Journal of Food and Drug Analysis

Volume 31 | Issue 2 Article 2

# What nature has to offer: Opportunities for immuno-oncology

REVIEW ARTICLE

# What nature has to offer: Opportunities for immuno-oncology

Rubina Kousar [a,b,c,d,1], Cheng-Han Lin [a,b,c,1], Bbumba Patrick [a,b,c], Miao He [e], Dong-Chuan Wu [a,f], Xing-Guo Li [a,b,c,*]

[a] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, 406040, Taiwan
[b] Research Center for Cancer Biology, China Medical University, Taichung, 406040, Taiwan
[c] Institute of Biochemistry and Molecular Biology, College of Life Sciences, China Medical University, Taichung, 406040, Taiwan
[d] Department of Biological Science and Technology, China Medical University, Taichung, 406040, Taiwan
[e] PhD Program in Cellular and Molecular Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA
[f] Translational Medicine Research Center, China Medical University Hospital, Taichung, 40402, Taiwan

**Abstract**

**Recent rapid development of cancer therapy has come about with the paradigm shift from the traditional goal of targeting cancer cells themselves, to reprograming the immune tumor microenvironment. Accumulating evidence shows that compounds that target epigenetic regulation, called epidrugs, play a crucial role in mediating the immunogenicity of cancer cells and in reshaping antitumor immunity. A large body of literature has recognized natural compounds as epigenetic modulators for their immunomodulatory effects and anticancer potential. Unifying our understanding of the role of these biologically active compounds in immuno-oncology may open new avenues for more effective cancer therapies. In this review, we explore how natural compounds modulate the epigenetic machinery to shape antitumor immune response, highlighting the promise offered by the Mother Nature that could be exploited therapeutically to improve outcomes for cancer patients.**

*Keywords:* Cancer immunotherapy, Epidrugs, Natural compounds

## 1. Introduction

Most cancers are fundamentally driven by the accumulation of genetic and epigenetic alterations. In addition to their abilities to confer cancer cell growth advantage, these changes play a critical role in caner initiation and progression by dynamically reshaping the tumor microenvironment and reprogramming immune cells [1,2]. These findings have created an emerging route to exploit the inherent flexibility and reversibility of epigenetic modulators of immune-oncology. In recent years, natural compounds have been recognized for their properties that reportedly modulate epigenetic and immune mechanisms in cancer [3,4]. Although the effects of these compounds on the immunogenicity of cancer cells, and the epigenetic mechanisms involved herein are still under investigation, what we have learned from Mother Nature may represent unique opportunities that can be exploited therapeutically to enhance antitumor immune response and ultimately improve clinical outcome. In this review, we first highlight the US Food and Drug Administration (FDA)-approved epidrugs that target epigenetic machinery and their mechanisms of action, then we provide an updated summary of the preclinical and clinical applications of natural compounds either alone or in combination with the current cancer therapies, and finally, we explore the potential epigenetic mechanisms of natural compounds that could be beneficially applied to immuno-oncology.

Received 28 December 2022; accepted 22 March 2023.
Available online 15 June 2023

* Corresponding author: Graduate Institute of Biomedical Sciences, Research Center for Cancer Biology, Institute of Biochemistry and Molecular Biology, College of Life Sciences, China Medical University, Taichung, 406040, Taiwan.
E-mail address: xli@mail.cmu.edu.tw (X.-G. Li).
[1] These authors contribute equally to this work.

https://doi.org/10.38212/2224-6614.3459

## 2. Epidrugs: toward epigenetic regulation of antitumor immunity

In 2018, James P Allison and Tasuku Honjo were awarded the Nobel Prize in Physiology or Medicine "for their discovery of cancer therapy by inhibition of negative immune regulation". Although this breakthrough in cancer immunotherapy has revolutionized cancer treatment, only a subset of patients elicit favorable responses and most immunologically cold solid tumors are not responsive [1,2]. The composition of tumor-infiltrating immune cells is highly heterogeneous and can be dynamically shaped by the tumor microenvironment (TME). Accumulating evidence points out that tumor-infiltrating lymphocytes (TILs) may serve as predictive biomarkers for clinical response to immunotherapies [5]. Indeed, current efforts have focused on turning "cold" tumors (T cell non-infiltrated) into "hot" tumors (T cell infiltrated) by epigenetically reprogramming the tumor microenvironment, which contributes to the cancer-permissive setting that impedes T cell infiltration and function [6]. Furthermore, an increasing amount of evidence demonstrates that infiltrating immune cells show epigenetic abnormalities that lead to prolonged antigen stimulation, progressive loss of T cell effector function, and increased expression of inhibitory receptors [7].

Consistent with the epigenetic alteration in immune cells, a distinct epigenetic state characterizes this exhaustion phenotype in exhausted T cells compared to effector and memory T cells [8]. It is thus not surprising that pharmacological and genetic disruption of exhaustion-associated epigenetic programs, such as genes involved in T cell effector functions, allowed these T cells to retain their effector functions and increased immune checkpoint blockade (ICB)-mediated T cell rejuvenation [8]. Given that most patients remain refractory to current ICB therapies, including anti-programmed cell death (PD-1), anti-PD1 ligand 1 (PD-L1) and anti-cytotoxic T lymphocyte antigen 4 (CTLA-4), these recent studies highlight that epigenetic drugs can work synergistically with immunotherapies to enhance the antitumor immune response by avoiding the onset of exhaustion and reprogramming exhausted cytotoxic T lymphocytes into effector phenotypes [7,10]. To date, there has been great interest in using epigenetic therapy to increase tumor immune infiltration, enhance antitumor immunogenicity, and reinvigorate T cell dysfunction [9,10].

In addition to the modulation of immune effectors, epigenetic modulators can also boost the immune system by reshaping the immune TME through multiple mechanisms. A prime example is cytokine signaling, known to be suppressed epigenetically in certain cancers. Notably, targeting DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) led to the reactivation of T helper 1 (Th1) cell-type cytokine expression, the induction of the secretion of these cytokines, and synergistic effects with PD-L1 blockade [11]. Moreover, epigenetic changes are also associated with the expression of antigen-presenting machinery-related genes, such as class I major histocompatibility complex (MHC-I) genes. It has been demonstrated that DNMT inhibitors can reactivate the expression of NOD-like receptor family CARD domain containing 5 (NLRC5), a gene encoding class I trans activator, thus leading to concomitant increases in class I MHC gene expression [12]. Finally, it is important to note that epigenetic modulation occurs in many other immune cell subsets in the TME, including myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), cancer-associated fibroblasts, and regulatory T cells (Treg), dysfunction of all of which contributes to impaired immunity [5].

So far, FDA has approved 3 types of 7 epigenetic modifying agents: DNMT, HDAC, and the recently approved enhancer of zeste homolog 2 (EZH2) inhibitors. We summarize in Table 1 the current clinical trials using FDA-approved epidrugs in combination with immunotherapies. We highlight in Fig. 1 the molecular mechanisms of these reagents to regulate antitumor immunity through modulation of TME and the crosstalk between TME, tumor cells, and epidrugs. Given the wide role of epigenetic regulators in modulating antitumor immunity, such as T-cell exhaustion, and the infiltration and function of immune cells, a large number of ongoing clinical trials are evaluating the efficacy of combining epigenetic agents with immunotherapies [13]. Future research is required to define the effects of epidrugs on tumor and immune cell populations, and these studies should provide novel insights into the molecular processes behind the responses to epigenetic treatments, which is crucial for the development of rationale combination therapies.

### *2.1. HDAC inhibitor*

HDACs have been observed to be abnormally regulated in a variety of malignancies. Oncogenes and tumor suppressor genes, which are strongly linked to cell division, apoptosis, differentiation, migration, and cancer angiogenesis, are affected by these alterations in how they are transcribed.

*Table 1. Cancer clinical trials of FDA-approved epidrugs in combination with immunotherapies.*

| FDA-approved drugs | Immunotherapy | Cancer type | Phase | NCT# |
|---|---|---|---|---|
| Domatinostat | Avelumab (PD-L1) | Gastrointestinal cancer | Phase 2 | NCT03812796 |
| Vorinostat | Pembrolizumab (PD-1) | Lymphomas | Phase 1 | NCT03150329 |
| | | Renal and bladder cancer | Phase 1 | NCT02619253 |
| | | Lung cancer | Phase 1,2 | NCT02638090 |
| | | Head and neck cancer | Phase 1,2 | NCT02538510 |
| | | Salivary gland cancer | Phase 1,2 | NCT02538510 |
| Azacitidine | Avelumab (PD-L1) | Diffuse large B-Cell lymphoma | Phase 3 | NCT02951156 |
| | Alemtuzumab (CD52) | Myeloid cancer | Phase 2 | NCT02497404 |
| | Pembrolizumab (PD-1) | Acute myeloid leukemia | Phase 2 | NCT03769532 |
| | | Pancreatic cancer | Phase 2 | NCT03264404 |
| Oral azacytidine (CC-486, ONUREG®) | Pembrolizumab (PD-1) | Ovarian cancer | Phase 2 | NCT02900560 |
| | | Lung cancer | Phase 2 | NCT02546986 |
| | | Melanoma | Phase 2 | NCT02816021 |
| Decitabine | Pembrolizumab (PD-1) | T cell lymphomas | Phase 2 | NCT03240211 |
| | | Lymphomas | Phase 1 | NCT03445858 |
| | | Myeloid leukemia | Phase 1 | NCT03969446 |
| | | Breast cancer | Phase 2 | NCT02957968 |
| Tazemetostat | Pembrolizumab (PD-1) | Bladder cancer | Phase 1,2 | NCT03854474 |
| Azacitidine, Venetoclax | Pembrolizumab (PD-1) | Acute myeloid leukemia | Phase 2 | NCT04284787 |
| Vorinostat, Temozolomide | Pembrolizumab (PD-1) | Glioblastoma | Phase 1 | NCT03426891 |
| Vorinostat, Tamoxifen | Pembrolizumab (PD-1) | Breast cancer | Phase 2 | NCT04190056 |
| | | | Phase 2 | NCT02395627 |
| Azacytidine, Romidepsin | Pembrolizumab (PD-1) | Pembrolizumab (PD-1) | Phase 1 | NCT02512172 |
| Azacytidine | Durvalumab (PD-L1), Tremelimumab (CTLA-4) | Head and neck cancer | Phase 1,2 | NCT03019003 |

Inhibition of HDACs has proven to be an effective strategy for cancer therapy. To date, a variety of HDAC inhibitors have been approved by the FDA or are undergoing clinical trials. With this data, scientists were able to single out 32 distinct HDAC inhibitors. Five of these drugs are well-established in clinical practice; 11 are experimental HDAC inhibitors; 11 are investigational compounds of a non-hydroxamate type; and 2 are investigational molecules that defy classification. They also add three more compounds that have been shown to have HDAC inhibitory action in clinical trials. In terms of effectiveness, safety, and quality of life evaluation, significant clinical trials of the two FDA-approved medications vorinostat and romidepsin have been conducted [14]. Nicotinamide, an allosteric non-competitive pan-inhibitor, and Sirt-specific inhibitors like EX-527, sirtinol, and cambinol are examples of Sirtuin (class III HDAC) inhibitors. It has been suggested that Sirt inhibitors might be beneficial in the treatment of cancer; however, none of these inhibitors have been authorized for use in clinical practice as yet. Additionally, approximately 10 other HDAC inhibitors have completed or are conducting clinical studies as monotherapy or in combination treatment for patients with solid tumors or hematologic malignancies [15]. For instance, phase III studies (NCT02115282 and NCT03538171) are now evaluating the therapeutic benefit of the benzamide inhibitor entinostat in patients with HR-positive, HER2-negative, locally progressed, or metastatic breast cancer. The hydroxamate HDAC inhibitor pracinostat was shown to be safe in patients with advanced hematological malignancies and to have moderate single-agent efficacy in a phase I dose-escalation multicenter study (NCT00741234) [16].

Besides their role on cancer cells, HDAC inhibitors can have a direct effect on immune cells. In terms of innate immunity, HDACs can control Toll-like receptor (TLR) signaling. Several TLR target genes are turned on by HDAC. Some of these genes make important inflammatory molecules, including pro-inflammatory cytokines (such as interleukin (IL)-6) or chemokines (C-X-C motif chemokine ligand 10 (CXCL10), Chemokine (C-C motif) ligand 7 (CCL7), and chemokine (C-C motif) ligand 2 CCL2). On the other hand, HDACs are thought to be involved in the negative regulation of TLR signaling. For example, HDACs directly shut down promoters of inflammatory genes. HDACs are also involved in adaptive immunity. It has been demonstrated that downregulation of antigen presentation and MHC-I expression are known resistance mechanisms to immunotherapy. This is because an effective anti-tumor immune response requires that cytotoxic CD8+ T cells recognize and kill cells displaying foreign antigens, including

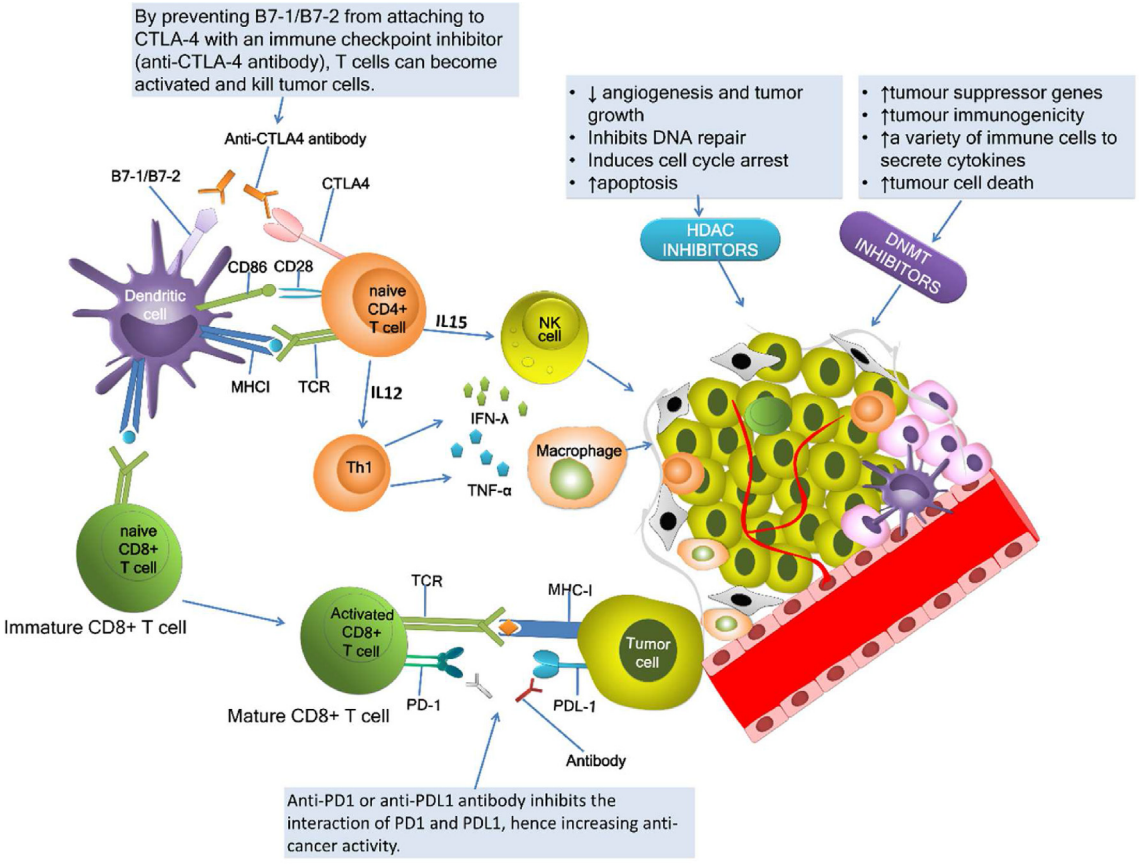


*Fig. 1. Schematic representation of the immunomodulatory mechanisms of FDA-approved epidrugs to regulate antitumor immunity through modulation of TME and the crosstalk between epidrugs, tumor cells and TME.*

tumor neoantigens, via MHC-I's presentation. By reducing the expression of transporter associated with antigen processing-1 (TAP-1), a molecule involved in the transport of peptides from the cytosol to the endoplasmic reticulum, which is implicated in the formation process of the MHC-I peptide complex, loss of histone acetylation has been implicated in the downregulation of MHC-I presentation in preclinical models. In a mouse model of merkel cell carcinoma, it was possible to improve MHC-I presentation with HDAC inhibitors, which increased the expression of TAP-1 and TAP-2 and improved tumor control [17].

### *2.1.1. HDAC inhibitor and immunotherapy*

In animal models of several malignancies, combination of HDAC inhibitors and immunotherapies have been shown to be effective. When compared to single-agent therapies, the HDAC inhibitor panobinostat, for instance, upregulates the expression of PD-L1 and PD-L2 in melanomas and boosts anti-PD-1 antibody therapy, resulting in decreased tumor growth and enhanced survival. Belinostat, another HDAC inhibitor, has been shown in a mouse model of hepatocellular carcinoma to enhance the antitumor effects of anti-CTLA-4 treatment, with increased IFN-γ generated by antitumor T cells and decreased regulatory T cells [18]. Furthermore, entinostat suppresses regulatory T cells in animal models of prostate and renal cancer and boosts the anticancer effects of IL-2 and vaccination treatment. Importantly, the first report of clinical trials combining IL-2 immunotherapy with HDAC inhibitors further supports the notion that the combination treatment improves objective response rate and median progression-free survival in patients with metastatic renal cell carcinoma (RCC).

## *2.2. DNMT inhibitor*

One of the most well-known epigenetic processes, DNA methylation regulates gene expression and is dysregulated in conditions like cancer. The most popular epigenetic medicines for cancer therapy are DNMT inhibitors, sometimes referred to as hypomethylating drugs. Hypomethylation, formation of double-stranded DNA breaks, cell cycle or G2 phase

arrest, and activation of immunological responses are the primary mechanisms by which DNMT inhibitors exercise their anticancer action [19]. DNMT inhibitors have been used in clinical oncology for some time, but only recently has it been discovered that they can not only target tumor cells but also have the potential to improve the effectiveness of ICB therapy by modulating the TME [20]. DNMT inhibitors typically work by blocking DNA methylation, such as reducing promoter hypermethylation and allowing abnormally repressed tumor suppressor genes, including P15 or CDKN2B, P16 or CDKN2A, MLH1, and RB to re-express. There are two different categories of DNMT inhibitors: nucleoside DNMT inhibitors and non-nucleoside DNMT inhibitors. While the non-nucleoside analogues are able to target the catalytic sites of DNMTs to influence their activity, nucleoside analogues function by integrating into the DNA and trapping DNMTs to DNA covalently [21]. Nucleoside analogues that have been approved by the FDA to treat acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are 5-aza cytidine and decitabine (5-aza-2′-deoxycytidine). Instead of integrating into DNA, most non-nucleoside DNMT inhibitors are small molecule medicines that target the catalytic sites directly. Green tea polyphenol, epigallocatechin-3-gallate (EGCG), curcumin, hydralazine, procainamide, RG-108, disulfiram, and SGI-1027 were among them. Only azacytidine (Vidaza; Celgene) and decitabine (5 aza 2′-deoxycytidine) are DNMT inhibitors (azanucleosides) currently licensed by the US FDA [22]. Guadecitabine (SGI-110), a next-generation DNMT inhibitor, is being tested for its capacity to withstand cytidine deaminase-mediated degradation, resulting in extended action *in vivo*. Of note, SGI-110 has potential immunomodulatory and anticancer properties since it is able to increase the expression of the HLA class I molecule on melanoma cells as well as the quantity of CD8+ T cells and CD20+ B cells [16,20].

#### 2.2.1. DNMT inhibitor and immunotherapy

Recent research has shown that DNMT inhibitors may enhance the effectiveness of immunotherapies in a variety of malignancies. Decitabine promotes the infiltration and anticancer activities of cytolytic CD8+ T lymphocytes in a syngeneic mouse ovarian cancer model, and the combination of decitabine with anti-CTLA-4 antibody displays synergistic antitumor effects and longer survival. The anticancer effects of anti-PD-1 antibodies are amplified by DNMT inhibition in animal models of breast, colon, and prostate cancer due to the overexpression of MHC-I genes, T-cell chemotaxis, and CD8+ T cell tumor invasion. Notably, a phase 2 clinical research examining the combination of the FDA-approved anti-PD-1 antibody nivolumab and azacitidine in AML patients showed that the combination is safe and results in promising objective response rates and overall survival results [23].

### 2.3. EZH2 inhibitor

EZH2 regulates gene expression epigenetically via H3K27me3, which plays a pivotal role in carcinogenesis. Targeting EZH2 has been proved efficacious for impeding tumor growth in many types of cancer. Moreover, inhibition of EZH2 suppressed cell proliferation in those tumor-bearing cancers, including brain cancer [24], breast cancer [25], and ovarian cancer [26]. It should be noted that not all tumors respond well to EZH2 inhibition. As over 50% of stage 3 and stage 4 breast cancer patients suffer from bone metastases, Dihua Yu et al. found that EZH2 inhibitor was futile to bone metastasis. However, treatment with focal adhesion kinase inhibitor, a downstream effector of EZH2 of breast cancer bone metastasis, significantly impedes the metastasis [27]. Furthermore, increasing evidence has shown that histone methylation plays an important role in modulating the immune response in cancer. Dual G9A/EZH2 inhibitor treatment inhibits lysine histone methyl transferases, activating immune response via the CXCL10-CXCR3 axis and suppressing tumor-induced regulatory T cells in ovarian cancer [26]. These results support that combination therapy with an EZH2 inhibitor is urgent for cancer therapy.

#### 2.3.1. EZH2 inhibitor and immunotherapy

EZH2 is essential for the function of tumor-specific effector T cells. However, TME leads to a decreased expression of EZH2 on T cells by limiting glucose uptake, which in turn causes T cells to malfunction. EZH2 expression in Tregs, Th and NK cells, however, has the potential to inhibit anticancer immunity. Moreover, expression of EZH2 in ovarian tumors inhibits the synthesis of CXCL9, a Th1-type chemokine that is essential for CD8+ T cell infiltration in malignancies. Intratumoral CD8+ T cells and EZH2 levels were adversely associated with ovarian tumors. IL-6, IL-10, and C-C motif chemokine ligand 20 (CCL20) are examples of cytokines that TAMs with the M2 phenotype may release to suppress the anti-tumor immune response and promote tumor development. In addition, EZH2 expression in effector T cells and DCs could promote anti-cancer immunity. For naïve T cells, EZH2 expression promotes survival, proliferation, and function of effector T cells.

Numerous clinical studies of EZH2 inhibitor in various cancer types are now underway. These include the EZH2 inhibitors GSK126, GSK2816126, EPZ005687, El1, GSK343, GSK926, tazemetostat, E7438/EPZ6438, EPZ011989, CPI-1205, CPI-169, ZLD1039, and PF-06821497 [28]. On Jan. 23, 2020, FDA approved tazemetostat (Tazverik®) as the first EZH2 inhibitor for epithelioid sarcoma. Tazemetostat is now being investigated for diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma, prostate cancer, mesothelioma, urothelial carcinoma, and rhabdoid tumors, among other diseases, in clinical trials with other EZH2 inhibitors [28]. In the foreseeable future, further EZH2 inhibitors are most likely to get approval. Clinical studies of combination therapy using EZH2 inhibitors and additional treatments such as immunotherapy, conventional chemotherapy, and targeted medicines are now being conducted, some of which have shown synergistic benefits [29]. One example is a combination of Tazemetostat and anti-PD-L1 (Pembrolizumab) for bladder cancer therapy that has initiated an early phase clinical trial (NCT03854474).

### *2.4. Combined epidrugs with immunotherapy*

As of now, HDAC inhibitors like romidepsin and panobinostat are being used to treat cutaneous and peripheral T-cell lymphomas as well as multiple myeloma in the clinic. However, single-agent treatment with DNMT or HDAC inhibitors produces a suboptimal response in solid tumors, potentially due to a reduced therapeutic index or poor bioavailability. In solid tumors, the combination of DNMT and HDAC inhibitors synergistically induces re-expression of tumor suppressor genes and inhibition of tumor growth in preclinical models. Therefore, combination therapy of DNMT and HDAC inhibitors would be effective in reversing abnormal gene DNA methylation and thus result in therapeutic benefit. With an extension cohort for patients with virally caused malignancies, they initiated a phase I (NCT01537744) research to ascertain the maximum tolerated dosage (MTD) of the combination of CC-486 and romidepsin for patients with advanced solid tumors. Even though the CC-486 and romidepsin combination was ultimately tolerated, no discernible anticancer action was found [30].

Remarkable responses are seen with pembrolizumab, a PD-1 antibody used for immunotherapy in patients with mismatch repair (MMR)-deficient colorectal cancer (CRC). However, most patients with advanced CRC have tumors that are resistant to PD-1 blockage because they are MMR proficient (MMR-p). Tumor suppressor genes are silenced by the actions of DNMTs and HDACs. Re-expression of silenced tumor suppressors is a mechanism through which preclinical combination HDAC/DNMT inhibition suppresses tumor development. Patients with advanced MMR-p CRC who had previously progressed on one course of chemotherapy were included in a single-institution, open-label, randomized pilot trial (NCT02512172). Pembrolizumab combined with the epigenetic medicines 5-azacitidine and romidepsin is well tolerated and safe for patients with advanced p-MMR colorectal cancer. To identify response predictors, it will be necessary to compare biopsies taken before and after therapy [31].

## 3. FDA-approved epidrugs: advantages and disadvantages

With the development of various epidrugs, a plethora of small molecule inhibitors targeting epigenetic regulators have progressed to clinical stage studies and have been investigated exhaustively to ascertain therapeutic benefits in diverse malignancies. We summarize below the advantages and disadvantages of these epigenetic-targeted reagents in cancer therapy. It is now well accepted that targeting epigenetic regulators has a profound and rational foundation in the molecular understanding of its mechanism. Furthermore, increasing evidence has validated epigenetic regulators as viable therapeutic targets in various cancer types. As a result, significant progress has been made in the development of epigenetic-targeted small molecule reagents. Thus far, these epidrugs have exhibited impressive therapeutic efficacy, particularly for hematological malignancies in preclinical and clinical trials. In addition, given the potential for epigenetic therapy to enhance antitumor efficacy of current therapies, such as chemotherapy, immunotherapy, and targeted therapy, there have been promising opportunities to develop novel treatment regimens combining epidrugs with standard treatment strategies. However, some challenges remain to be faced and solved for successful epigenetic drug discovery, including selectivity, efficacy and resistance.

The first problem is selectivity in normal versus cancer cells. It is generally considered that epigenetic regulation of gene expression is an essential component in a wide variety of physiological processes in normal cells. Given the complexity of tumorigenesis, aberrant regulation of epigenetic machinery may affect multiple aspects in cancer, leading to cancer growth, invasion and metastasis. It

is therefore not surprising that some cancers may develop addiction or higher sensitivity to certain perturbation of epigenetic regulation as compared to other cancer types or their normal counterparts. Thus, it is important to identify the most important epigenetic aberrations for different cancers, which may provide cancer-specific vulnerabilities.

The second challenge is limited efficacy in solid tumors. Up to now, the most impressive results of epigenetic therapy has been obtained in hematological malignancies, but not in solid tumors, which has attracted tremendous research efforts to understand the role of TME, particularly, the immunosuppressive environment in solid tumors. Considering the distinct properties of hematological malignant cells versus solid tumor cells, future studies will have to incorporate appropriate strategies to tackle the TME to improve the therapy success.

The third challenge is acquired drug resistance. Like most small molecule compounds, drug resistance is a common problem and more importantly, single agent may not work well for advanced solid tumors. Given that epigenetic alterations may affect the sensitivity of other therapies, such as chemotherapy, radiotherapy, or immunotherapy, it would be interesting to test whether epigenetic-targeted therapy could be used an important adjunctive therapy. In the near future, results from the ongoing clinical trials on the combination of epidrugs and standard therapies will provide valuable information.

## 4. The renaissance of natural compounds in cancer therapy: an update

It has been increasingly evident that dietary supplements and natural compounds exhibit potent antitumor activities. These effects are mediated in part by modulation of epigenetic machinery to reprogram the TME to affect antitumor immunity. Here, we highlight the most up-to-date information on the preclinical and clinical trials using naturally occurring compounds alone or in combination with current cancer therapies, such as chemotherapy, targeted therapy, and immunotherapy. We summarize the ongoing cancer clinical trials of natural compounds, as of November 2022, either alone or in combination therapy in Tables 2 and 3, respectively. So far, there are 77 clinical trials testing the antitumor efficacy of 26 natural compounds in various cancer types, among which we highlight the most recent results of these clinical studies.

### 4.1. Curcumin

Due to its extensive pharmacological and biological qualities, curcumin, a natural chemical derived from the rhizomes of *Curcuma longa*, has come under intensive examination. Given the antibacterial, anti-inflammatory, antioxidant, anti-fungal, anti-antineoplastic, and pro-apoptotic properties of curcumin, the enormous potential of curcuminoids in the therapeutic management of numerous chronic diseases like cancer has already been shown by a multitude of literature. When exposed to the toxic effects of conventional therapies, it displays both chemoprotective and chemo sensitive, as well as radioprotective and radiosensitive, qualities. Curcumin has been shown to have anti-cancer effects in animal studies, and many more trials are underway to assess its safety, feasibility, and recurrence rate in cervical cancer patients receiving chemoradiation treatment (ClinicalTrials.gov Identifier: NCT02554344; NCT04266275; NCT04294836; NCT02944578). Curcumin's potential to alleviate the itchiness and discomfort caused by radiation was highlighted in another research involving patients with breast cancer [32]. Curcumin, an FDA-approved chemotherapeutic medication, has synergistic benefits in combination treatment outcomes. The majority of these combination therapies are currently in the pre-clinical trial stage of testing. For instance, curcumin helps 5-FU (fluorouracil, a popular chemotherapy treatment authorized by the FDA), destroy the stomach tumor [33]. Additionally, curcumin suppresses the growth of liver cancer synergistically. Of note, a phase 3 clinical study for curcumin is being conducted in conjunction with breast cancer. Finally, scientists have recently used

*Table 2. Clinical trials of natural compounds in combination with cancer immunotherapies.*

| Natural compounds | Immunotherapy | Cancer type | Phase | NCT# |
|---|---|---|---|---|
| Romidepsin | Pembrolizumab (PD-1) | Colorectal cancer | Phase 1 | NCT02512172 |
| | Pembrolizumab (PD-1) | Lymphoma | Phase 2 | NCT03192059 |
| | Pembrolizumab (PD-1) | Multiple lymphoma | Phase 1,2 | NCT03278782 |
| | Pembrolizumab (PD-1) | Solid tumors | Phase 1 | NCT01537744 |
| Vitamin D | Pembrolizumab (PD-1) | Cervical cancer | Phase 1 | NCT03331562 |
| Curcumin | Pembrolizumab (PD-1) | Endometrial cancer, Uterine cancer | Phase 2 | NCT03192059 |

*Table 3. Pre-clinical studies of natural compounds in combination with other cancer therapies.*

| Natural compounds | Cancer therapy | Cancer model | *In vitro* and *in vivo* results | Ref |
|---|---|---|---|---|
| Nano-curcumin | Nano-vaccine | Breast cancer: ***in vivo*** | ***In vivo***: amplified the level of systemic host T-cell immunity, promoted the infiltration of CD8+ T lymphocytes in tumor, and attenuated the local tumor recurrence and pulmonary metastasis | [34] |
| | Nano-docetaxel | Breast cancer: ***in vitro*** and ***in vivo*** | ***In vitro***: nanosuspensions showed higher cytotoxicity on MCF-7 cell line compared to their suspensions;<br>***In vivo***: sensitization of tumor cells and inhibition of p-gp showed greater tumor inhibition rate of up to 70% | [96] |
| | Doxorubicin | Colorectal cancer: ***in vitro*** and ***in vivo*** | ***In vitro:*** poly (ethylene glycol) -coated curcumin/doxorubicin hydrochloride nanoparticles (PEG−CRC/DOX NPs), well localized within DOX-resistant cancer cells, induced apoptosis and suppressed the major efflux proteins;<br>***In vivo***: HCT-8/DOX-resistant tumor xenograft showed improved bioavailability of the PEG −CRC/DOX NPs, and suppressed tumor growth significantly | [97] |
| Curcumin | Gemcitabine (Gemzar) | Pancreatic cancer: **clinical trial** | Phase 3 clinical trial: Recruiting | NCT 00486460 |
| | Metformin | Liver cancer: ***in vitro*** and ***in vivo*** | ***In vitro***: co-treatment of metformin and curcumin induced tumor cells apoptosis and suppressed invasion, metastasis of HCC cells and angiogenesis of HUVECs;<br>***In vivo***: co-administration of metformin and curcumin significantly inhibited HCC tumor growth than administration with metformin or curcumin alone in a xenograft mouse model | [98] |
| | 5-FU (Fluorouracil) | Gastric cancer: ***in vitro*** and ***in vivo*** | Curcumin enhances the anticancer effect of 5-fluorouracil (5-FU) against gastric cancer ***in vitro*** and ***in vivo*** | [33] |
| Resveratrol | Doxorubicin | Breast cancer: ***in vitro*** and ***in vivo*** | ***In vitro***: resveratrol markedly enhanced Dox-induced cytotoxicity in breast cancer cells;<br>***In vivo***: treatment with a combination of resveratrol and Dox significantly inhibited xenograft tumor volume by 60%, relative to the control group | [35] |
| | Gemcitabine (Gemzar®) | Pancreatic cancer: ***in vitro*** and ***in vivo*** | ***In vitro***: resveratrol inhibited pancreatic cancer cell proliferation, synergized the apoptotic effects of gemcitabine;<br>***In vivo***: in an orthotopic model of human pancreatic cancer, resveratrol significantly suppressed the growth of the tumor, which was further enhanced by gemcitabine | [36] |
| | Temozolomide (Temodar®) | Brain cancer: ***in vitro*** and ***in vivo*** | ***In vitro***: temozolomide (TMZ) induced both apoptotic cell death and cytoprotective autophagy, which was suppressed by resveratrol, resulting in a decrease in autophagy and an increase in apoptosis in a synergistic manner;<br>***In vivo***: mouse xenograft study showed that coadministration of resveratrol and TMZ reduced tumor volumes | [37] |
| | 5-FU (Fluorouracil) | Skin cancer: ***in vitro*** and ***in vivo*** | ***In vitro***: resveratrol augmented the growth inhibitory effect of 5-FU on TE-1 and A431 cancer cells;<br>***In vivo***: the tumor regression rate in the combination group increased significantly after four weeks of treatment | [99] |
| | | Bladder cancer: ***in vitro*** | ***In vitro***: combination treatment with rapamycin and resveratrol induced cell death specifically in TSC1−/− MEF cells, and not in wild-type MEFs. Similarly, resveratrol alone or in combination with rapamycin induced cell death in human bladder cancer cell lines | [100] |
| | Sirolimus (Rapamune, Rapamycin) | Thyroid cancer: ***in vitro*** and ***in vivo*** | ***In vitro***: compared to single use of rapamycin or resveratrol, co-administration had a synergistic effect in inhibiting proliferation and invasion/migration of papillary thyroid cancer cells and inducing apoptosis. Resveratrol is sensitizing the anti-tumor effects of rapamycin;<br>***In vivo***: co-administration significantly enhanced the anti-tumor effects than use of any one drug, as shown by the reduction of the tumor growth rate in the xenograft model | [101] |

(*continued on next page*)

*Table 3. (continued)*

| Natural compounds | Cancer therapy | Cancer model | *In vitro* and *in vivo* results | Ref |
|---|---|---|---|---|
| | Sirolimus (Rapamune, Rapamycin) | Lymphoma: *in vivo* | *In vivo*: the combination of rapamycin treatment with resveratrol in the TSC2-null xenograft tumor model inhibited PI3K/Akt/mTORC1 signaling and activated apoptosis | [102] |
| EGCG | Doxorubicin | Bladder cancer: *in vitro* | *In vitro*: EGCG enhanced the anti-tumor effect of DOX in bladder cancer via NF-κB/MDM2/p53 pathway | [103] |
| | Doxorubicin | Lung cancer: *in vivo* | *In vivo*: Treatment of metastatic human lung tumor xenograft/severe combined immunodeficient (SCID) mice with doxorubicin-loaded anti-beta1 Fab immunoliposomes resulted in a significant suppression of tumor growth; the immunoliposomes prevented the metastatic spread of the tumor to the liver and adrenal glands and increased the median survival time of the tumor-bearing mice | [104] |
| | Doxorubicin | Liver cancer: *in vitro* and *in vivo* | *In vitro*: administration of DOX in combination with ECG or EGCG markedly enhanced intracellular DOX accumulation and inhibited HCC cell proliferation;<br>*In vivo*: administration of DOX with ECG or EGCG at lower doses significantly inhibited hepatoma growth in a xenograft mouse model, compared with treatment with either agent alone at the same dose | [105] |
| | 5-FU (Fluorouracil) | Gastric cancer: *in vitro* | *In vitro*: EGCG inhibited gastric cancer growth and reverses 5-FU resistance of gastric cancer | [106] |
| | 5-FU (Fluorouracil) | Colorectal cancer: *in vitro* | *In vitro*: compared to 5-FU or EGCG treatment alone, the combination of both significantly promotes cancer cell apoptosis and DNA damage | [107] |
| | Gemcitabine (Gemzar) | Pancreatic cancer: *in vitro* and *in vivo* | *In vitro:* EGCG affected glycolysis by suppressing the extracellular acidification rate through the reduction of the activity and levels of the glycolytic enzymes; EGCG sensitized gemcitabine to inhibit pancreatic cancer cell growth;<br>*In vivo*: EGCG and gemcitabine, given alone, reduced pancreatic tumor xenograft growth by 40% and 52%, respectively, whereas the EGCG/gemcitabine combination reduced tumor growth by 67% | [108] |
| | Gemcitabine (Gemzar) | Pancreatic, colon, and lung cancer: *in vitro* | *In vitro*: EGCG sensitized gemcitabine, 5-FU, and doxorubicin in multiple cancer cell lines, including pancreatic, colon, and lung cancer cells | [109] |
| | Gemcitabine (Gemzar) | Lung cancer: *in vitro* and *in vivo* | EGCG synergizes with Gefitinib to inhibit the proliferation of Gefitinib-resistant NSCLC cells *in vitro* and suppress tumor growth in an *in vivo* xenograft mouse model | [110] |
| Romidepsin (FDA approved) | Duvelisib (Copiktra®) | Lymphoma: **clinical trial** | Phase I clinical trial; Active, not recruiting | NCT 02783625 |
| Sodium Butyrate | Cisplatin (Platinol) | Gastric cancer: *in vitro* and *in vivo* | *In vitro:* Cisplatin combined with sodium butyrate increased the apoptosis of GC cells;<br>*In vivo*: in the nude mouse xenograft tumor model, sodium butyrate in combination with cisplatin markedly inhibited the growth of the tumor more effectively than either single agent | [111] |
| | Docetaxel | Lung cancer: *in vitro* and *in vivo* | Combined therapy of sodium butyrate and docetaxel additively inhibited proliferation and promotes apoptosis of A549 lung adenocarcinoma cells via suppressing Gli1 expression *in vitro* and *in vivo* | [112] |
| | Gemcitabine (Gemzar) | Pancreatic cancer: *in vitro* and *in vivo* | *In vitro*: beside slowing proliferation, butyrate enhanced gemcitabine effectiveness against human pancreatic cancer cell lines;<br>*In vivo*: when administered to a PDAC mouse model, alone or combined with gemcitabine, butyrate markedly reduced the cancer-associated stromatogenesis, preserved intestinal mucosa integrity and affected fecal microbiota composition by increasing short chain fatty acids producing bacteria and decreasing some pro-inflammatory microorganisms | [45] |
| EGCG | Resveratrol | Head and neck cancer: *in vitro* and *in vivo* | *In vitro:* combination of EGCG and resveratrol has synergistic growth inhibitory in head and neck cancer cell lines;<br>*In vivo*: The combination significantly inhibited growth of xenografted head and neck tumors in nude mice | [113] |

nanoparticle technology to overcome the poor drug-likeness of curcumin. It has been shown that nano-curcumin maintains its anti-tumor action while also improving bioavailability. Similar to regular curcumin, nano-curcumin has the potential to eradicate cancer by boosting tumor immunogenicity and sensitizing drug-resistant tumor cells. This combination has been authorized by the FDA [34].

### 4.2. Resveratrol

Numerous studies have documented the anti-tumor efficacy of resveratrol in pre-clinical and clinical trials of single therapy. Importantly, resveratrol in combination with other cancer treatments have recently been shown to have a synergistic impact on some tumors [35]. Additionally, the findings of the pre-clinical trials demonstrate that in cold tumors, such as breast cancer [35], pancreatic cancer [36], and brain cancer [33,37], the combination strategy reduced tumor volume more than that of the single therapy. Furthermore, resveratrol has been mentioned as a possible immunomodulatory reagent. As a result, resveratrol plus immunotherapy may turn a cold tumor into a hot tumor which is then susceptible to immunotherapy. The most recent research found that resveratrol lowers T cell PD-1 expression [38]. However, it has been demonstrated that resveratrol reduces tumor volume in the hot tumor, such as via enhancing NK cell IFN- production in a mouse melanoma model, despite the lack of data about the interaction of resveratrol and classical immune blockade, PD-L1/PD-1 pathway [39]. Additionally, it lowers the number of melanoma colonies *in vivo* while increasing CXCL10 and IFN- levels in the tumor microenvironment [40].

### 4.3. EGCG

Epigallocatechin gallate (EGCG) is the primary active ingredient in the green tea polyphenol preparation known as Polyphenon E (Poly E). A recent study examined Poly E's effectiveness and safety in preventing cancer in those who had rectal aberrant crypt foci (ACF), which are thought to be the origins of colorectal cancer. Participants were considered eligible if they had a history of advanced colorectal adenomas or malignancies and if they had fewer than five rectal ACF at the time of their preregistration chromoendoscopy. In the clinical experiment with the identifier NCT01606124, participants (N = 39) were assigned at random to receive 6 months of oral Poly E daily. The main outcome was the percentage of rectal ACF decrease at chromoendoscopy compared to baseline. At six months, adenoma recurrence rates were comparable. The amount of medication taken was not considerably different. Adverse effects (AE) from Poly E were well tolerated and did not significantly vary. In summary, Poly E for 6 months did not substantially lower the number of rectal ACF. At the dosage tested, Poly E was well tolerated and had little toxicity [41].

Furthermore, in pre-clinical studies, immune modification by EGCG has been widely documented. Although these findings point to potential application outcomes, numerous challenges still exist, such as low stability and limited solubility. Importantly, combination treatment has shown better anti-tumor effectiveness in pre-clinical testing than single regimens. Combining EGCG with doxorubicin demonstrates that EGCG helps reduce doxorubicin dosages while maintaining the same benefit in the treatment of bladder cancer, prostate cancer, and liver cancer [103–105]. Since doxorubicin's cardiotoxicity was well documented in clinical studies, EGCG serves as the perfect adjuvant to shield against unintended consequences. Additionally, drug-resistant cancers are sensitized by EGCG in multiple cancer types, including pancreatic, colon and lung cancer cells [109]. Mechanistically, this sensitization may be through concentration-dependent suppression of ERK phosphorylation by EGCG, which leads to increased sensitivity to current chemotherapeutics, including gemcitabine, 5-flourouracil, and doxorubicin [109]. Furthermore, according to recent research, co-treatments with EGCG and FDA-approved chemotherapeutic medicines reduce the development of cold tumors including breast cancer and pancreatic cancer [110].

### 4.4. Trichostatin A

Through its HDAC inhibitor action, trichostatin A (TSA) has been investigated in pre-clinical trials for its potential to prevent tumor growth and boost the immune system in a variety of cancer types. TSA is seen as a possible adjuvant to treat resistant cancer cells in light of the rising incidence of drug resistance to chemotherapy. Through the inhibition of the ERK pathway, TSA synergistically enhances the anti-tumor activity of paclitaxel in cases of bladder cancer [42]. Recent research by Xiaolei Li et al. showed that co-administering TSA with anti-PD-L1 immunotherapy increased PD-L1 on the surface of tumor cells [43]. Additionally, using TSA in conjunction with immune treatment and radiation therapy seems to enhance the prognosis for head and neck cancer, according to the cancer genome

dataset. These pre-clinical study findings support the significance of TSA to assist in the tumor removal in cancer treatment, despite the lack of TSA use in the clinical experiments yet [44].

### 4.5. Butyrate

In several *in vitro* and *in vivo* investigations, butyrate has anti-cancer efficacy via its HDAC inhibitory action. Increasing research in recent years has suggested the potential of combination therapy with chemotherapy drugs in the treatment of gastric cancer [57] and lung cancer [58], despite the fact that the most recent clinical trial database shows insufficient study numbers in both the single treatment and co-treatment of butyrate. In pancreatic cancer, mainly considered a cold tumor, butyrate and gemcitabine combination also augments gemcitabine-induced apoptosis. However, the same article *in vivo* study shows no significant difference in combination treatment as compared to single administration. Despite that, the co-treatment impedes stroma genesis by decreasing extracellular matrix and fibrosis formation [45].

## 5. Natural compounds as epigenetic modulators in immune-oncology

The adaptive and innate immune systems work together to maintain a highly proficient immune surveillance machinery that can recognize and eradicate malignant cells. The functions of, and cross-regulation between, the adaptive immunity, mediated by T and B cells, and innate immunity, mediated by dendritic cells (DCs), monocytes and macrophages, and natural killer (NK) cells, are essential for anticancer immunity through interactions and production of cytokines, chemokines, and growth factors. Accumulating evidence from multiple experimental models demonstrates a multifaceted role of natural compounds in the epigenetic modulation of antitumor immunity [1,4].

### 5.1. Antitumor adaptive immunity

The adaptive immune responses mediated by T and B cells are initiated and shaped in part by the innate immune responses. The detection of antigen by T cell antigen receptors is the first signal in a three-signal model that activates T cells. The second signal is co-stimulation by the antigen presenting cell (APC). As for the third signal, it comes from innate immunological activation-derived inflammatory cytokines that may either act directly on the T cell or indirectly by boosting costimulatory molecules on the APC. Antigen may also activate B cells in two ways: through interactions with T cells or independently of them [46].

#### 5.1.1. CD4+ T cells

For quite some time, the cytokines secreted by CD4+ T helper (Th) cells and, more recently, the expression of important transcription factors, have been the primary determinants of subset identification. Understanding Th differentiation and function has been aided by the original paradigm of IFN-producing Th1 cells and IL-4-producing Th2 cells. Recently, a group of T cells called Th17 cells, which are distinguished by their ability to produce IL-17, has been identified based on observations that could not be addressed using the traditional Th1/Th2 paradigm alone. The importance of Th17 cells is exemplified by the fact that the response of the body to infections, cancer, and the development of autoimmune illnesses all require Th17 cells. At the same time, regulatory/suppressor CD4+ and CD8+ T cells are crucial immune regulatory mechanisms that control CD4+ T cell responses in both immunological stimulation and immune suppression [47].

*5.1.1.1. CD4+ T cells in immune stimulation*. Many natural compounds modulate CD4+ T cells in immune stimulation. Kespohl et al. suggest that butyrate enhances immunological response at high concentrations (1 mM). By causing H3 acetylation at *T-bet* and *Ifny* genes, butyrate epigenetically regulates Treg differentiation and prevents Treg production [48]. Additionally, butyrate promotes the development of naive CD4+ T cells into Th1 and Th17 cells and boosts IFN- production in CD4+ T cells. Overall, butyrate promotes CD4+ T cells in a dose-dependent way leading to immunological activating or immune suppressive [49]. Curcumin inhibits cell-cell interaction and suppresses the expression of Foxp3 and CTLA-4, which dampens the tumor-induced suppressive activity of Tregs. Curcumin also inhibits the release of inhibitory cytokines and reduces the number of Tregs that secrete them [50]. Importantly, following investigations of individuals with human colon and lung cancer corroborated the significance of curcumin as an immune-modulator. Treatment with curcumin dramatically decreases regulatory T cells, impairs Foxp3 expression, boosts IFN- production, and converts regulatory T cells to Th1 cells [51].

*5.1.1.2. CD4+ T cells in immune suppression*. On the other hand, many natural compounds, such as butyrate, curcumin, EGCG, sulforaphane, and trichostatin A, also have the capacity to decrease the

immune system's response to CD4+ T cells. Through reducing the expression of Rort, a crucial transcription factor for Th17 differentiation, butyrate prevents the formation of Th17 cells. According to research by J-Q Liu's team, curcumin prevents the proliferation of CD4+ T cells, including Th1, Th17, and Treg cells, by upregulating the pro-apoptotic protein Bax and downregulating the anti-apoptotic protein Bcl-2 in CD4+ T cells [52]. Additionally, curcumin reduces the synthesis of IL-12 by macrophages, which dampens Th1 response and prevents Th17 cells from secreting IL-17 [53]. But additional research is needed to determine curcumin's overall impact on immune response.

Another mechanism by which natural compounds modulate CD4+ T cell function in immune suppression is through the phosphorylation of STAT3, a key transcription factor for Th17 development. Curcumin modulates STAT3 phosphorylation in patients with psoriatic arthritis and psoriasis, and curcumin therapy results in anti-inflammatory response [54]. Another report also found that curcumin impairs the DC-dependent differentiation of Th1 and Th17 cells *in vivo* and causes anti-inflammatory phenotype in DCs with enhanced STAT3 phosphorylation [55]. Moreover, through inhibition of IL-2, proliferation of CD4+ T cells in mice was directly suppressed by EGCG [56]. Tyrosine and serine phosphorylation of STAT1, which is required for naïve T cell development into Th1, is also inhibited by EGCG, as was shown in a recent research [57]. Jie Liang et al. reported that sulforaphane inhibits STAT3 phosphorylation in contrast to curcumin, which enhances phosphorylation of STAT3, but studies are required to determine whether this is connected to the Th17 cell's inhibition by sulforaphane (SFN), a naturally occurring substance found in cruciferous vegetables and well-defined DNMT and HDAC inhibitor [58]. Cristian Doas's finding on Treg modulation demonstrates that TSA promotes H3 acetylation at the Foxp3 gene promoter, which aids in Treg development and is significantly greater in differentiated Treg than in naïve T cells. Additionally, mice with TSA-induced Treg have increased levels of ectonucleotidases CD39 and CD73, which furthers their immunosuppression [59]. On the other hand, a previous study found that TSA treatment decreases the amount of Foxp3 mRNA and protein, as well as CD4+CD25+Foxp3+ Treg [60]. Finally, Cao K. et al. also found that TSA and anti-CTLA4, when used together, increased the filtration of the CD4+ T lymphocyte population, which led to synergistic anti-tumor effects in an animal model with melanoma [61].

*5.1.2. CD8+ T cell*

CD8+ cytotoxic T lymphocytes (CTLs) are highly sought after for their ability to destroy cancer cells. TME factors, such as immune-related tolerance and immunosuppression, support adaptive immune-resistance during cancer development, leading to malfunction and depletion of CTLs. When it comes to eliminating cancer cells that display major MHC class I molecules, CD8+ T lymphocytes are crucial Whereas DCs, NKs, and CD4+ T cells are key players in the priming process that sets the stage up for this task [62].

*5.1.2.1. CD8+ T cell in immune stimulation*. The gut microbiota produces metabolites such as short-chain fatty acids (SCFAs) that regulate the energy homeostasis and impact immune cell function of the host. Recently, innovative approaches based on the oral administration of SCFAs have been discussed for therapeutic modification of immune responses. So far, although most studies have investigated the SCFA-mediated effects on CD4+ T cells and APCs, butyrate and, to a lesser degree, propionate directly modulate gene expression of CD8+ CTLs and Tc17 cells. Importantly, butyrate was a mediator of the molecular transition of Tc17 cells towards the CTL phenotype through increased IFN- and granzyme B production by CTLs, irrespective of its interaction with particular SCFA-receptors GPR41 and GPR43. Strong HDAC inhibition by butyrate in CD8+ T lymphocytes affected the expression of effector molecule genes. As a result, sodium valproate and TSA, two pan-HDAC inhibitors, had an equivalent effect on CD8+ T cells. Furthermore, via modifying cellular metabolism and mTOR activity, increased acetate concentrations were also able to boost IFN-production in CD8+ T cells. These discoveries may have important effects on antiviral immunity as well as cancer adoptive immunotherapy [63].

*5.1.2.2. CD8+ T cells in immune suppression*. Romidepsin has anti-tumor actions against a wide variety of solid cancers. Although romidepsin has been shown to increase PD-L1 expression in tumor cells, its effect on anti-tumor immune responses is recently emerging. In one study examining romidepsin for its potential to inhibit tumor growth and impact immune responses in colorectal cancer, the growth of CT26 and MC38 cells was suppressed by romidepsin, which also produced a G0/G1 cell cycle arrest and elevated apoptosis. Romidepsin upregulated BRD4 and enhanced H3 and H4 acetylation, leading to higher PD-L1 expression *in vivo* and *in vitro*. Furthermore, romidepsin enhanced the

proportion of FOXP3+ Tregs and lowered the ratio of Th1/Th2 cells and the percentage of IFN-+ CD8+ T cells in the peripheral blood and the TME in mice with subcutaneous transplant tumors and animals with colitis-associated cancer (CAC). The anti-tumor effects of romidepsin were amplified, and the impact on CD4+ and CD8+ T cells was partly reversed, when the drug was combined with an anti-PD-1 antibody. Therefore, combining romidepsin with anti-PD-1 immunotherapy is a promising strategy for treating colon cancer [64]. Similarly, when EGCG was added to mouse spleen cells, it stopped T cells from responding. Cocultures of APCs and T cells that had been preincubated with or without EGCG showed that EGCG stopped the growth of T cells that were caused by antigens, mostly through a direct effect on T cells. Furthermore, when anti-CD3/CD28-purified mouse T cells were tested in the presence of EGCG (2.5–15 mmol/L), EGCG dose-dependently stopped cell division and progression through the cell cycle, and that this effect was stronger in CD4+ T cells than in CD8+ T cells. Notably, IL-2 levels in cell cultures treated with EGCG were higher than those in the control cells untreated. However, intracellular staining showed no difference in IL-2 synthesis, but EGCG-treated cells expressed less IL-2 receptor (IL-2R) compared with untreated control cells. Thus, EGCG directly inhibits T cell proliferative response to both polyclonal and antigen-specific stimulation. Future studies should determine the effect of EGCG on CD4+ cell subsets to assess its application in T cell-mediated immune suppression [52,56].

### 5.2. Antitumor innate immunity

Pattern-recognition receptors (PRRs) that detect danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) are essential for the activation of the innate immune response. A significant class of PAMPs that function in certain subcellular localizations and are detected by a wide variety of PRRs are pathogen-derived nucleic acids. Because nucleic acids are abundant in cells, the PRRs that correspond to them have evolved to be tightly controlled and segregated [65].

#### 5.2.1. DCs

DCs serve critical functions in both fostering immunological defense and maintaining immune tolerance. They constitute the vital connection between innate immunity and adaptive immunity. Different DC subsets must be transported between lymphoid and nonlymphoid tissues in order for DCs to activate and control immune response and inflammation. DC chemotaxis and migration are induced by interactions between chemokines and their receptors, which are controlled by a variety of intracellular processes, including tissue-specific metabolic remodeling, protein modification, epigenetic reprogramming, and cytoskeletal rearrangement. An imbalance of immune responses, including autoimmune reactions, infectious disorders, allergy diseases, and malignancies, may occur from dysregulation of DC migration, aberrant placement of DCs, or even DC activation [66].

Due to microbial fermentation of dietary fiber, butyrate, a prominent SCFA in the mammalian gastrointestinal system, is the first important HDACi that has been discovered. A number of lines of evidence suggest that butyrate may exercise its anti-cancer and chemo-preventive effects by boosting the anti-tumor immune response, in part through dampening DC's efficacy [67]. In this study, butyrate reduces the production of pro-inflammatory chemokines such as CCL3, CCL4, CCL5, CXCL9, CXCL10, and CXCL11 that are secreted by DCs [68]. This modulation of DCs by butyrate may be attributed to its role as a HDAC inhibitor. In addition, butyrate has also been shown to suppress immunological response by increasing IL-10 Treg activity. In this work, butyrate promotes net histone acetylation in human DCs, driving retinaldehyde dehydrogenase 1 (RALDH1) production. In turn, Retinoic acid (RA), which is produced from vitamin A by RALDH1, is transported to Treg, where RA binds to the RA receptor, causing Foxp3- Treg1 to secrete IL-10, an immunosuppressive cytokine of T cell proliferation [69]. Similarly, EGCG also prevents DCs from mounting an immunological response. Generally, either lipopolysaccharide (LPS) or inflammatory cytokines stimulate the maturation of DCs through boosting expression of CD80, CD83, and CD86, leading to T cell activation. According to Yoneyama's study, EGCG prevents DCs from maturing by reducing CD83 expression in human DC progenitors [70]. Mechanistically, this regulation may be attributed to the histone methyltransferases (HMT) activity of EGCG, although the mechanism is not fully understood [71]. Consistent with the notion that histone methylation modifications occur throughout DC development and maturity, the maturation of a common DC progenitor (CDP) into a conventional DC is accompanied by increased levels of histone H3 lysine 4 monomethylation (H3K4me1) and H3 lysine 4 trimethylation (H3K4me3) [72]. According to Muhammad Jasim Uddin et al., SFN inhibits LPS-induced immunological responses in DCs via suppressing the expression of CD40, CD80, and CD86. They further

showed that the immune suppression by SFN is mediated by the downregulation of HDAC10 and DNMT3a by SFN [88]. Additionally, their findings indicated that SFN also enhances the accumulation of cellular-resident TNF-, which may eliminate pathogens by activating the NF-B signaling pathway, supporting the idea that SFN functions as an immune stimulant in this context [88]. Consistent with a pleiotropic effect of SFN on immunological response, SFN blocks DCs maturation by downregulation of CD40, CD80, and CD86, which in turn reduces co-stimulatory signals involved in CD4+ T cell activation. Alternatively, MHC-SLA1, a TLR4-induced cell surface molecule located on DCs is upregulated by SFN, through an decrease of MHC-SLA1 promoter DNA methylation, which leads to the augmentation of MHC-SLA1 expression, then T cell recognition and T cell cytotoxicity [73]. Finally, curcumin, one of the most extensively studied natural phytochemicals, play multiple roles as an epigenetic modulator that have recently been reviewed [74]. As shown by the work of Francesca Milano et al., curcumin upregulates CD86 to enhance DCs maturation, which in turn stimulates the favorable immunological response. Although the specific mechanisms by which curcumin activates CD86 were not revealed in this report, there are studies suggesting that the function of curcumin is related to m6A regulation [75], DNA methylation, and histone methylation [76].

*5.2.2. TAMs*

The presence of TAMs is generally associated with therapy failure and poor prognosis in solid tumors. These findings have sparked tremendous interest in the development of macrophage antagonists, many of which have already demonstrated combinatorial efficacy when combined with immunotherapy. Given the effects of many natural compounds on macrophages polarization, further studies are required to test whether this leads to enhanced antitumor immunity. By altering cytokine production, Sadaf Shiri's team found that dendrosomal formation of curcumin (DNC) stimulates a strong macrophage immune response and prevents metastatic breast cancer in mice [77]. By increasing the expression of STAT4/IL-12 and decreasing the expression of STAT3/IL-10, DNC treatment changed the balance of M1/M2 macrophages into a majority of M1. But it is still not clear how DNC controls STAT4/IL-12 and STAT3/IL-10. Additionally, curcumin blocks STAT3 activation by preventing phosphorylation of STAT3 at tyrosine 705 [78]. Importantly, curcumin's ability to prevent phosphorylation of STAT3 is responsible for its effects on macrophages. Additionally, the findings of William F. Carson 4th′s team may help explain why STAT4 and IL-12 are modulated in the first place. Using a mouse model, they demonstrated that curcumin regulates macrophages to elicit a favorable immunological response via increasing H3K4me3 in STAT4 [79]. Similarly, according to Ji-Young Jang et al., EGCG suppresses the polarization of M2 macrophages and controls the release of the immune suppressive cytokines IL-6 and TGF-β. They further proposed that miR-16 upregulation is the key mechanism of this regulation. According to their model, EGCG upregulates miR-16 in breast cancer cells, which is then transported via exosome to M2 macrophages [80].

On the other hand, SFN reduces immunological response through macrophages through a variety of mechanisms. Recent research by Haidy A. Saleh et al. shows that SFN is a strong immune suppressor against LPS/IFN-stimulated macrophages by reducing the induction of miR-146a and miR-155 trigged by LPS/IFN [81]. These findings may lead to the hypothesis that SFN might serve as a promising reagent to reduce inflammation. Similarly, SFN reduces the LPS-induced DNMT3a elevation in porcine macrophages, which lowers the amount of CD14 metalation and inhibits CD14 in the LPS-induced inflammatory pathway [82].

*5.2.3. Natural killer cells (NKs)*

NKs are a distinct class of innate lymphoid cells with the capacity to recognize and destroy cancerous and virally-infected cells, thus playing a crucial role in anticancer immunity due to their various cytotoxicity mechanisms to modify the immune response via cytokine production. Following the groundbreaking achievements of chimeric antigen receptor (CAR)-T cell therapy and the development of technologies that may transform cells into potent antitumor weapons, interest in NKs as a potential immunotherapy option has surged tremendously in recent years [83].

Curcumin, a potent cytotoxic natural compound against a variety of cancers, exerts multiple roles in the modulation of antitumor immunity. In glioblastoma and cervical cancer models, TAMs exhibited a dramatic switch from the pro-tumor and immunosuppressive M2-like phenotype to the tumoricidal and pro-inflammatory M1 phenotype when treated with a CD68 antibody-curcumin adduct, phytosomal curcumin, or TriCurin, a synergistic formulation of curcumin, resveratrol, and EGCG. Additionally, TAMs release the chemokine MCP-1 (also known as CCL2), which leads to the recruitment of activated macrophages and NKs

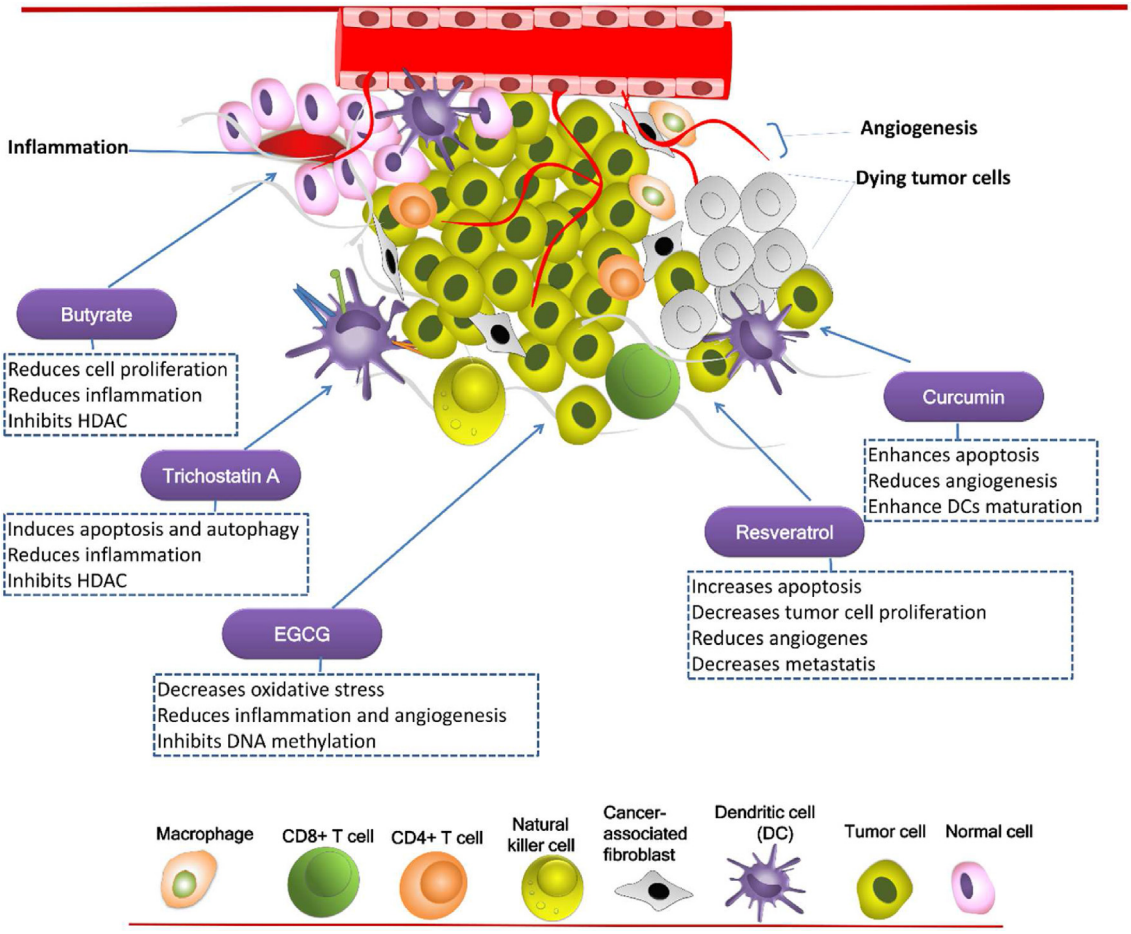


*Fig. 2. Schematic representation of the anticancer activities of natural compounds. On the one hand, natural compounds have shown anticancer activities by directly targeting cancer cells. On the other hand, they have immunomodulatory properties in immune cells in part by modulation of epigenetic machinery. The rational combination of natural compounds with current therapies, such as chemotherapy, immunotherapy and targeted therapy, may hold great promise to maximize the therapeutic benefits through enhancing antitumor immunity.*

from the bloodstream into the tumor. Because activated NKs destroy tumor cells and also release IFN, which binds to its receptor on microglia and macrophages to maintain the M1 phenotype, thus further amplifying the effects of curcumin. Furthermore, the M1-like microglia/macrophages destroy tumor cells by secreting the deadly chemical nitric oxide (NO), but activated NKs also kill cancer cells, cancer stem cells, and dormant macrophages (M0-type) by establishing immunological synapses. This is in line with the notion that M2 and M0-type macrophages are both killed by activated NKs whereas M1-like macrophages are spared [84].

Not only curcumin, EGCG and resveratrol are other natural compounds that have the capacity to activate NKs. As for green tea, tea catechin and saponin are key players in Th1/Th2 polarization that controls B-cell development. In one study, mice were given an oral dose of a standardized green tea extract (EGTE), and the effects on innate and

*Table 4. Comparison between FDA-approved epidrugs and natural compounds regarding their benefits and challenges of clinical applications.*

| | FDA-approved epidrugs | Natural compounds |
|---|---|---|
| Molecular targets | Well defined | Targets and mechanisms not fully characterized; pleiotropic activities |
| Specificity and selectivity | Higher | Lower |
| Pro-immunogenic vs immunosuppressive roles | Context-dependent immune-modulation | Context-dependent immune-modulation |
| Biomarkers | Some biomarkers are available for certain epidrugs | Biomarkers still yet to be identified to predict the therapeutic response and to stratify patients |

adaptive immunity were assessed by looking at the activity of phagocytes, B&T lymphocytes and NK cells [85]. As for resveratrol, it increased the protein and mRNA expression of MICA and MICB in breast cancer cells, two MHC-I genes that are crucial ligands for immune effector cells to recognize tumor cells. As a result, this facilitated both *in vitro* and *in vivo* lysis of breast cancer cells by NKs. Mechanistically, direct binding sites of miR-17 were found at the 3′-untranslated regions of MICA and MICB. In breast cancer cells transfected with a miR-17 inhibitor or mimic, respectively, MICA and MICB expression was increased or reduced accordingly. Furthermore, c-Myc was identified as the upstream regulator. Clinically, in two sets of breast cancer tumor specimens, the expression of miR-17 was negatively linked with the expression of MICA and MICB and overall survival. Taken together, by inhibiting the c-Myc/miR-17 pathway in breast cancer cells, resveratrol upregulates MICA and MICB and promotes the cytolysis of breast cancer cells by NK cells, stimulating the antitumor immunity [86].

It should be noted that the function of NKs can also be suppressed by natural compounds. Lucas E. Rossi's team demonstrated that TSA reduces NKG2D, an immune-activating signal receptor that binds to IL-5 to trigger an immunological response, which in turn prevents NKs from secreting IFN-γ [87]. Although the mechanism of this immune suppression remains unclear, a recent study, on the other hand, showed that HDAC inhibitors do not influence NKG2D, suggesting that TSA may regulate NKG2D via a different route [88]. Furthermore, Elisa C. Toffoli et al. showed that romidepsin, a natural HDAC1/2 inhibitor, increases the expression of NKG2DL in lung cancer, proving the significance of HDAC1/2 in making tumor cells susceptible to NKs detection [89]. According to an investigation by Alba Fernández-Sánchez et al., curcumin reduces NK cells' NKG2D expression, which prevents them from secreting IFN-γ. As for the epigenetic mechanism, curcumin induced histone H3 lysine 9 acetylation (H3K9Ac) at NKG2D gene, which may mediate its effects in NKs [90]. Furthermore, in NKs treated with valproic acid (VPA), a synthetic HDAC inhibitor, NKG2D is downregulated, accompanied by a decreased level of H3K9Ac. This finding is in agreement with an earlier report showing that HDAC inhibitors suppress NK cell function through a variety of mechanisms, including lowering cytotoxicity [91] and downregulating NKp46 and NKp30, two NK cell activating receptors [91]. Curcumin's anti-cancer potential is intriguing, but its high toxicity and limited water solubility are major drawbacks that can't be overlooked. Milan Fiala et al. proposed that a mixture of omega-3 fatty acids and curcuminoids may prevent NK cell deteriorating and thus compensate for the removal of IFN-γ release. This research sheds new information on a potentially fruitful strategy for overcoming the challenges of HDACi-induced immunosuppression of NK cells [92].

## 6. Future perspectives

The recent recognition of the role that more and more natural chemicals and their derivatives play as epigenetic modulators in immune-oncology has opened new avenues for drug discovery and therapeutics, which has led to an increasing number of ongoing clinical trials for cancer treatment. This demonstrates the possibility of integrating these drugs with existing epigenetic and immunological strategies (Fig. 2). The complicated interaction between these natural chemicals and tumor biology will need to be further explored in order to maximize the therapeutic possibility that these novel techniques have created, with a focus on translational implications and basic biological processes. We present the main challenges and future perspectives below (Table 4).

First, although the pleiotropic activities of most natural compounds offer desirable antitumor effects by targeting multiple oncogenic pathways, they impose an important barrier to a precise understanding of their biological targets and mechanisms involved herein. Not surprisingly, many of these agents are classified as pan-assay interference compounds, such as curcumin and EGCG [93]. As a result, additional study is required to investigate their selectivity and specificity. Importantly, future preclinical and clinical models should examine these medicines thoroughly to determine their effectiveness. In particular, when compared to currently available class-specific or isoform-specific epigenetic modulators, adequate comparative analysis may show to be a promising method. Similarly, the notion that the epigenetic markers may counteract one other is crucial to keep in mind as tumors advance. More systematic studies may be necessary to better understand the structure-activity correlation of these natural compounds to their immune-epigenetic functions in relevant tumor models, especially with the advent of emerging single-cell sequencing technologies that permit quantitative characterization of the diverse epigenetic landscape in various states.

Second, as shown by HDACi, many epigenetic modulators have immune-suppressive properties.

While Class I HDACi promote the activities of NKs and CD8 T cells, inhibition of Class II HDACs increases the quantity and effectiveness of Treg [94], Because of this, there is now a problem with drawing broad conclusions from specific experiments. Additionally, curcumin provided a good example of how different amounts of naturally occurring substances may have opposite effects on the immune system, stimulating or suppressing the immune system, respectively. Furthermore, the same epigenetic modulator, as is the case with natural substances, might play different roles of pro-immunogenic or immunosuppressive effects on different subpopulations of immune cells. The overall clinical prognosis in many cancer types may depend on the equilibrium between these antagonistic qualities of natural chemicals in the setting of the TME.

Finally, a growing body of evidence from preclinical models across a wide range of tumor types suggests that combining epigenetic treatment with existing immunotherapies yields better antitumor response than monoregimen alone. From the research discussed here, it is clear that natural substances, either alone or in conjunction with other epigenetic or immunogenic treatments, have a significant role in altering the immune milieu epigenetically. To move forward, one challenge is to select the appropriate patients best fit for the combinational immunotherapy to achieve the best clinical outcomes. It has been reported that patients with a high tumor mutational burden and diagnosed with advanced cutaneous squamous cell carcinoma (SCC) have more clinical benefits from the current immunotherapy. These results are consistent with those of cemiplimab clinical studies, which demonstrated a 65% and 61% persistent disease control in patients with advanced cutaneous SCC with metastases in Phase I and Phase II, respectively. However, the prognostic value of SCC tumor PD-L1 status is controversial. In the clinical study using nivolumab, it has been shown that the improvement of overall survival observed in the nivolumab group was not associated with the PD-L1 expression. Other studies have also shown no association between PD-L1 expression and immunotherapy efficiency [95]. Taken together, defining the molecular roles of distinct natural compounds and their physiological/pathological targets in the context of immune-oncology holds promise to understand the various immunoregulatory activities of these naturally active products. More importantly, knowledge of these studies will provide mechanistic rationale for the selection and stratification of patients who are mostly likely to respond to therapy. Therefore, the growing list of ongoing clinical trials will offer valuable information to address the abovementioned questions in the near future. Future studies on the biology and pharmacology of natural compounds should allow the discovery of novel biomarkers for therapeutic response and the best approaches to manipulating the immune microenvironment to tip the balance in favor of antitumor and chemopreventive benefits as medicine moves towards the development of custom-tailored precision therapies.

## Author contributions

D.-C. W. and X.-G. L. conceptualization; R. K., C.-H. L., M. H. and X.-G. L. writing original draft; R. K., D.-C. W. and X.-G. L. writing-review and editing.

## Funding

X.-G. L. was supported by the Andrew McDonough B+ (Be Positive) Foundation's Childhood Cancer Research Award, the Children's Cancer Research Fund's Emerging Scientist Award, the National Science and Technology Council (MOST 110-2311-B-039-001 and MOST 111-2320-B-039-050), and China Medical University (CMU109-YT-03). X.-G. L. was an Infinite Love for Kids Fighting Cancer Independent Investigator and Bear Necessities Pediatric Cancer Foundation Independent Investigator (No: 19IN31).

### Conflict of interest

The authors declare no conflicts of interest with the contents of this article.

## Acknowledgements

We thank current and former members (Kristie Stiles) of the Li laboratory and Dr. Ho Yuan-Soon (Graduate Institute of Biomedical Sciences, Institute of Biochemistry and Molecular Biology, College of Life Sciences, China Medical University) for helpful discussions.

## References

[1] Hogg SJ, Beavis PA, Dawson MA, Johnstone RW. Targeting the epigenetic regulation of antitumour immunity. Nat Rev Drug Discov 2020;19:776–800.
[2] Villanueva L, Álvarez-Errico D, Esteller M. The contribution of epigenetics to cancer immunotherapy. Trends Immunol 2020;41:676–91.
[3] Schnekenburger M, Dicato M, Diederich MF. Anticancer potential of naturally occurring immunoepigenetic modulators: a promising avenue? Cancer 2019;125:1612–28.
[4] Irshad R, Husain M. Natural products in the reprogramming of cancer epigenetics. Toxicol Appl Pharmacol 2021;417:115467.

[5] Spranger S. Mechanisms of tumor escape in the context of the T-cell-inflamed and the non-T-cell-inflamed tumor microenvironment. lnt Immunol 2016;28:383−91.
[6] Van der Woude LL, Gorris MA, Halilovic A, Figdor CG, de Vries IJM. Migrating into the tumor: a roadmap for T cells. Trends Cancer 2017;3:797−808.
[7] Sen DR, Kaminski J, Barnitz RA, Kurachi M, Gerdemann U, Yates KB, et al. The epigenetic landscape of T cell exhaustion. Science 2016;354:1165−9.
[8] Ghoneim HE, Fan Y, Moustaki A, Abdelsamed HA, Dash P, Dogra P, et al. De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation. Cell 2017;170:142−57.
[9] Topper MJ, Vaz M, Marrone KA, Brahmer JR, Baylin SB. The emerging role of epigenetic therapeutics in immuno-oncology. Nat Rev Clin Oncol 2020;17:75−90.
[10] Yau HL, Ettayebi I, De Carvalho DD. The cancer epigenome: exploiting its vulnerabilities for immunotherapy. Trends Cell Biol 2019;29:31−43.
[11] Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature 2015;527:249−53.
[12] Yoshihama S, Roszik J, Downs I, Meissner TB, Vijayan S, Chapuy B, et al. NLRC5/MHC class I transactivator is a target for immune evasion in cancer. Proc Natl Acad Sci USA 2016;113:5999−6004.
[13] Feng S, De Carvalho DD. Clinical advances in targeting epigenetics for cancer therapy. FEBS J 2022;289:1214−39.
[14] Bondarev AD, Attwood MM, Jonsson J, Chubarev VN, Tarasov VV, Schiöth HB. Recent developments of HDAC inhibitors: emerging indications and novel molecules. Br J Clin Pharmacol 2021;87:4577−97.
[15] Pan D, Lu X. New therapeutic avenue of epigenetic modulations in cancer. Transl Breast Cancer Res 2020;1:2. 2.
[16] Abaza YM, Kadia TM, Jabbour EJ, Konopleva MY, Borthakur G, Ferrajoli A, et al. Phase 1 dose escalation multicenter trial of pracinostat alone and in combination with azacitidine in patients with advanced hematologic malignancies. Cancer 2017;123:4851−9.
[17] Borcoman E, Kamal M, Marret G, Dupain C, Castel-Ajgal Z, Le Tourneau C. HDAC inhibition to prime immune checkpoint inhibitors. Cancers 2021;14:66.
[18] Llopiz D, Ruiz M, Villanueva L, Iglesias T, Silva L, Egea J, et al. Enhanced anti-tumor efficacy of checkpoint inhibitors in combination with the histone deacetylase inhibitor Belinostat in a murine hepatocellular carcinoma model. Cancer Immunol Immunother 2019;68:379−93.
[19] Liu GH, Chen T, Zhang X, Ma XL, Shi HS. Small molecule inhibitors targeting the cancers. MedComm 2022;3:181.
[20] Yang S-C, Wang W-Y, Zhou J-J, Wu L, Zhang M-J, Yang Q-C, et al. Inhibition of DNMT1 potentiates antitumor immunity in oral squamous cell carcinoma. Int Immunopharm 2022;111:109−13.
[21] Zhang S, Gong Y, Li C, Yang W, Li L. Beyond regulations at DNA levels: a review of epigenetic therapeutics targeting cancer stem cells. Cell Prolif 2021;54:12963.
[22] Nepali K, Liou J-P. Recent developments in epigenetic cancer therapeutics: clinical advancement and emerging trends. J Biomed Sci 2021;28:1−58.
[23] Daver N, Garcia-Manero G, Basu S, Boddu PC, Alfayez M, Cortes JE, et al. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/refractory acute myeloid leukemia: a nonrandomized, open-label, phase II StudyAzacitidine and nivolumab in relapsed AML. Cancer Discov 2019;9:370−83.
[24] Stazi G, Taglieri L, Nicolai A, Romanelli A, Fioravanti R, Morrone S, et al. Dissecting the role of novel EZH2 inhibitors in primary glioblastoma cell cultures: effects on proliferation, epithelial-mesenchymal transition, migration, and on the pro-inflammatory phenotype. Clin Epigenet 2019;11:1−17.
[25] Bao Y, Oguz G, Lee WC, Lee PL, Ghosh K, Li J, et al. EZH2-mediated PP2A inactivation confers resistance to HER2-targeted breast cancer therapy. Nat Commun 2020;11:1−17.
[26] Spiliopoulou P, Spear S, Mirza H, Garner I, McGarry L, Grundland-Freile F, et al. Dual G9A/EZH2 inhibition stimulates antitumor immune response in ovarian high-grade serous carcinoma. Mol Cancer Therapeut 2022;21: 522−34.
[27] Zhang L, Qu J, Qi Y, Duan Y, Huang Y-W, Zhou Z, et al. EZH2 engages TGFβ signaling to promote breast cancer bone metastasis via integrin β1-FAK activation. Nat Commun 2022;13:1−16.
[28] Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. J Hematol Oncol 2020;13:1−12.
[29] Kang N, Eccleston M, Clermont P-L, Latarani M, Male DK, Wang Y, et al. EZH2 inhibition: a promising strategy to prevent cancer immune editing. Epigenomics 2020;12: 1457−76.
[30] Gaillard SL, Zahurak M, Sharma A, Durham JN, Reiss KA, Sartorius-Mergenthaler S, et al. A phase 1 trial of the oral DNA methyltransferase inhibitor CC-486 and the histone deacetylase inhibitor romidepsin in advanced solid tumors. Cancer 2019;125:2837−45.
[31] Murphy AG, Walker R, Lutz ER, Parkinson R, Ahuja N, Zheng L, et al. Epigenetic priming prior to pembrolizumab in mismatch repair-proficient advanced colorectal cancer. Am J Clin Oncol 2019:591. 591.
[32] Ryan Wolf J, Gewandter JS, Bautista J, Heckler CE, Strasser J, Dyk P, et al. Utility of topical agents for radiation dermatitis and pain: a randomized clinical trial. Support. Care Cancer 2020;28:3303−11.
[33] Yang H, Huang S, Wei Y, Cao S, Pi C, Feng T, et al. Curcumin enhances the anticancer effect of 5-fluorouracil against gastric cancer through down-regulation of COX-2 and NF-κB signaling pathways. J Cancer 2017;8:3697.
[34] Liu X, Feng Z, Wang C, Su Q, Song H, Zhang C, et al. Co-localized delivery of nanomedicine and nanovaccine augments the postoperative cancer immunotherapy by amplifying T-cell responses. Biomaterials 2020;230:119−649.
[35] Kim TH, Shin YJ, Won AJ, Lee BM, Choi WS, Jung JH, et al. Resveratrol enhances chemosensitivity of doxorubicin in multidrug-resistant human breast cancer cells via increased cellular influx of doxorubicin. Biochim Biophys Acta 2014; 1840:615−25.
[36] Harikumar KB, Kunnumakkara AB, Sethi G, Diagaradjane P, Anand P, Pandey MK, et al. Resveratrol, a multitargeted agent, can enhance antitumor activity of gemcitabine in vitro and in orthotopic mouse model of human pancreatic cancer. Int J Cancer 2010;127:257−68.
[37] Lin CJ, Lee CC, Shih YL, Lin TY, Wang SH, Lin YF, et al. Resveratrol enhances the therapeutic effect of temozolomide against malignant glioma in vitro and in vivo by inhibiting autophagy. Free Radic Biol Med 2012; 52:377−91.
[38] Han X, Zhao N, Zhu W, Wang J, Liu B, Teng Y. Resveratrol attenuates TNBC lung metastasis by down-regulating PD-1 expression on pulmonary t cells and converting macrophages to M1 phenotype in a murine tumor model. Cell Immunol 2021;368:104−423.
[39] Lee Y, Shin H, Kim J. In vivo anti-cancer effects of resveratrol mediated by NK cell activation. Innate Immun 2021;13: 94−106.
[40] Davoodvandi A, Darvish M, Borran S, Nejati M, Mazaheri S, Tamtaji OR, et al. The therapeutic potential of resveratrol in a mouse model of melanoma lung metastasis. Int Immunopharm 2020;88:106−905.
[41] Sinicrope FA, Viggiano TR, Buttar NS, Song LM, Schroeder KW, Kraichely RE, et al. Randomized phase II trial of Polyphenon E versus placebo in patients at high risk of recurrent colonic NeoplasiaPolyphenon E for prevention of colorectal cancer. Cancer Prev Res 2021;14:573−80.
[42] Hsu F-S, Wu J-T, Lin J-Y, Yang S-P, Kuo K-L, Lin W-C, et al. Histone deacetylase inhibitor, trichostatin A, synergistically enhances paclitaxel-induced cytotoxicity in urothelial carcinoma cells by suppressing the ERK pathway. Int J Mol Sci 2019;20:1162.

[43] Li X, Su X, Liu R, Pan Y, Fang J, Cao L, et al. HDAC inhibition potentiates anti-tumor activity of macrophages and enhances anti-PD-L1-mediated tumor suppression. Oncogene 2021;40:1836–50.
[44] Dai D, Guo Y, Shui Y, Li J, Jiang B, Wei Q. Combination of radiosensitivity gene signature and PD-L1 status predicts clinical outcome of patients with locally advanced head and neck squamous cell carcinoma: a study based on the cancer genome atlas dataset. Front Mol Biosci 2021;8:775562.
[45] Panebianco C, Villani A, Pisati F, Orsenigo F, Ulaszewska M, Latiano TP, et al. Butyrate, a postbiotic of intestinal bacteria, affects pancreatic cancer and gemcitabine response in in vitro and in vivo models. Biomed. Pharmacother 2022;151:113–63.
[46] Deets KA, Vance RE. Inflammasomes and adaptive immune responses. Nat Immunol 2021;22:412–22.
[47] Crawford MP, Sinha S, Renavikar PS, Borcherding N, Karandikar NJ. CD4 T cell-intrinsic role for the T helper 17 signature cytokine IL-17: effector resistance to immune suppression. Natl Acad Sci U.S.A 2020;117:19408–14.
[48] Kespohl M, Vachharajani N, Luu M, Harb H, Pautz S, Wolff S, et al. The microbial metabolite butyrate induces expression of Th1-associated factors in CD4+ T cells. Front Immunol 2017;8:1036.
[49] Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR–S6K pathway. Mucosal Immunol 2015;8:80–93.
[50] Zhao GJ, Lu ZQ, Tang LM, Wu ZS, Wang DW, Zheng JY, et al. Curcumin inhibits suppressive capacity of naturally occurring CD4+ CD25+ regulatory T cells in mice in vitro. Int Immunopharm 2012;14:99–106.
[51] Xu B, Yu L, Zhao L-Z. Curcumin up regulates T helper 1 cells in patients with colon cancer. Am J Transl Res 2017;9:1866.
[52] Liu J, Yan Y, Liu J, Wang Y, Wang X. Curcumin prevents experimental autoimmune encephalomyelitis by inhibiting proliferation and effector CD4+ T cell activation. Eur Rev Med Pharmacol Sci 2019;23:9108–16.
[53] Kang B, Song Y, Kim KM, Choe YK, Hwang S, Kim TS. Curcumin inhibits Th1 cytokine profile in CD4+ T cells by suppressing interleukin-12 production in macrophages. Br J Pharmacol 1999;128:380–4.
[54] Skyvalidas DN, Mavropoulos A, Tsiogkas S, Dardiotis E, Liaskos C, Mamuris Z, et al. Curcumin mediates attenuation of pro-inflammatory interferon γ and interleukin 17 cytokine responses in psoriatic disease, strengthening its role as a dietary immunosuppressant. Nutr Res 2020;75:95–108.
[55] Brück J, Holstein J, Glocova I, Seidel U, Geisel J, Kanno T, et al. Nutritional control of IL-23/Th17-mediated autoimmune disease through HO-1/STAT3 activation. Sci Rep 2017;7:1–17.
[56] Pae M, Ren Z, Meydani M, Shang F, Meydani SN, Wu D. Epigallocatechin-3-gallate directly suppresses T cell proliferation through impaired IL-2 utilization and cell cycle progression. J Nutr 2010;140:1509–15.
[57] Farooqi AA, Pinheiro M, Granja A, Farabegoli F, Reis S, Attar R, et al. EGCG mediated targeting of deregulated signaling pathways and non-coding RNAs in different cancers: focus on JAK/STAT, Wnt/β-Catenin, TGF/SMAD, NOTCH, SHH/GLI, and TRAIL mediated signaling pathways. Cancers 2020;12:951.
[58] Liang J, Jahraus B, Balta E, Ziegler JD, Hübner K, Blank N, et al. Sulforaphane inhibits inflammatory responses of primary human T-cells by increasing ROS and depleting glutathione. Front Immunol 2018;9:2584.
[59] Doñas C, Fritz M, Manríquez V, Tejón G, Bono MR, Loyola A, et al. Trichostatin A promotes the generation and suppressive functions of regulatory T cells. Clin Dev Immunol 2013.
[60] Liu Z, Zhang C, Sun J. Deacetylase inhibitor trichostatin A down-regulates Foxp3 expression and reduces CD4+ CD25+ regulatory T cells. Respons Commun 2010;400:409–12.
[61] Cao K, Wang G, Li W, Zhang L, Wang R, Huang Y, et al. Histone deacetylase inhibitors prevent activation-induced cell death and promote anti-tumor immunity. Oncogene 2015;34:5960–70.
[62] Farhood B, Najafi M, Mortezaee K. CD8+ cytotoxic T lymphocytes in cancer immunotherapy: a review. Cell Physiol 2019;234:8509–21.
[63] Luu M, Weigand K, Wedi F, Breidenbend C, Leister H, Pautz S, et al. Regulation of the effector function of CD8+ T cells by gut microbiota-derived metabolite butyrate. Sci Rep 2018;8:1–10.
[64] Shi Y, Fu Y, Zhang X, Zhao G, Yao Y, Guo Y, et al. Romidepsin (FK228) regulates the expression of the immune checkpoint ligand PD-L1 and suppresses cellular immune functions in colon cancer. Cancer Immunol. Immunotherapy 2022;70:61–73.
[65] Zhao S, Habib SL, Senejani AG, Sebastian M, Kidane D. Role of base excision repair in innate immune cells and its relevance for cancer therapy. Biomedicines 2022;10(3):557.
[66] Liu J, Zhang X, Cheng Y, Cao X. Dendritic cell migration in inflammation and immunity. Cell Mol Immunol 2021;18:2461–71.
[67] Seidel C, Schnekenburger M, Dicato M, Diederich M. Histone deacetylase modulators provided by mother nature. Genes Nutr 2012;7:357–67.
[68] Nastasi C, Candela M, Bonefeld CM, Geisler C, Hansen M, Krejsgaard T, et al. The effect of short-chain fatty acids on human monocyte-derived dendritic cells. Sci Rep 2015;5:1–10.
[69] Kaisar MM, Pelgrom LR, Van der Ham AJ, Yazdanbakhsh M, Everts B. Butyrate conditions human dendritic cells to prime type 1 regulatory T cells via both histone deacetylase inhibition and G protein-coupled receptor 109A signaling. Front Immunol 2017;8:1429.
[70] Yoneyama S, Kawai K, Tsuno NH, Okaji Y, Asakage M, Tsuchiya T, et al. Epigallocatechin gallate affects human dendritic cell differentiation and maturation. J Allergy Clin Immunol 2008;121:209–14.
[71] Ciesielski O, Biesiekierska M, Balcerczyk A. Epigallocatechin-3-gallate (EGCG) alters histone acetylation and methylation and impacts chromatin architecture profile in human endothelial cells. Molecules 2020;25:2326.
[72] Lin Q, Chauvistré H, Costa IG, Gusmao EG, Mitzka S, Hänzelmann S, et al. Epigenetic program and transcription factor circuitry of dendritic cell development. Nucleic Acids Res 2015;43:9680–93.
[73] Qu X, Neuhoff C, Cinar MU, Pröll M, Tholen E, Tesfaye D, et al. Epigenetic modulation of TLR4 expression by sulforaphane increases anti-inflammatory capacity in porcine monocyte-derived dendritic cells. Biology 2021;10:490.
[74] Focaccetti C, Izzi V, Benvenuto M, Fazi S, Ciuffa S, Giganti MG, et al. Polyphenols as immunomodulatory compounds in the tumor microenvironment: friends or foes? Int J Mol Sci 2019;20:1714.
[75] Balzarolo M, Engels S, de Jong AJ, Franke K, van den Berg TK, Gulen MF, et al. m6A methylation potentiates cytosolic dsDNA recognition in a sequence-specific manner. Open Biol 2021;11:210030.
[76] Sulek JE, Robinson SP, Petrossian AA, Zhou S, Goliadze E, Manjili MH, et al. Role of epigenetic modification and immunomodulation in a murine prostate cancer model. Prostate 2017;77:361–73.
[77] Shiri S, Alizadeh AM, Baradaran B, Farhanghi B, Shanehbandi D, Khodayari S, et al. Dendrosomal curcumin suppresses metastatic breast cancer in mice by changing m1/m2 macrophage balance in the tumor microenvironment. Asian Pac J Cancer Prev APJCP 2015;16:3917–22.
[78] Alexandrow MG, Song LJ, Altiok S, Gray J, Haura EB, Kumar NB. Curcumin: a novel stat 3 pathway inhibitor for chemoprevention of lung cancer. Eur J Cancer Prev 2012;21:407.

[79] Carson WF, Cavassani KA, Soares EM, Hirai S, Kittan NA, Schaller MA, et al. The STAT4/MLL1 epigenetic axis regulates the antimicrobial functions of murine macrophages. J Immunol 2017;199:1865−74.
[80] Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, et al. The miR-15a−miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med 2008;14:1271−7.
[81] Saleh HA, Ramdan E, Elmazar MM, Azzazy HM, Abdelnaser A. Comparing the protective effects of resveratrol, curcumin and sulforaphane against LPS/IFN-γ-mediated inflammation in doxorubicin-treated macrophages. Sci Rep 2021;11:1−16.
[82] Yang Q, Pröll MJ, Salilew-Wondim D, Zhang R, Tesfaye D, Fan H, et al. LPS-induced expression of CD14 in the TRIF pathway is epigenetically regulated by sulforaphane in porcine pulmonary alveolar macrophages. Innate Immun 2016;22:682−95.
[83] Laskowski TJ, Biederstädt A, Rezvani K. Natural killer cells in antitumour adoptive cell immunotherapy. Nat Rev Cancer 2022;22:557−75.
[84] Mukherjee S, Baidoo JN, Fried A, Banerjee P. Using curcumin to turn the innate immune system against cancer. Biochem Pharmacol 2020;176:113−824.
[85] Kuo C-L, Chen T-S, Liou S-Y, Hsieh C-C. Immunomodulatory effects of EGCG fraction of green tea extract in innate and adaptive immunity via T regulatory cells in murine model. Immunopharmacol Immunotoxicol 2014; 36:364−70.
[86] Pan J, Shen J, Si W, Du C, Chen D, Xu L, et al. Resveratrol promotes MICA/B expression and natural killer cell lysis of breast cancer cells by suppressing c-Myc/miR-17 pathway. Oncotarget 2017;8(39):65743.
[87] Rossi LE, Avila DE, Spallanzani RG, Ziblat A, Fuertes MB, Lapyckyj L, et al. Histone deacetylase inhibitors impair NK cell viability and effector functions through inhibition of activation and receptor expression. J Leukoc Biol 2012;91: 321−31.
[88] Ni L, Wang L, Yao C, Ni Z, Liu F, Gong C, et al. The histone deacetylase inhibitor valproic acid inhibits NKG2D expression in natural killer cells through suppression of STAT3 and HDAC3. Sci Rep 2017;7:1−9.
[89] Toffoli EC, Sheikhi A, Höppner YD, de Kok P, Yazdanpanah-Samani M, Spanholtz J, et al. Natural killer cells and anti-cancer therapies: reciprocal effects on immune function and therapeutic response. Cancers 2021;13:711.
[90] Fernández-Sánchez A, Baragano Raneros A, Carvajal Palao R, Sanz AB, Ortiz A, Ortega F, et al. DNA demethylation and histone H3K9 acetylation determine the active transcription of the NKG2D gene in human CD8+ T and NK cells. Epigenetics 2013;8:66−78.
[91] Ogbomo H, Michaelis M, Kreuter J, Doerr HW, Cinatl Jr J. Histone deacetylase inhibitors suppress natural killer cell cytolytic activity. FEBS Lett 2007;581:1317−22.
[92] Halder RC, Almasi A, Sagong B, Leung J, Jewett A, Fiala M. Curcuminoids and ω-3 fatty acids with anti-oxidants potentiate cytotoxicity of natural killer cells against pancreatic ductal adenocarcinoma cells and inhibit interferon γ production. Front Physiol 2015;6:129.
[93] Baell JB. Feeling nature's PAINS: natural products, natural product drugs, and pan assay interference compounds (PAINS). J Nat Prod 2016;79:616−28.
[94] Kroesen M, Gielen P, Brok IC, Armandari I, Hoogerbrugge PM, Adema GJ. HDAC inhibitors and immunotherapy; a double edged sword? Oncotarget 2014;5: 6558.
[95] Burcher KM, Lantz JW, Gavrila E, Abreu A, Burcher JT, Faucheux AT, et al. Relationship between tumor mutational burden, PD-L1, patient characteristics, and response to immune checkpoint inhibitors in head and neck squamous cell carcinoma. Cancers 2021;13:5733.
[96] Sahu BP, Hazarika H, Bharadwaj R, Loying P, Baishya R, Dash S, et al. Curcumin-docetaxel co-loaded nanosuspension for enhanced anti-breast cancer activity. Expert Opin Drug Deliv 2016;13:1065−74.
[97] Rejinold NS, Yoo J, Jon S, Kim Y-C. Curcumin as a novel nanocarrier system for doxorubicin delivery to MDR cancer cells: in vitro and in vivo evaluation. ACS Appl Mater Interfaces 2018;10:28458−70.
[98] Zhang HH, Zhang Y, Cheng YN, Gong FL, Cao ZQ, Yu LG, et al. Metformin incombination with curcumin inhibits the growth, metastasis, and angiogenesis of hepatocellular carcinoma in vitro and in vivo. Mol Carcinog 2018;57:44−56.
[99] Dun J, Chen X, Gao H, Zhang Y, Zhang H, Zhang Y. Resveratrol synergistically augments anti-tumor effect of 5-FU in vitro and in vivo by increasing S-phase arrest and tumor apoptosis. Exp Biol Med 2015;240:1672−81.
[100] Alayev A, Salamon RS, Schwartz NS, Berman AY, Wiener SL, Holz MK. Combination of rapamycin and resveratrol for treatment of bladder cancer. J Cell Physiol 2017;232:436−46.
[101] Bian P, Hu W, Liu C, Li L. Resveratrol potentiates the antitumor effects of rapamycin in papillary thyroid cancer: PI3K/AKT/mTOR pathway involved. Arch Biochem Biophys 2020;689:108−461.
[102] Alayev A, Salamon RS, Sun Y, Schwartz NS, Li C, Yu JJ, et al. Effects of combining rapamycin and resveratrol on apoptosis and growth of TSC2-deficient xenograft tumors. Am J Respir Cell Mol Biol 2015;53:637−46.
[103] Luo K-W, Zhu X-h, Zhao T, Zhong J, Gao H-c, Luo X-L, et al. EGCG enhanced the anti-tumor effect of doxorubicine in bladder cancer via NF-κB/MDM2/p53 Pathway. Front Cell Dev Biol 2020;8:606. 123.
[104] Sugano M, Egilmez NK, Yokota SJ, Chen F-A, Harding J, Huang SK, et al. Antibody targeting of doxorubicin-loaded liposomes suppresses the growth and metastatic spread of established human lung tumor xenografts in severe combined immunodeficient mice. Cancer Res 2000;60:6942−9.
[105] Liang G, Tang A, Lin X, Li L, Zhang S, Huang Z, et al. Green tea catechins augment the antitumor activity of doxorubicin in an in vivo mouse model for chemoresistant liver cancer. Int J Oncol 2010;37:111−23.
[106] Tang H, Zeng L, Wang J, Zhang X, Ruan Q, Wang J, et al. Reversal of 5-fluorouracil resistance by EGCG is mediate by inactivation of TFAP2A/VEGF signaling pathway and down-regulation of MDR-1 and P-gp expression in gastric cancer. Oncotarget 2017;8:82842.
[107] La X, Zhang L, Li Z, Li H, Yang Y. (−)-Epigallocatechin Gallate (EGCG) enhances the sensitivity of colorectal cancer cells to 5-FU by inhibiting GRP78/NF-κB/miR-155-5p/MDR1 pathway. J Agric Food Chem 2019;67:2510−8.
[108] Wei R, Hackman RM, Wang Y, Mackenzie GG. Targeting glycolysis with epigallocatechin-3-gallate enhances the efficacy of chemotherapeutics in pancreatic cancer cells and xenografts. Cancers 2019;11:1496.
[109] Wei R, Wirkus J, Yang Z, Machuca J, Esparza Y, Mackenzie GG. EGCG sensitizes chemotherapeutic-induced cytotoxicity by targeting the ERK pathway in multiple cancer cell lines. Arch Biochem Biophys 2020;692:108−546.
[110] Meng J, Chang C, Chen Y, Bi F, Ji C, Liu W. EGCG overcomes gefitinib resistance by inhibiting autophagy and augmenting cell death through targeting ERK phosphorylation in NSCLC. OncoTargets Ther 2019;12:6033.
[111] Li Y, He P, Liu Y, Qi M, Dong W. Combining sodium butyrate with cisplatin increases the apoptosis of gastric cancer in vivo and in vitro via the mitochondrial apoptosis pathway. Front Pharmacol 2021:12.
[112] Chen M, Jiang W, Xiao C, Yang W, Qin Q, Mao A, et al. Sodium butyrate combined with docetaxel for the treatment of lung adenocarcinoma A549 cells by targeting Gli1. OncoTargets Ther 2020;13:8861.
[113] Amin A, Wang D, Nannapaneni S, Lamichhane R, Chen ZG, Shin DM. Combination of resveratrol and green tea epigallocatechin gallate induces synergistic apoptosis and inhibits tumor growth in vivo in head and neck cancer models. Oncol Rep 2021;45:1−10.