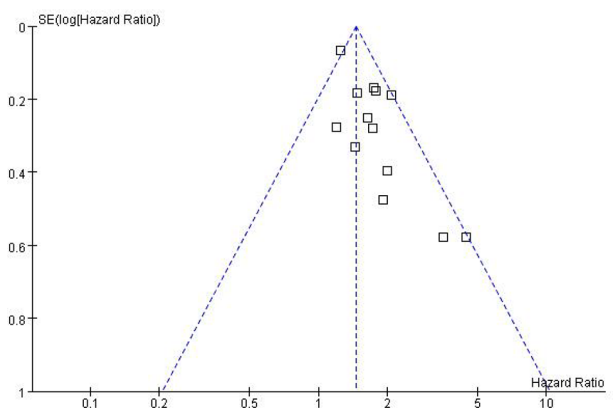

FIGURE 6 | Funnel Plot of OS.

However, this study also has some limitations. First, our research is essentially based on a meta-analysis of available data from published literature. Although we have made a lot of efforts to collect as much information as possible, many important details of the included studies, such as heterogeneous populations, tumor types, and patient characteristics have limited our further analysis to a certain extent and affected our results. In addition, due to the rare sequencing evidence, we have not been able to discuss the microbiome changes of patients receiving ABX before and/or during ICI treatment. This requires metagenomic analysis on the basis of sufficient samples to resolve. Second, there is the potential publication bias in this study, although it cannot significantly influence the conclusions. We attribute this limitation to three reasons: ①Incorporating more positive results research, rather than negative/contrary results; ②Sample size; ③Features of follow-up and included population. Third, in retrospective analysis, inherent factors such as patient selection, treatment methods, and drug type/dose affect the heterogeneity of the study. On the basis of sufficient literature, this restriction is expected to be improved through stricter inclusion. Fourth, we did not investigate the correlation between ABX administration and ICI adverse events, which is worth emphasizing in future work. Fifth, due to the study design, impact of other pertinent clinical variables such as age, gender, BMI, PPI use, etc, could not be examined. Finally, in terms of tumor types, our current research mainly focuses on lung cell carcinoma, renal cell carcinoma and melanoma, so we should pay more attention to other solid tumors, such as gastrointestinal or esophageal tumors in the future.

In conclusion, this study evaluated the effect of concomitant ABX use on ICI efficacy in advanced cancer patients by systematically reviewing the relevant literature. The findings demonstrated that ABX use during ICIs treatment of cancer may significantly shorten PFS and OS, and adversely affect the drugs efficacy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

AUTHORS CONTRIBUTIONS

We declare that all authors made fundamental contributions to the manuscript. All authors contributed to the study conception

and design. Database search and data analysis was conducted by SJ, SG, and QC. Study selection and data extraction were performed by CZ, MC, YY, and SZ. The manuscript was written by SJ and SG. NS and MD reviewed the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by The Key Program of Harbin Medical University Cancer Hospital Haiyan Fund (no. JJZD2019-03) and The General Program of Harbin Medical University Cancer Hospital Haiyan Fund (no. JJMS2021-25).

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frontiers in Pharmacology

ORIGINAL RESEARCH
published: 17 February 2022
doi: 10.3389/fphar.2022.845261

Adjuvant Chemotherapy in pT2N0M0 Gastric Cancer: Findings From a Retrospective Study

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OPEN ACCESS

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: 29 December 2021
Accepted: 04 February 2022
Published: 17 February 2022

Citation:
Mei Y, Feng X, Feng T, Yan M, Zhu Z, Li T and Zhu Z (2022) Adjuvant Chemotherapy in pT2N0M0 Gastric Cancer: Findings From a Retrospective Study. Front. Pharmacol. 13:845261. doi: 10.3389/fphar.2022.845261

Background: There is no global consensus on adjuvant chemotherapy (ACT) for pT2N0M0 gastric cancer. We conducted a retrospective study to reveal the role of ACT in such patients.

Methods: Patients with pT2N0M0 gastric cancer who underwent radical resection with D2 lymphadenectomy for primary gastric cancer between January 2012 and May 2016 were included. Kaplan–Meier and Cox regression were used to evaluate overall survival (OS), disease-specific survival (DSS) and predictors of prognosis. Stratified analysis based on high-risk factors was conducted.

Results: Of enrolled 307 patients, 111 patients underwent surgery alone and 196 patients received ACT. Surgery alone (HR = 2.913, 95% CI: 1.494-5.682, p = 0.002) and total gastrectomy (HR = 2.445, 95% CI: 1.279-4.675, p = 0.007) were independently associated with decreased OS. With the median follow-up of 73.1 months, the 5-year OS rate was 87.9% and 5-year DSS rate was 91.8%. Patients receiving ACT showed a better 5-year OS rate (92.9 *vs*. 79.3%, $p < 0.001$) and DSS rate (96.8 vs. 83.0%, $p < 0.001$) than patients underwent surgery alone. Patients receiving monotherapy (n = 130) had a relatively poor prognosis compared to patients receiving dual-drug (n = 66) without a significant difference (92.3 *vs*. 93.9%, p = 0.637). In patients without high-risk factors based on the Chinese Society of Clinical Oncology (CSCO) Guidelines, ACT also provided survival benefit (96.0 vs 82.9%, p = 0.038).

Conclusions: ACT was accompanied with higher 5-year OS and DSS rates of patients with pT2N0M0 gastric cancer. Patients with pT2N0M0 gastric cancer, regardless of high-risk factors based on the CSCO guidelines, might be considered candidates for ACT. In regard to the therapy regimen, monotherapy might be the optimal choice, considering the adverse events.

Keywords: gastric cancer, pT2N0M0, adjuvant chemotherapy, surgery alone, prognosis

INTRODUCTION

Gastric cancer is the fourth leading cause of death from malignant tumors worldwide and the third main cause of cancer death in China (Cao et al., 2021; Navashenaq et al., 2021; Sun et al., 2021; Zeng and Jin, 2021). Despite the incidence of gastric cancer has reduced, gastric cancer related mortality has not changed (Sukri et al., 2020; Varon et al., 2021). Benefiting from advances in medical technology and the popularity of endoscopy, more and more gastric cancer is diagnosed at a relatively early stage. pT2N0M0 gastric cancer is defined as tumors infiltrating the muscularis propria without regional lymph node metastasis or distant metastasis based on the 8th edition of the AJCC TNM staging system for gastric cancer (Amin et al., 2017; Brierley et al., 2017).

Surgery is the only potential chance of cure for gastric cancer, but a certain percentage of patients relapse after curative surgery, which leads to a poor prognosis. Adjuvant chemotherapy (ACT) or chemoradiotherapy has been demonstrated to be beneficial in numerous clinical trials worldwide (Macdonald et al., 2001; Sasako et al., 2011; Noh et al., 2014; Park et al., 2015). Nevertheless, these trials did not report whether patients with less advanced disease would benefit from adjuvant therapy. There are few studies on patients with pT2N0M0 gastric cancer.

Consensus guidelines provide disparate recommendations. Based on the National Comprehensive Cancer Network (NCCN) Guidelines (version 1.2021, Gastric Cancer), options for pT2N0M0 gastric cancer patients after D2 lymph node dissection include surveillance or ACT. Patients with poorly differentiated or high-grade cancer, lymphovascular invasion, neural invasion or aged <50 years are candidates for ACT (National and Comprehensive, 2020). Meanwhile, observation without adjuvant therapy after curative resection is recommended for stage I (including T2N0M0) gastric cancer according to the Japanese Gastric Cancer Treatment Guidelines 2018 (5th edition) (Japanese Gastric Cancer A, 2020). ACT may decrease the risk of metastasis in high-risk pT2N0M0 patients, such as those aged <40 years or with high-grade or poorly differentiated tumor and nervous, lymphovascular invasion, based on the Chinese Society of Clinical Oncology (CSCO) Guidelines (version 1.2021, Gastric Cancer); however, it is unclear whether there is survival benefit of ACT for stage I gastric cancer ((Wang et al., 2021)).

Based on the 8th edition of the TNM staging system of gastric cancer, pT2N0M0 gastric cancer belongs to stage IB, which has good prognosis, with 5-year survival rate of approximately 80–90% after curative surgery ((He et al., 2018; Ji et al., 2018)). The recurrence rates for pT2N0M0 gastric cancer after resection range from 3 to 9% (Jin et al., 2015; Park et al., 2016). Considering the number of patients with stage I gastric cancer is increasing, several retrospective studies focused on the role of ACT in patients with pT2N0M0 gastric cancer and evaluated the high-risk factors of relapse and death; however, they reported diverse opinions on the effect of ACT on pT2N0M0 gastric cancer.

Since it was an open question whether ACT would benefit patients with pT2N0M0 gastric cancer, we aimed to determine the effect of ACT after curative resection in this study.

MATERIALS AND METHODS

Patients

All patients who underwent radical resection with D2 lymphadenectomy for primary gastric cancer and were ultimately diagnosed with pT2N0M0 gastric cancer based on the 8th edition of the AJCC TNM staging system for gastric cancer at Ruijin Hospital, Shanghai Jiao Tong University School of Medicine between January 2012 and May 2016 were reviewed. Patients with less than 16 harvested lymph nodes, other primary malignancies, prior gastric surgery, R1 or R2 surgical margins, age over 80 years, with postoperative complications; who died within 30 days of surgery; who were lost to follow-up and who received preoperative treatment were excluded. All surgeons had experience doing gastric surgery (>100 procedures per year) and the standard operating procedures were based on the principles of surgery of CSCO Guidelines. Finally, a total of 307 patients were included in this study (**Figure 1**). This study was approved by the Ruijin Hospital Ethics Committee, Shanghai Jiao Tong University School of Medicine, China (No. 2018-151).

Evaluation of Clinical Pathological Variables

Clinical pathological characteristics, including age, sex, tumor size, location, Borrmann type, differentiation, histopathology, invasion depth, number of examined lymph nodes, lymphovascular invasion, perineural invasion, resection patterns and treatment regimen after surgery were analyzed. Age was converted to categorical variable, and the cutoff value (40 years) was decided based on the high-risk factors according to the CSCO guidelines (Wang et al., 2019a). Tumor location was classed as the upper, middle, or lower third of the stomach. Tumor histopathology was reviewed based on the WHO classification of the digestive system tumors, 5th edition (WHO Classification of Tumours Editorial Board 2019). Histological type was divided into two groups: differentiated type (including well differentiated and moderately differentiated tubular adenocarcinoma) and undifferentiated type (including mucinous adenocarcinoma, signet ring cell carcinoma and poorly differentiated adenocarcinoma). The tumor invasion depth was divided into the superficial muscularis propria (sMP) layer and the deep muscularis propria (dMP) layer according to pathological examination (Sun et al., 2009). This category was based on the type of muscularis propria fibers; the transverse and longitudinal muscle layers were classified as the sMP and dMP layers, respectively. Lymphovascular invasion was defined as malignant cells appearing in a vascular wall structure or tubular space lined by endothelial cells. Perineural invasion was diagnosed when tumor cells were present in the perineural space of nerves. Total or subtotal gastrectomy was conducted

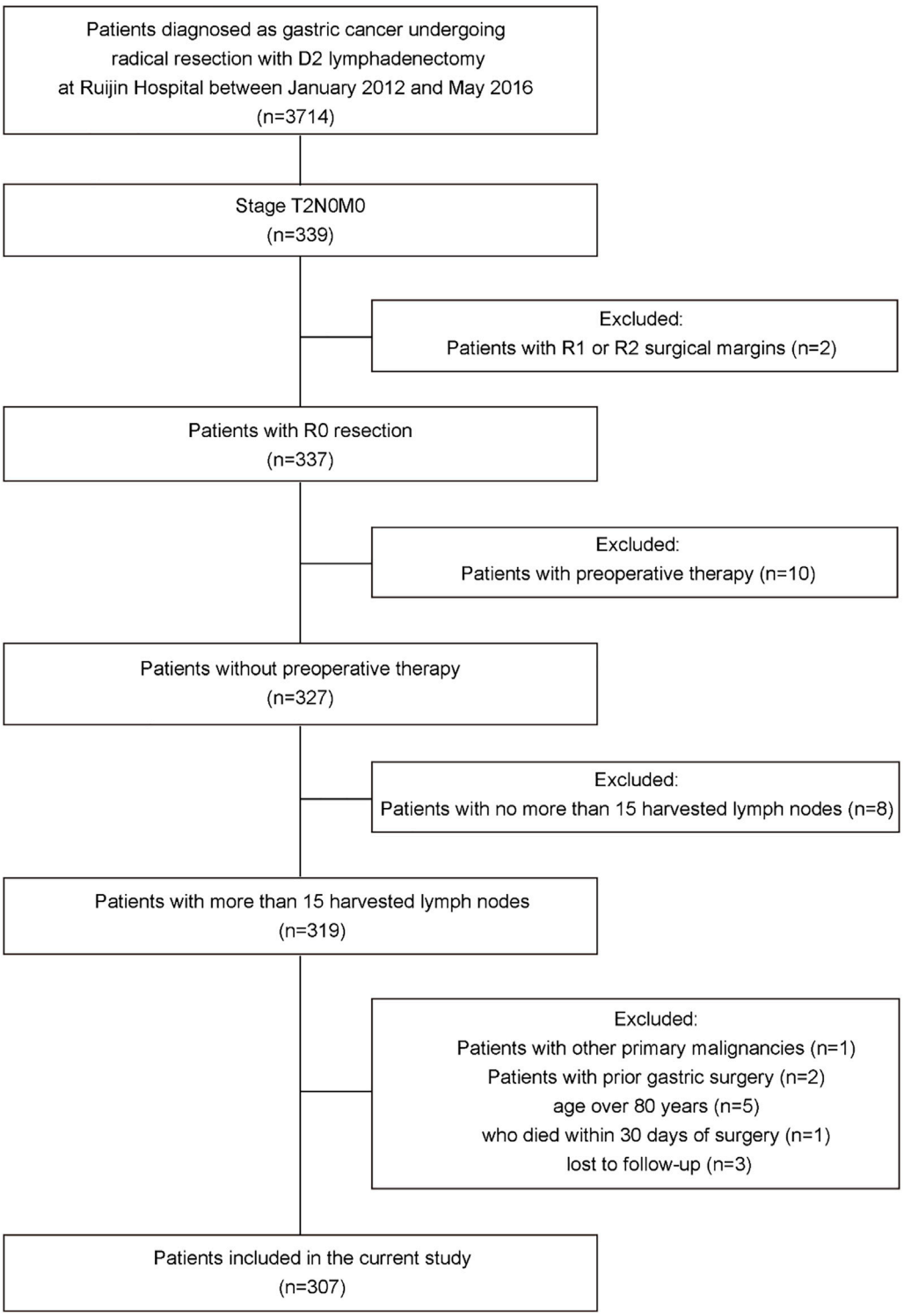

FIGURE 1 | Flowchart of patient selection process.

based on the tumor location. Two independent, experienced pathologists reviewed hematoxylin-eosin (H&E)-stained slides from each case. If the diagnosis of the two pathologists was inconsistent, a third pathologist was needed.

Treatment After Surgery

All patients received postoperative examinations within 3–4 weeks after surgery and patients who received ACT start therapy within 4–6 weeks after surgery. All patients

TABLE 1 | Clinical pathological characteristics of patients with pT2N0M0 gastric cancer.

Variables	Total (*n* = 307)	With high-risk factors (*n* = 216)	Without high-risk factors (*n* = 91)	*p* Value
Age (years)				0.022*
Median (IQRs)	63 (56, 71)	63 (55, 71)	64 (60, 71)	
<40	12 (3.9)	12 (5.6)	0	
≥40	295 (96.1)	204 (94.4)	91 (100)	
Sex				0.006*
Male	216 (70.4)	142 (65.7)	74 (81.3)	
Female	91 (29.6)	74 (34.3)	17 (18.7)	
Location				0.913
Upper	55 (17.9)	40 (18.5)	15 (16.5)	
Middle	46 (15.0)	32 (14.8)	14 (15.4)	
Lower	206 (67.1)	144 (66.7)	62 (68.1)	
Size (cm)				0.989
≤2.5	155 (50.5)	109 (50.5)	46 (50.5)	
>2.5	152 (49.5)	107 (49.5)	45 (49.5)	
Borrmann				0.661
I	39 (12.7)	26 (12.0)	13 (14.3)	
II	111 (36.2)	76 (35.2)	35 (38.5)	
III	157 (51.1)	114 (52.8)	43 (47.3)	
Differentiation				<0.001*
Differentiated	96 (31.3)	5 (2.3)	91 (100)	
Undifferentiated	211 (68.7)	211 (97.7)	0	
Histopathology				<0.001*
Tub	96 (31.3)	5 (2.3)	91 (100)	
Por	160 (52.1)	160 (74.1)	0	
Sig	32 (10.4)	32 (14.8)	0	
Muc	19 (6.2)	19 (8.8)	0	
Depth				0.356
sMP	163 (53.1)	111 (51.4)	52 (57.1)	
dMP	144 (46.9)	105 (48.6)	39 (42.9)	
Examined LNs (Median (IQRs))	22 (18, 29)	21 (18, 29)	23 (18, 28)	0.425
PNI	23 (7.5)	23 (10.6)	0	0.001*
LVI	33 (10.7)	33 (15.3)	0	<0.001*
Gastrectomy				0.652
Distal	224 (73.0)	156 (72.2)	68 (74.7)	
Total	83 (27.0)	60 (27.8)	23 (25.3)	
Postoperative treatment				0.035*
ACT	196 (63.8)	146 (67.6)	50 (54.9)	
SA	111 (36.2)	70 (32.4)	41 (45.1)	
ACT type				0.018*
Monotherapy	130 (66.3)	90 (61.6)	40 (80.0)	
Dual drug	66 (33.7)	56 (38.4)	10 (20.0)	

p < 0.05 was considered statistically significant.

High-risk factors including patients aged <40 years or with high-grade or poorly differentiated tumor and nervous, lymphovascular invasion, according to the CSCO Guidelines (version 1.2018, Gastric Cancer); Tub, tubular adenocarcinoma; Por, poorly differentiated adenocarcinoma; Sig, signet ring cell carcinoma; Muc, mucinous adenocarcinoma; sMP, superficial muscularis propria layer; dMP, deep muscularis propria layer; LNs, lymph nodes; IQRs, interquartile ranges; PNI, perineural invasion; LVI, lymphovascular invasion; ACT, adjuvant chemotherapy; SA, surgery alone.

included in my study was with adequate organ function for chemotherapy and PS 0-1. Decisions to administer ACT to patients with pT2N0M0 gastric cancer were based on the preference of surgeons or oncologists. Some doctors approve the Japanese guidelines, thereby they do not recommend postoperative chemotherapy for pT2N0M0 gastric cancer patients; some doctors follow the Chinese guidelines, so they recommend postoperative chemotherapy for patients with high-risk factors based on the CSCO guidelines. Patients with younger age, undifferentiated tumor, perineural or lymphovascular invasion were more likely to receive dual-drug regimen. Patients were given S-1 as monotherapy, while the dual-drug regimen included XELOX or SOX. S-1 was given as follows: 40mg/m2 p. o. b. i.d. day 1-day 14, Q3W for 1 year (Sasako et al., 2011). XELOX was given as six 3-week cycles of capecitabine (1,000 mg/m2 p. o. b. i.d. days 1–14) plus oxaliplatin (130 mg/m2 iv. day 1) (Noh et al., 2014). SOX was given as six 3-week cycles of S-1 (40 mg/m2 p. o. b. i.d. days 1–14) plus oxaliplatin (130 mg/m2 iv. day 1) (Park et al., 2021). Adverse events were assessed by

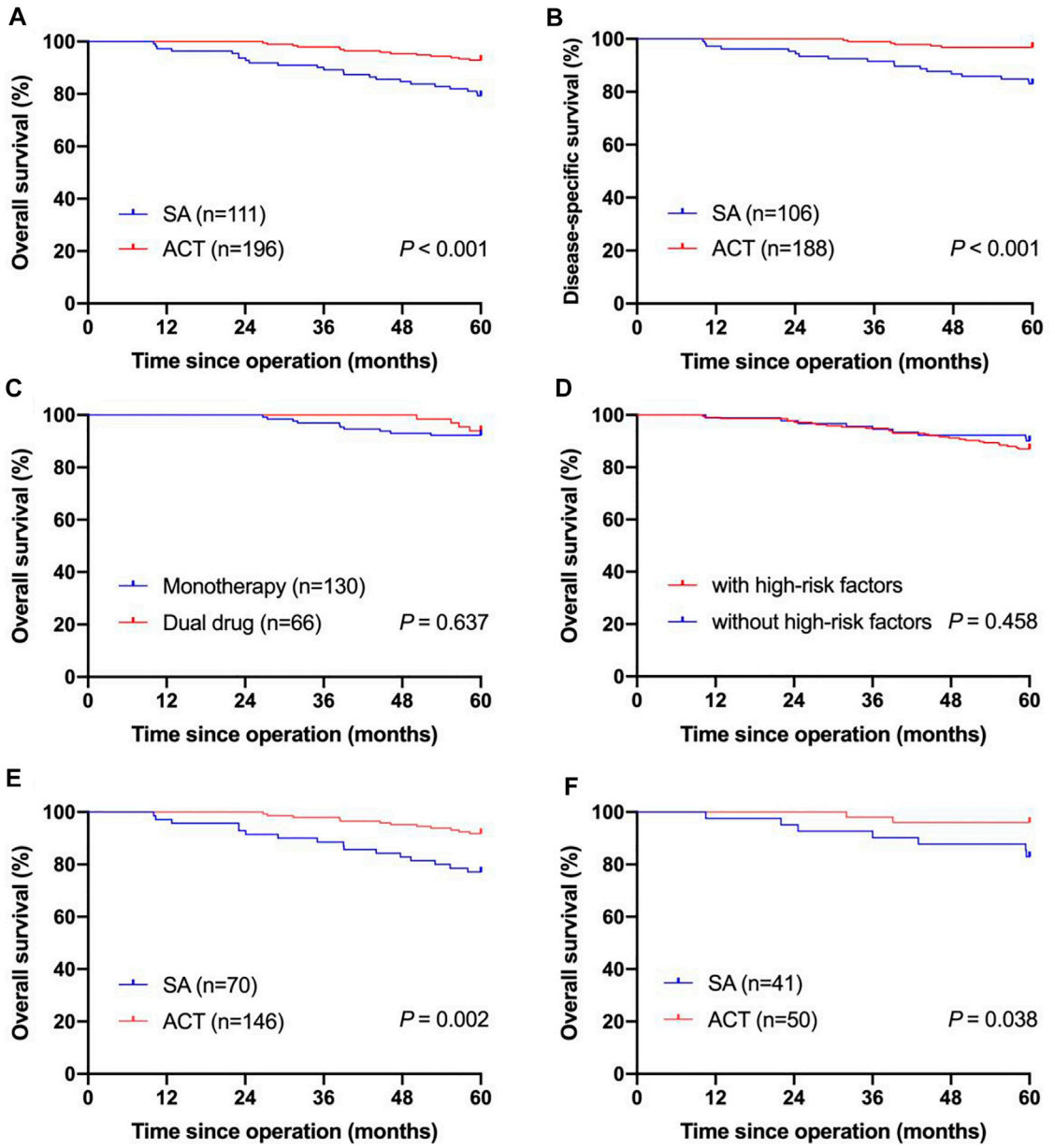

FIGURE 2 | Kaplan-Meier curves for survival of pT2N0M0 gastric cancer patients. **(A)** Kaplan-Meier curves of pT2N0M0 gastric cancer patients underwent SA and patients receiving ACT in the OS analysis; **(B)** Kaplan-Meier curves of pT2N0M0 gastric cancer patients underwent SA and patients receiving ACT in the DSS analysis; **(C)** Kaplan-Meier curves of pT2N0M0 gastric cancer patients who received monotherapy and patients who received the dual-drug regimen in the OS analysis; **(D)** Kaplan-Meier curves of pT2N0M0 gastric cancer patients with high-risk factors and patients without high-risk factors in the OS analysis; **(E)** Kaplan-Meier curves of pT2N0M0 gastric cancer patients with high-risk factors who underwent SA and who received ACT in the OS analysis; **(F)** Kaplan-Meier curves of pT2N0M0 gastric cancer patients without high-risk factors who underwent SA and who received ACT in the OS analysis; SA, surgery alone; ACT, adjuvant chemotherapy; OS, overall survival; DSS, disease-specific survival.

the Common Terminology Criteria for Adverse Events (version 5.0). Dose reduction or interruption were allowed if patients had adverse events of grade 3 or 4. Patients underwent surgery alone accepted no anticancer therapy until recurrence. When cancer relapse was observed, first-line treatment was administered.

Follow-Up

Outpatient follow-up was conducted every 3 months in the first 2 years and every 6 months for the next 3 years and included a physical examination, blood tests, and tumor markers. Chest-abdomen-pelvis CT and endoscopy were performed every 6 months. Liver MRI, bone scans and PET were optional. The

TABLE 2 | Univariate and Cox-regression of overall survival of patients with pT2N0M0 gastric cancer.

Variables	5-year OS rate (%)	*p* Value	Cox-regression		
			HR	95% CI	*p* Value
Age (years)		0.665			
<40	91.7				
≥40	87.8				
Sex		0.126			
Male	86.1				
Female	92.3				
Location		0.773			
Upper	85.5				
Middle	87.0				
Lower	88.8				
Size (cm)		0.399			
≤2.5	86.5				
>2.5	89.5				
Borrmann		0.627			
I	89.7				
II	85.6				
III	89.2				
Differentiation		0.339			
Differentiated	90.6				
Undifferentiated	86.7				
Histopathology		0.776			
Tub	90.6				
Por	86.3				
Sig	87.5				
Muc	89.5				
Depth		0.327			
sMP	89.6				
dMP	86.1				
Examined LNs		0.556			
LVI		0.273			
Negative	87.2				
Positive	93.9				
PNI		0.166			
Negative	88.7				
Positive	78.3				
Gastrectomy		0.001*			
Distal	91.5				
Total	78.3		2.445	1.279-4.675	0.007*
Postoperative treatment		<0.001*			
SA	79.3		2.913	1.494-5.682	0.002*
ACT	92.9				
ACT type		0.637			
Monotherapy	92.3				
Dual drug	93.9				

*p < *0.05 was considered statistically significant.*

Tub, tubular adenocarcinoma; Por, poorly differentiated adenocarcinoma; Sig, signet ring cell carcinoma; Muc, mucinous adenocarcinoma; sMP, superficial muscularis propria layer; dMP, deep muscularis propria layer; LNs, lymph nodes; PNI, perineural invasion; LVI, lymphovascular invasion; ACT, adjuvant chemotherapy; SA, surgery alone; HR, hazard ratio; 95% CI, 95% confidence interval.

follow-up lasted at least 5 years after surgery or until censoring date or death.

Statistical Analysis

Continuous variable is shown as median with interquartile ranges (IQRs), and categorical variable is presented as number with proportions. Categorical variable was analyzed using Fisher's exact test or chi square test. DSS was defined as the time of surgery to death from gastric cancer. The 5-year OS and DSS rates were calculated using the Kaplan-Meier curve, and differences were analyzed by the log-rank test. Independent predictors of survival were found by Cox-regression survival analysis. Hazard ratio (HR) > 1 was related to a higher hazard of death. A p value < 0.05 was considered as statistically significant. SPSS version 22.0 for Windows (IBM Corporation, Armonk, NY, United States) was used for statistical analysis.

RESULTS

Clinical Pathological Features of Patients With pT2N0M0 Gastric Cancer

A total of 307 patients with pT2N0M0 gastric cancer were enrolled in this study. The clinical pathological characteristics

are shown in **Table 1**. The age ranged from 29 to 80 years, with a median age of 63 years. Most of patients were male (n = 216, 70.4%). The median tumor size was 2.5 cm. Size was converted to categorical variable, and the cutoff value was median size. Tumors were more likely located in the lower 1/3 of the stomach (n = 206, 67.1%) and presented as the Borrmann III type (n = 151, 51.1%) and undifferentiated type (n = 211, 68.7%). The median number of harvested lymph nodes was 22 with a range from 16 to 68. Twenty-three patients had perineural invasion and 33 patients had lymphovascular invasion. 63.8% of patients received ACT, 130 patients received monotherapy and 66 patients were given dual drug treatment.

Long-Term Outcomes and Effect of ACT on Prognosis in pT2N0M0 Gastric Cancer Patients

As of May 2021, the median follow-up was 73.1 months, ranging from 10 to 112.9 months. In our study, 5-year OS rate of all patients was 87.9%. Kaplan–Meier survival analysis showed that the 5-year OS rate was higher in patients who received ACT (92.9%) compared with those who underwent surgery alone (79.3%, $p < 0.001$, **Figure 2A**). In the Cox-regression analysis, independent predictors of decreased OS were surgery alone (HR = 2.913, 95% CI: 1.494-5.682, $p = 0.002$) and total gastrectomy (HR = 2.445, 95% CI: 1.279-4.675, $p = 0.007$, **Table 2**).

The 5-year DSS rate of enrolled patients was 91.8% when excluding 13 patients who did not die from gastric cancer. **Supplementary Table S1** shows the clinical pathological characteristics of patients enrolled in the DSS analysis. Patients receiving ACT showed a better 5-year DSS rate (96.8 vs. 83.0%, $p < 0.001$) than patients underwent surgery alone with significant difference (**Figure 2B**). In the Cox-regression analysis, surgery alone (HR = 5.052, 95% CI: 1.993-12.809, $p = 0.001$) and total gastrectomy (HR = 2.820, 95% CI: 1.256-6.329, $p = 0.012$) were independently associated with decreased OS (**Supplementary Table S2**). **Supplementary Table S4** shows the dominant recurrence sites in patients who died of gastric cancer relapse.

Effect of the ACT Regimen on Prognosis in pT2N0M0 Gastric Cancer

Of 196 patients received ACT, 130 patients received monotherapy, and 66 patients received dual-drug chemotherapy. **Supplementary Table S3** shows the clinical pathological variables of patients who were given different chemotherapy regimens. The clinical pathological characteristics between the two groups were comparable, except for age, differentiation, lymphovascular invasion and perineural invasion. Most patients with lymphovascular invasion or perineural invasion received dual-drug chemotherapy, and the 5-year OS rate of the dual-drug subgroup reached 93.9%, while the monotherapy subgroup had a relatively poor prognosis, without a significant difference (92.3%, $p = 0.637$, **Figure 2C**).

Grade 5 adverse events did not occur. The main grade 3 or 4 adverse events were anemia (9.2%), anorexia (6.9%) diarrhea (4.6%) in the monotherapy group and neutropenia (15.2%), peripheral neuropathy (12.1%), anorexia (7.6%) and anemia (4.5%) in the dual-drug regimen group.

Stratification by High-Risk Factors According to the CSCO Guidelines

According to the CSCO guidelines, high-risk factors include patients aged <40 years or with high-grade or poorly differentiated tumor and nervous, lymphovascular invasion. Seventy-three patients had high-risk factors and thirty-four patients did not have high-risk factors. The clinical pathological features of patients stratified by high-risk factors was showed in **Table 1**. The 5-year OS rate was lower in patients with high-risk factors (87.0%) compared with those without high-risk factors (90.1%), whereas the difference was not statistically significant ($p = 0.458$, **Figure 2D**).

In patients with high-risk factors, gastrectomy type and postoperative therapy were concerned with prognosis in the univariate analysis. In patients with high-risk factors, the 5-year OS rate of patients received ACT was significantly higher than that of patients underwent surgery alone (91.8 vs 77.1%, $p = 0.002$, **Figure 2E**). In the Cox-regression analysis, surgery alone (HR = 3.130, 95% CI: 1.480-6.620, $p = 0.003$) and total gastrectomy (HR = 3.303, 95% CI: 1.571-6.947, $p = 0.002$) were independently associated with decreased OS (**Table 3**). In patients without high-risk factors, the 5-year OS rate of patients received ACT was also significantly higher than that of patients underwent surgery alone (96.0 vs 82.9%, $p = 0.038$, **Figure 2F**). Thus, ACT could not only increase the 5-year survival rate of patients with high-risk factors, but also increase the 5-year survival rate of patients without high-risk factors.

In patients without high-risk factors, 40 patients received monotherapy, 10 patients received dual-drug regimen. The 5-year OS rate was 95.0% for the monotherapy subgroup and 100% for the dual-drug subgroup without significant difference ($p = 0.477$, **Table 3**). In patients with high risk factors, 90 patients received monotherapy, 56 patients received dual-drug regimen. The 5-year OS rate was 91.1% for the monotherapy subgroup and 92.9% for the dual-drug subgroup without significant difference ($p = 0.664$, **Table 3**).

DISCUSSION

ACTS-GC trial (Sasako et al., 2011) demonstrated that patients with stage II/III gastric cancer could significantly benefit from adjuvant S-1. CLASSIC trial (Noh et al., 2014) also showed survival benefit of adjuvant XELOX for stage II/III gastric cancer patients. ARTIST II trial (Park et al., 2021) showed that adjuvant SOX was more effective than S-1 in patients with node positive, stage II/III gastric cancer. Exiting prospective randomized clinical trials demonstrating the benefit of ACT could not explain whether all gastric cancer patients (especially stage IB gastric cancer) would benefit from ACT. Although the prognosis of pT2N0M0 gastric cancer is

TABLE 3 | Univariate and Cox-regression of overall survival of patients with pT2N0M0 gastric cancer stratified by high-risk factors.

Variables	With high-risk factors					Without high-risk factors	
	5-year OS rate	*P* value	Cox-regression			5-year OS rate	*P* value
			HR	95% CI	*P* value		
Age (years)		0.601				—	—
< 40	91.7%						
≥ 40	86.8%						
Sex		0.122					0.534
Male	84.5%					89.2%	
Female	91.9%					94.1%	
Location		0.479					0.797
Upper	82.5%					93.3%	
Middle	84.4%					92.9%	
Lower	88.9%					88.7%	
Size (cm)		0.420					0.754
≤ 2.5	85.3%					89.1%	
> 2.5	88.8%					91.1%	
Borrmann		0.654					0.311
I	92.3%					84.6%	
II	85.5%					85.7%	
III	86.8%					95.3%	
Differentiation		0.399				—	—
Differentiated	100%						
Undifferentiated	86.7%						
Histopathology		0.829				—	—
Tub	100%						
Por	86.3%						
Sig	87.5%						
Muc	89.5%						
Depth		0.536					0.410
sMP	88.3%					92.3%	
dMP	85.7%					87.2%	
Examined LNs		0.927					0.210
LVI		0.211				—	—
Negative	85.8%						
Positive	93.9%						
PNI		0.223				—	—
Negative	88.1%						
Positive	78.3%						
Gastrectomy		0.001*					0.552
Distal	91.7%					91.2%	
Total	75.0%		3.303	1.571-6.947	0.002*	87.0%	
Postoperative treatment		0.002*					0.038*
SA	77.1%		3.130	1.480-6.620	0.003*	82.9%	
ACT	91.8%					96.0%	
ACT type		0.664					0.477
Monotherapy	91.1%					95.0%	
Dual drug	92.9%					100%	

*p < *0.05 was considered statistically significant.*

High-risk factors including patients aged <40 years or with high-grade or poorly differentiated tumor and nervous, lymphovascular invasion, according to the CSCO Guidelines (version 1.2018, Gastric Cancer); Tub, tubular adenocarcinoma; Por, poorly differentiated adenocarcinoma; Sig, signet ring cell carcinoma; Muc, mucinous adenocarcinoma; sMP, superficial muscularis propria layer; dMP, deep muscularis propria layer; LNs, lymph nodes; PNI, perineural invasion; LVI, lymphovascular invasion; ACT, adjuvant chemotherapy; SA, surgery alone; HR, hazard ratio; 95% CI, 95% confidence interval.

relatively good in general, postoperative relapse still occurs in some patients with various recurrence sites.

In the current study, we found a good prognosis of pT2N0M0 gastric cancer, with the 5-year OS rate of 87.9% and 5-year DSS rate of 91.8%, similar to other studies ((In et al., 2016; Park et al., 2016)).

Some retrospective studies identified risk factors in stage I gastric cancer patients. The authors of a Korean study focusing on stage I gastric cancer reported that age, sex, stage IB, lymphatic vessel invasion, nerve invasion and a high serum carcinoembryonic antigen level, were independent prognostic factors (Caccialanza et al., 2016; Liu et al., 2016). A population-based study using the Surveillance, Epidemiology, and End Results (SEER) database demonstrated that older age, proximal tumor location, high tumor grade and large tumor size were independent factors of poor disease-related survival (Gold et al., 2013). Other studies found that several clinical pathological factors were significantly associated with a high risk of relapse and

death in pT2N0M0 gastric cancer patients and suggested that patients with high-risk factors receive ACT. A further Chinese study identified the upper 1/3 of the stomach, large tumor diameter, perineural and lymphovascular invasion as independent risk factors associated with decreased OS rates (Wang et al., 2018). Another study also reported that lymphatic vessel and nerve invasion and tumor size were independent risk factors (Caccialanza et al., 2016; Liu et al., 2016).

Our study found that total gastrectomy and surgery alone were independent risk factors for survival. Other studies also found many other risk factors associated with a poor prognosis. The main reason for this inconsistency was study heterogeneity, with differences in race, surgical practice and initial prognosis.

A single-center study from the CLASSIC trial (Caccialanza et al., 2016; Liu et al., 2016) found a marked loss in body composition parameters (muscle, visceral fat and subcutaneous fat) significantly predicted short disease-free survival and OS among patients who underwent gastrectomy. Malnutrition was considered as poor prognostic factor in cancer patients (Caccialanza et al., 2016; Liu et al., 2016). Fujiya demonstrated that persistent postoperative malnutrition was frequently observed in patients who underwent total gastrectomy (Fujiya et al., 2018). These studies might explain why patients who received total gastrectomy had poor prognoses in our study, although we could not evaluate the nutrition index.

Despite a lack of prospective studies that explored the benefit of ACT in less advanced gastric cancer, there were some retrospective studies exploring the effect of ACT such patients. Based on the 8th edition of the TNM staging system of gastric cancer, stage IB gastric cancer includes pT1N1M0 and pT2N0M0. Wang used the SEER database to explore the difference between T1N1M0 and T2N0M0 and found that patients with T2N0M0 gastric cancer may not benefit from adjuvant treatment (Wang et al., 2019b). Recently, Jin et al.(Jin et al., 2021) found that pT2N0 gastric cancer patients with non-signet ring cell carcinoma, tumor size >3 cm and examined lymph nodes≤15 may be particularly appropriate candidates for ACT. In our study, there was no significant difference in OS between patients with signet ring cell carcinoma and patients with other histopathology type.

Since 1997, the retrieval of at least 15 lymph nodes has been recommended for adequate gastric cancer staging, and several studies have found that lymphadenectomy with <15 lymph nodes removed was an adverse independent prognostic factor for OS. A SEER study demonstrated that OS was dependent on the number of harvested lymph nodes; in patients with node-negative T1-2 gastric cancer, every additional 10 lymph nodes harvested increased the 5-year survival rate of 7.6% (Smith et al., 2005). Haejin found that their subgroup of T2N0M0 gastric cancer patients who underwent suboptimal lymphadenectomy benefitted from chemoradiotherapy rather than chemotherapy (Coburn et al., 2008; Du et al., 2011). Due to a lack of patients who received postoperative radiotherapy, the differences in radiotherapy and chemoradiotherapy roles could not be established in our study. Other studies failed to show the number of removed lymph nodes as an independent prognostic factor (Coburn et al., 2008; Du et al., 2011). One large population-based study demonstrated that surgery with adequate lymph node removing alone (≥15 lymph nodes) predicted better prognosis compared with adjuvant therapy in patients with stage I or node-negative gastric cancer (Dudeja et al., 2012). Our study found that the number of harvested lymph nodes was not associated with prognosis, which may be related to excluding patients with fewer than 15 harvested lymph nodes.

Several studies on patients with pT2 gastric cancer focused on the invasion depth. Some studies have showed that pT2 gastric cancer patients showing invasion into dMP had a relatively poor prognosis than those only invasion sMP (Zhang et al., 2016; Park et al., 2021), while others reported no significant difference in the prognosis between the two groups (Son et al., 2007; Nakamura et al., 2019). In our study, the difference in the 5-year OS rate between the sMP and dMP subgroups was not significant (89.6 *vs.* 86.1%, $p = 0.327$).

Regarding the therapy regimen, monotherapy and dual-drug therapy showed no significant difference. ACTS-GC trial and CLASSIC trial demonstrated that ACT with S-1 or XELOX was safe. In our study, the main grade 3 or 4 adverse events were neutropenia, peripheral neuropathy in the dual-drug group and anemia, anorexia in the monotherapy group. According to the ACTS-GC trial ((Sakuramoto et al., 2007)), the most common adverse events of grade 3 or grade 4 were anorexia (6.0%), nausea (3.7%), and diarrhea (3.1%) in the S-1 group. According to the CLASSIC trial ((Bang et al., 2012)), the main grade 3 or 4 adverse events were neutropenia (22%), thrombocytopenia (8%), nausea (8%), and vomiting (7%) in the XELOX group. According the ARTIST II study ((Nagaraja et al., 2019; Li et al., 2020; Low et al., 2021; Nakazawa et al., 2021)), the most common adverse events of grade 3 or 4 were peripheral neuropathy (12%), anemia (8%) and anorexia (4%) in the SOX group. The common dose-limiting toxicity of oxaliplatin is peripheral neuropathy, which affects 90% patients ((Kweekel et al., 2005)). The incidence of peripheral neuropathy is considered to be related to the prolonged use of oxaliplatin ((Baek et al., 2010)). Thus, we recommend monotherapy to prevent toxicity and discomfort. However, other studies, which aim to explore the role of ACT in stage IB gastric cancer, failed to analyse the difference between monotherapy and dual-drug therapy.

According to the CSCO guidelines, patients with pT2N0M0 gastric cancer with high-risk factors (age <40 years or with high-grade or poorly differentiated tumor and nervous, lymphovascular invasion) are recommended to receive ACT to reduce the risk of recurrence. Then, we divided patients with pT2N0M0 gastric cancer into two subgroups (with high-risk factors and without high-risk factors) and evaluated whether the effect of postoperative therapy was diverse. ACT indeed provided survival benefits to patients with high-risk factors, while patients without high-risk factors also benefitted from ACT, which was inconsistent with the CSCO guidelines. Regarding the therapy regimen, monotherapy and dual-drug therapy showed no significant difference; thus, considering possible adverse events, we recommend monotherapy regardless of high-risk factors.

There were no studies exploring the role of ACT stratified by high-risk factors based on the CSCO guidelines.

Although patients with gastric cancer received ACT after radical gastrectomy, some patients still experienced relapse. Timely detection of recurrence, as well as identification of patients at high risk of relapse after surgery or completion of adjuvant therapy are major challenges in the treatment of gastric cancer. Drug resistance is the major factor of treatment failure and relapse and numerous studies aim to investigate the mechanisms of drug resistance ((Nagaraja et al., 2019; Li et al., 2020; Low et al., 2021; Nakazawa et al., 2021)). Over the past few decades, predictive biomarkers have received increasing attention in diagnosis, treatment, and prognosis of gastric cancer. Studies have found many predictive biomarkers for the precision treatment of gastric cancer (Petrillo and Smyth, 2020). In 2014, The Cancer Genome Atlas (Cancer Genome Atlas Resea, 2014) proposed a molecular classification of gastric cancer into 4 subtypes: chromosomal instability, Epstein-Barr virus positive, genomically stable and microsatellite instability (MSI). An et al.(An et al., 2012) found that in stage II/III gastric cancer, patients with microsatellite stable and MSI-low type significantly benefited from 5-FU-based ACT, while patients with MSI-high type did not benefit from 5-FU-based ACT. Findings from the MAGIC trial (Smyth et al., 2017) showed that mismatch repair deficiency (dMMR) and MSI-high were associated with good prognosis in patients treated with surgery alone, whereas in gastric cancer patients treated with perioperative chemotherapy, dMMR and MSI-high were associated with worse prognosis. Post hoc analysis of CLASSIC trial (Choi et al., 2019) showed that MSI-high was independent prognostic factor and ACT significantly improved disease-free survival in MSS group while no benefit was found in the MSI-high group. MSI status could be used for precision treatment of gastric cancer in the future.

A prospective randomized trial comparing surgery alone with ACT in stage IB gastric cancer patients with at least one risk factor for recurrence (male sex, age>65 years, perineural and lymphovascular invasion) is now ongoing (ClinicalTrials.gov identifier NCT01917552), and this large-scale prospective trial is expected to compensate for previous research shortcomings and yield satisfactory results. Although the trial is based on the 6th edition of the AJCC staging system, it also includes pT2N0M0 gastric cancer based on the 8th edition of the AJCC staging system.

Nevertheless, there are several potential limitations in this study. The number of patients with pT2N0M0 gastric cancer was relatively small since it was a single-center study, the resultant effects may have been underestimated, and the results should be interpreted with caution. In addition, this was a retrospective study, and there were likely patient and tumor baseline characteristic imbalances between the treatment groups. Finally, the role of radiotherapy was not analysed due to a lack of patients who received postoperative radiotherapy. Therefore, the conclusions of this study need to be verified by prospective study with a large sample size.

CONCLUSION

ACT was accompanied with higher 5-year OS and DSS rates of patients with pT2N0M0 gastric cancer. Patients with pT2N0M0 gastric cancer, regardless of high-risk factors based on the CSCO guidelines, might be considered candidates for ACT. In regard to the therapy regimen, monotherapy might be the optimal choice, considering the adverse events.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ruijin Hospital Ethics Committee, Shanghai Jiao Tong University School of Medicine, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YM and XF designed the study, collected the patient data and drafted the paper. TF checked all the statistical calculations. MY, ZZ, TL, and ZZ participated in the design of the study and assisted in the collection of the data and edited the final paper. All authors read and approved the paper for publication.

FUNDING

This work was supported by the Multicenter Clinical Trial of Shanghai Jiao Tong University of Medicine (No. DLY201602) and the Medical Engineering Cross Research Fund of Shanghai Jiao Tong University (No. YG2021QN14).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.845261/full#supplementary-material

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CASE REPORT
published: 12 April 2022
doi: 10.3389/fphar.2022.870823

Case Report: Prolonged Anorexia With Nausea Caused by Immune Checkpoint Inhibitors for Malignant Melanoma Treated Using Kampo Medicines Bukuryoingohangekobokuto and Ninjin'yoeito

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OPEN ACCESS

Edited by:
David A. Gewirtz, Virginia Commonwealth University, United States

Reviewed by:
Masahiro Ohsawa, Nagoya City University, Japan
Yoshiharu Motoo, Komatsu Sophia Hospital, Japan

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Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *07 February 2022*
Accepted: *18 March 2022*
Published: *12 April 2022*

Citation:
Takayama S, Arita R and Ishii T (2022) Case Report: Prolonged Anorexia With Nausea Caused by Immune Checkpoint Inhibitors for Malignant Melanoma Treated Using Kampo Medicines Bukuryoingohangekobokuto and Ninjin'yoeito. Front. Pharmacol. 13:870823. doi: 10.3389/fphar.2022.870823

Immune checkpoint inhibitors (ICIs) are indicated for several cancers, including malignant melanoma. Anorexia and nausea resulting in malnutrition are side effects of ICIs. In such cases, conventional drugs are used for symptom relief, but the symptoms may persist. We report a case of advanced malignant melanoma with prolonged anorexia and nausea, which occurred after nivolumab administration, and was successfully treated using Kampo medicines. A 75-year-old man with nasal bleeding visited our hospital. A nasal scope revealed an obstructive tumor in the left nasal concha. Tissue biopsy showed malignant melanoma, and computed tomography showed metastasis to the liver and bone. Thus, the patient was diagnosed with stage IV malignant melanoma. He received radiotherapy (30 Gy) and nivolumab with ipilimumab four times, followed by nivolumab administration alone. During the administration of nivolumab, he complained of severe anorexia and nausea, with a numeric rating scale (no symptoms, 0; severe symptoms, 10) score of 10. He could not consume food because of these symptoms, even after nivolumab administration was discontinued. His blood pressure was 92/59 mmHg, his performance status (PS; no fatigue, 0; bedridden or disabled, 4) was 4, and his body weight gradually decreased from 60 to 39 kg in a month. The patient showed malnutrition and dehydration and experienced anxiety and depression. Nivolumab was terminated, and conventional symptomatic drugs were prescribed, but the symptoms persisted. We then prescribed 9.0 g/day of ninjin'yoeito (TJ-108, Tsumura and Co.) to allow recovery from anorexia and subsequently added bukuryoingohangekobokuto (TJ-116, Tsumura and Co.) to treat the persistent nausea. After treatment with these two Kampo medicines, the patient's appetite gradually recovered. Along with the recovery of nutritional status, his PS improved to 0, his anxiety and depressive state improved, and his body weight increased to 60 kg. The patient remained in good condition without cancer recurrence. The patient's clinical course shows the usefulness of Kampo medicine as supportive care for symptom relief and maintenance of nutritional and mental status during cancer treatment.

Keywords: immune checkpoint inhibitors, side effect, anorexia, recover, kampo medicine

INTRODUCTION

Several immune checkpoint inhibitors (ICIs) have been developed to treat cancers, including malignant melanoma. Side effects of nivolumab have been reported, including appetite loss and nausea (JAPIC, 2014), with an incidence of >5.0%. Anorexia and nausea, resulting in poor nutritional status, are general problems associated with treatment using ICIs. Conventional drugs for symptom relief are used in these cases; however, symptoms are occasionally refractory to such treatment. Prolonged appetite loss due to nausea causes malnutrition, which leads to frailty, and influences mental status.

In contrast, the use of Kampo medicines for patients with cancer was reported to be >70% among physicians in core cancer treatment hospitals (Ito et al., 2012). The application of Kampo medicines, including ginseng, such as rikkunsito (RKT), has been reported in the treatment of anorexia as a side effect of cancer treatment (Yoshiya et al., 2020). We previously reported long-term survival and improvement in quality of life in patients with advanced cancer, including pancreatic cancer, brain cancer, and esophageal cancer, which was supported with Kampo medicines (Shimizu et al., 2021a; Shimizu et al., 2021b; Suzuki et al., 2021; Takayama and Ishii, 2022). Ninjin'yoeito (NYT) allows recovery from anorexia with frailty, and bukuryoingohangekobokuto (BRGHT) is used for persistent nausea with anxiety.

Kampo medicines is used according to slight and minor symptoms, considering the patient's body composition, constitution, and characteristics. According to symptoms and physical findings, RKT is used for the treatment of anorexia with qi (energy) deficiency and fluid retention; NYT is used to treat anorexia and malnutrition with qi deficiency and blood deficiency; and BRGHT is used to treat anorexia, nausea, vomiting, and anxiety with qi deficiency, qi counterflow, phlegm, and dampness in the traditional medicine setting.

Here, we report a case of advanced malignant melanoma with severe appetite loss and nausea, which occurred after ICIs administration and was recovered through Kampo medicine.

CASE DESCRIPTION

A 75-year-old man with a history of myocardial infarction and habituation to smoking complained of nasal bleeding and visited our hospital. A nasal scope revealed an obstructive tumor in the left nasal concha (**Figure 1A**). Tissue biopsy showed malignant melanoma, and computed tomography (**Figure 2A**) showed the obstructed tumor at the left nasal concha with metastases in the liver and bone; thus, he was diagnosed with stage IV malignant melanoma. Programmed death-ligand 1 (PD-L1) test using a biopsy showed positive results, suggesting that anti-cancer drugs and ICIs could effectively treat the advanced-stage malignant melanoma. The patient received radiotherapy (30 Gy) and nivolumab with ipilimumab four times, followed by the sole administration of nivolumab. Subsequently, the patient complained of severe anorexia with nausea, with a numerical rating scale (NRS; no symptoms, 0; severe symptoms, 10) score of 10. He also complained of taste disorder. The patient was unable to eat because of these symptoms. At the first visit to our outpatient clinic, his blood pressure was 92/59 mmHg, and his performance status (PS; no fatigue, 0; bedridden or disabling, 4) (Date, 1999) was 4. His body weight gradually decreased from 60 to 39 kg in a month. His body mass index decreased to 15.7, with a blood test showing total protein (TP) 6.0 g/dL, albumin (Alb) 3.2 g/dL, creatinine (Cr) 1.38 mg/dL, and lymphocyte count 1780/μL. The patient was positive for malnutrition and dehydration, and he experienced anxiety and depression. Anorexia, nausea, and renal failure were suspected to be adverse reactions to the ICIs. Hence, nivolumab was terminated; but the symptoms persisted. Conventional drugs, such as mosapride citrate hydrate, metoclopramide, and lansoprazole, were prescribed, but they did not eliminate the symptoms. Despite the tumor reduction (**Figures 1B**, **2B**), his PS, mental status, and quality of life decreased after cancer remission.

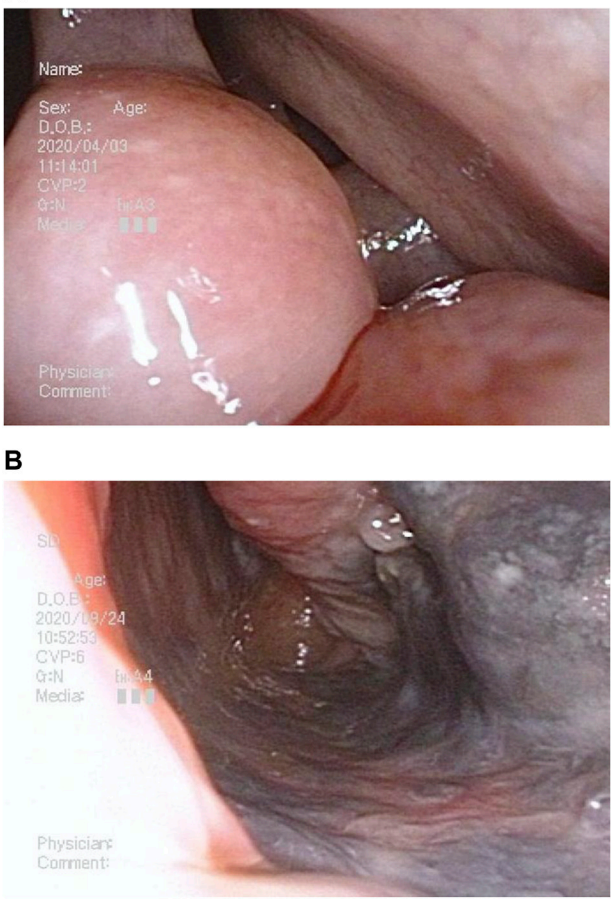

FIGURE 1 | Nasal scope at the first visit and after radiotherapy and chemotherapy. **(A)** First visit: left nasal concha occupied by the tumor. **(B)** Tumor remission after radiotherapy followed by nivolumab and ipilimumab therapy.

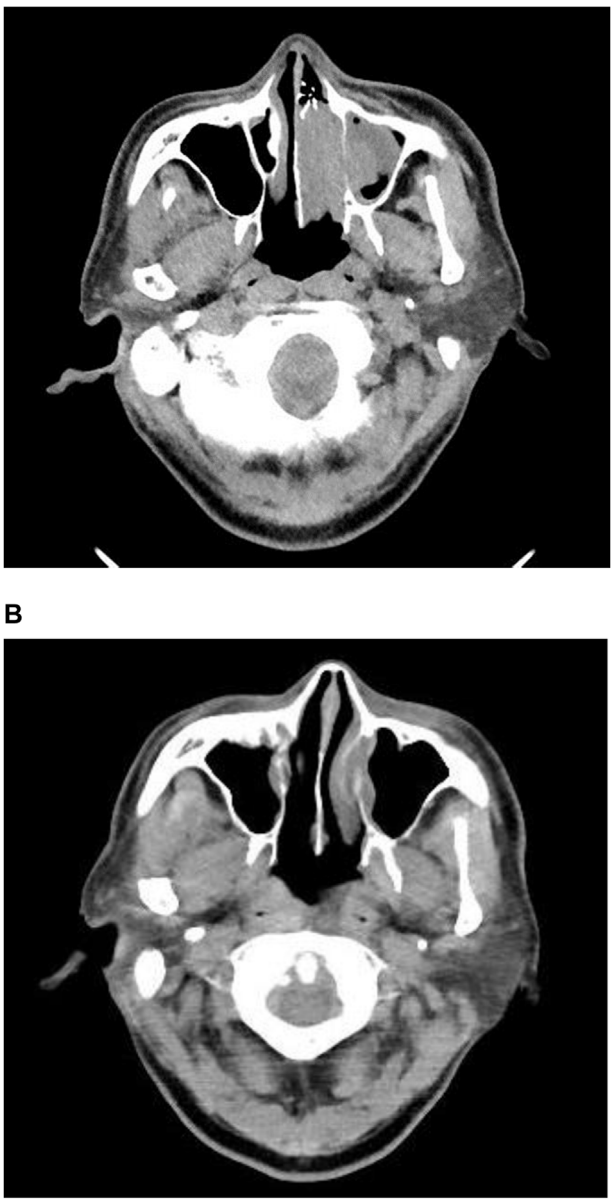

FIGURE 2 | Contrast computed tomography at the first visit and after radiotherapy and chemotherapy **(A)**. First visit: The left nasal concha was obstructed by the tumor. **(B)** Tumor remission after radiotherapy followed by nivolumab and ipilimumab therapy.

According to traditional medicine diagnosis, persistent nausea and anorexia, with a weak pulse and hypochondrium stuffiness, showed qi deficiency with fluid retention. We prescribed 7.5 g/day of RKT (TJ-43, Tsumura and Co., see STORK http://mpdb.nibiohn.go.jp/stork/) to alleviate the nausea. Two weeks after this prescription, the NRS score for nausea had decreased from 10 to 7. His anxiety slightly reduced because he could now consume solid food, but the improvement was insufficient. According to traditional medicine diagnosis, residual anorexia and malnutrition with a weak pulse showed qi deficiency and blood deficiency. Thus, we added 9.0 g/day of NYT (TJ-108, Tsumura and Co., see STORK http://mpdb.nibiohn.go.jp/stork/) to allow recovery from anorexia and malnutrition. Because his pulse remained weak and water brash from the stomach to esophagus persisted, indicating qi deficiency, qi counterflow, phlegm, and dampness in the traditional medicine setting, we changed RKT to BRGHT (TJ-116, Tsumura and Co., see STORK http://mpdb.nibiohn.go.jp/stork/) to treat the persistent anorexia, nausea, vomiting, and anxiety. After taking these two Kampo medicines, his appetite gradually recovered, and nutritional status indicators such as TP, Alb, and body weight gradually increased. His PS improved from 4 to 0, and his anxiety and depressive state also improved, with his body weight returning to 60 kg. **Figure 3** shows the clinical course of the treatment.

After the re-administration of nivolumab under Kampo treatment, the patient remained in good condition, with no cancer recurrence.

DISCUSSION

Recently, ICIs, such as human monoclonal anti-human PD-1 antibody, have been developed for several cancers, including malignant melanoma. Anorexia, nausea, and poor nutritional status are side effects of cancer treatment, including the use of ICIs. Prolonged anorexia and nausea cause malnutrition and mental disorders, leading to frailty during cancer treatment. Conventional treatment is used as supportive care to relieve these symptoms, but the symptoms occasionally persist. Delayed or prolonged effects and adverse reactions with ICIs have also been known. Furthermore, severe anorexia might be induced by combining nivolumab and ipilimumab rather than nivolumab alone.

In Japan, physicians can prescribe 148 types of Kampo medicines under the national health insurance system. Hospitalized patients undergoing cancer treatment *via* gastrointestinal and gynecological surgery receive several Kampo medicines (Sugimine et al., 2021). Recently, randomized controlled trials (RCTs) showed the efficacy and safety of Kampo medicines, including in cancer treatment (Motoo et al., 2021).

Frailty and cachexia related to cancer are serious problems. Anamorelin hydrochloride is a ghrelin mimetic agent for the treatment of cancer cachexia. However, its indication is limited to patients with unresectable advanced or recurrent non-small cell lung cancer, gastric cancer, pancreatic cancer, and colorectal cancer. Anamorelin hydrochloride is not indicated in cases of malignant melanoma, such as the present case. Supportive care during cancer treatment, including using Kampo medicine, is occasionally indicated to relieve symptoms such as fatigue, nausea, appetite loss, constipation, diarrhea, and abdominal pain. **Table 1** shows the list of Kampo medicines, including ginseng, for symptom relief in cancer treatment.

In the present case, we first prescribed RKT, but it was not efficacious. NYT was added, but the patient's serum potassium level decreased to hypokalemic levels. RKT and NYT include licorice, which is a possible crude drug for pseudoaldosteronism. The combined use of RKT and NYT includes a daily dose of 2.0 g licorice. One of the most well-known adverse reactions to Kampo

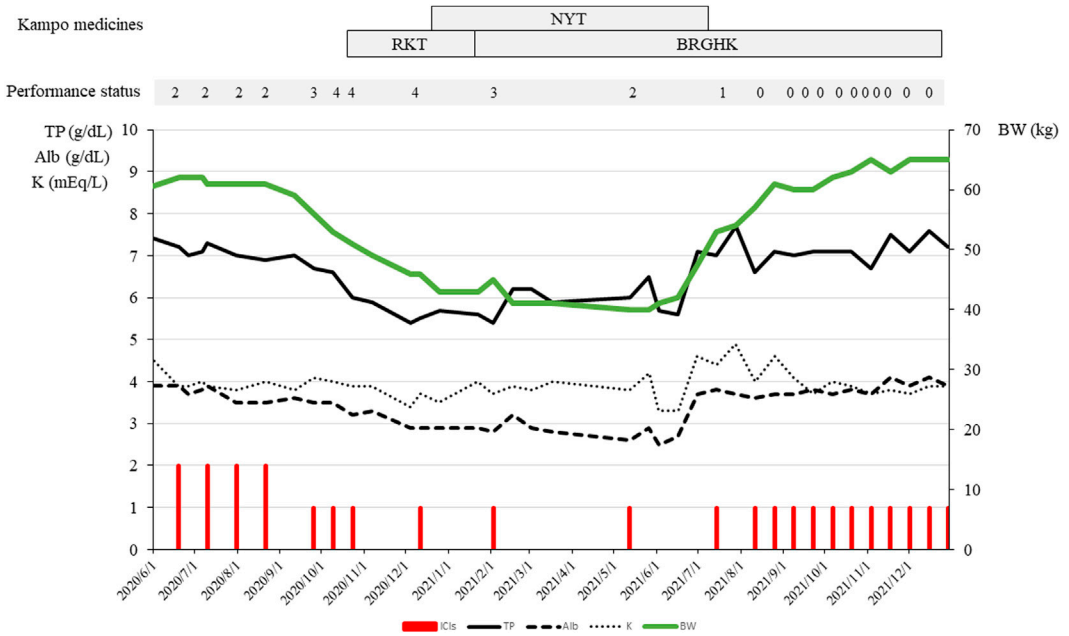

FIGURE 3 | Clinical course of the treatment. Performance status is rated from 0 to 4 for fatigue (lethargy, malaise, and asthenia); 0: none, 1: increased fatigue over baseline, but not altering normal activities; 2: moderate or causing difficulty in performing some activities; 3: severe or loss of ability to perform some activities; 4: bedridden or disabled (Date, 1999).

medicines is pseudoaldosteronism, caused by glycyrrhizin, which is included in several Kampo medicines (Arai et al., 2020). A report of patients with pseudoaldosteronism in Japan showed that even small amounts of licorice (less than 2.0 g) could cause pseudoaldosteronism, and older age is considered a risk factor for the disease (Yoshino et al., 2014). **Table 1** shows the number of crude drugs used in RKT, BRGHT, and NYT. The combination of BRGHT and NYT includes almost all the crude RKT drugs in large amounts. In addition, crude drugs that promote gastrointestinal movement are added. However, the amount of licorice is limited to 1.0 g per day, and pseudoaldosteronism can be avoided. Thus, we switched to BRGHT and NYT for recovery from anorexia and nausea.

BRGHT, composed of nine crude drugs, is a Kampo medicine used to treat nausea and anxiety. Further, 7.5 g of TJ-116 BRGHT includes the crude drugs shown in **Tables 1**, **2** (STORK, http://mpdb.nibiohn.go.jp/stork/). We previously reported that BRGHT could inhibit corticotropin-releasing hormone receptor 2, dopamine receptors D2 and D3, neuropeptide Y receptor type 2, and acetylcholinesterase, which synergistically improves gastric emptying (Mogami et al., 2020). BRGHT administration also reduces the frequency of aspiration pneumonia in patients with brain damage (Takayama et al., 2021).

NYT is a Kampo medicine, composed of 12 crude drugs, used to recover from disease, fatigue, and anorexia. Additionally, 9.0 g of TJ-108 NYT includes the crude drugs shown in **Tables 1**, **2** (STORK, http://mpdb.nibiohn.go.jp/stork/). A review of RCTs of NYT demonstrated the usefulness of NYT in the treatment of cancer and related conditions (Takayama et al., 2019). Other studies have reported increased food intake *via* the activation of orexigenic OX1R-expressing neurons in the hypothalamus (Miyano et al., 2020), maintenance of nutritional status in patients with wasting conditions (Sasatani et al., 2020), and the activation of both ghrelin-responsive and ghrelin-unresponsive neuropeptide Y pathways for the treatment of anorectic conditions, which are associated with cancer or frailty (Goswami et al., 2019). These studies support the potential of NYT in cancer patients with anorexia, malnutrition, and mental disorders.

RKT is also used to treat chemotherapy-induced anorexia. The mechanisms of appetite improvement through ghrelin signaling have been reported in several studies (Asakawa et al., 2001; Nakazato et al., 2001; Takeda et al., 2008). Yoshiya et al. (2020) reported that RKT could mitigate chemotherapy-induced anorexia and ameliorate acylated ghrelin levels in the plasma, decreasing anorexia during the delayed phase of cisplatin-based chemotherapy in cancer patients.

RKT is used to improve nausea and anorexia *via* ghrelin signaling, while NYT is used to improve anorexia, malnutrition, and anxiety. BRGHT improves both upper gastrointestinal motility *via* multiple signaling (D2, D3, neuropeptide Y, and acetylcholinesterase) and anxiety. Over 60% of metastatic melanoma survivors treated with ICIs experience anxiety while waiting for test results, fear of recurrence, and death (Lai-Kwon et al., 2019). Anxiety could result in appetite loss, gastrointestinal impairment, and malnutrition. The patient in the presented case may have had anxiety because of metastatic melanoma and long-term chemotherapy. Therefore, the combination of NYT and BRGHT would be more effective for improving malnutrition, upper gastrointestinal dysfunction, and anxiety. According to the reports above and the present case, the combined use of BRGHT and NYT allows recovery from appetite loss, nausea, and malnutrition during cancer treatment.

TABLE 1 | Amounts of crude drugs in Kampo medicines, RKT, BRGHT, and NYT (top of the table); and symptoms and conditions of application (bottom).

Crude Drugs	RTK (TJ-43)	BRGHT (TJ-116)	NYT (TJ-108)	BRGHT (TJ-116) with NYT (TJ-108)
JP Ginseng	4.0 g	3.0 g	3.0 g	6.0
JP Atractylodes lancea rhizome	4.0 g	4.0 g		4.0
JP Atractylodes rhizome	—	—	4.0 g	4.0
JP Citrus unshiu peel	2.0 g	3.0 g	2.0 g	5.0
JP Poria sclerotium	4.0 g	5.0 g	4.0 g	9.0
JP Ginger	0.5 g	1.0 g	—	1.0
JP Pinellia tuber	4.0 g	6.0 g		6.0
JP *Glycyrrhiza*	1.0 g	—	1.0 g	1.0
JP Jujube	2.0 g	—	—	—
JP Magnolia bark	—	4.0 g	—	4.0
JP Perilla herb	—	2.0 g	—	2.0
JP Immature orange	—	1.5 g	—	1.5
JP Japanese angelica root	—	—	1.5 g	1.5
JP *Astragalus* root	—	—	4.0 g	4.0
JP Rehmannia root	—	—	4.0 g	4.0
JP Cinnamon bark	—	—	2.5 g	2.5
JP Polygala root	—	—	2.0 g	2.0
JP Peony root	—	—	2.0 g	2.0
JP Schisandra fruit	—	—	1.0 g	1.0
Symptoms and conditions of application	Weak stomach, loss of appetite, full stomach pit, fatigue, anemia, cold limbs Gastritis, gastric atony, gastroptosis, maldigestion, anorexia, gastric pain, and vomiting	Depressed feelings, feeling of foreign body in the throat and esophagus Palpitation, dizziness, nausea, heartburn, decreased urine volume, anxiety neurosis, nervous gastritis, and hyperemesis gravidarum Water brash and gastritis	Declined constitution after recovery from disease, fatigue, malaise, anorexia, perspiration during sleep, cold limbs, and anemia	—

TABLE 2 | Plant names and part of each ingredient described in the present study.

Ingredient in English	Plant name (Latin)	Plant part (Latin)
JP Atractylodes Lancea Rhizome	*Atractylodes lancea* De Candolle, or *Atractylodes chinensis* Koidzumi *Compositae*)	*Rhizoma*
JP Atractylodes rhizome	*Atractylodes japonica* Koidzumi ex Kitamura or *Atractylodes macrocephala* Koidzumi (*Atractylodes ovata* De Candolle) (*Compositae*)	*Rhizoma*
JP Cinnamon bark	*Cinnamomum cassia* Blume (*Lauraceae*)	*Cortex*
JP Citrus unshiu peel	*Citrus unshiu* Marcowicz, or *Citrus reticulata* Blanco (*Rutaceae*)	*Pericarpium*
JP Ginger	Zingiber officinale Roscoe (*Zingiberaceae*)	*Rhizoma*
JP Ginseng	Panax ginseng C. A. Meyer (*Panax schinseng* Nees) (Araliaceae)	*Radix*
JP *Glycyrrhiza*	*Glycyrrhiza uralensis* Fischer, or *Glycyrrhiza glabra* Linné *Leguminosae*)	*Radix*
JP Immature orange	*Citrus aurantium* Linné var. daidai Makino, *Citrus aurantium* Linné, or *Citrus natsudaidai* Hayata (*Rutaceae*)	*Fructus immaturus*
JP Japanese angelica root	*Angelica acutiloba* Kitagawa, or *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino (*Umbelliferae*)	*Radix*
JP Jujube	*Zizyphus jujuba* Miller var. *inermis* Rehder (*Rhamnaceae*)	*Fructus*
JP Magnolia bark	*Magnolia obovata* Thunberg (*Magnolia hypoleuca* Siebold et Zuccarini), *Magnolia officinalis* Rehder et Wilson, or *Magnolia officinalis* Rehder et Wilson var. *biloba* Rehder et Wilson (*Magnoliaceae*)	*Cortex*
JP Perilla herb	*Perilla frutescens* Britton var. *crispa* W. Deane (*Labiatae*)	*Herba*
JP Pinellia tuber	*Pinellia ternata* Breitenbach (*Araceae*)	*Tuber*
JP Polygala root	*Polygala tenuifolia* Willdenow (*Polygalaceae*)	*Radix*
JP Poria sclerotium	*Wolfiporia cocos* Ryvarden et Gilbertson (*Poria cocos* Wolf) (*Polyporaceae*)	—
JP Schisandra fruit	*Schisandra chinensis* Baillon (*Schisandraceae*)	*Fructus*

Interstitial lung disease (ILD) or interstitial pneumonitis (IP) are important adverse events of ICIs. The occurrence rate of these adverse events is reported as 7.2% for nivolumab with ipilimumab (Opdiva, 2020). In the present case, computed tomography and blood sampling did not show the existence of ILD or IP. The ILD or IP occurrence rate with RKT has been reported to be 0% (Suzuki et al., 2020), and with NYT and BRGHT, it has never been reported. However, the other Kampo medicine has been reported as a possible cause of ILD or IP (Arai et al., 2020); hence, when using Kampo medicine together with ICIs, careful follow-up for adverse events is recommended.

We believe the improvement of anorexia and malnutrition is related to the use of Kampo medicine; however, cancer remission is also a reason for the patient's recovery.

CONCLUSION

In a patient with advanced malignant melanoma, cancer was treated using ICIs. Side effects such as anorexia and nausea with poor nutritional status were resolved using Kampo medicines, BRGHT and NYT. The clinical course of the patient in our report shows the usefulness of Kampo medicine as supportive care for symptom relief and the maintenance of nutritional and mental status during cancer treatment.

PATIENT PERSPECTIVE

This case report was approved by the ethics committee of the Graduate School of Medicine, Tohoku University, Sendai, Miyagi, Japan, on 7 February 2022 (protocol identification number: No. 24377).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This case report was approved by the ethical committee of the Graduate School of Medicine, Tohoku University, Sendai, Miyagi, Japan. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ST treated the patient and wrote the manuscript. RA created the tables, and TI provided suggestions regarding the manuscript.

FUNDING

This study was supported by the Tsumura and Co., a pharmaceutical company in Kampo medicines in Japan.

ACKNOWLEDGMENTS

We would like to thank Akiko Kuwabara for clinical research coordination.

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Conflict of Interest: All authors belong to the Department of Kampo and Integrative Medicine, Tohoku University Graduate School of Medicine, a joint research course with TSUMURA and Co., a pharmaceutical company of Kampo medicines in Japan.

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