

## REVIEW

# Epi-immunotherapy for cancers: rationales of epi-drugs in combination with immunotherapy and advances in clinical trials

Yang Xu<sup>1,2</sup>  | Ping Li<sup>3</sup> | Yang Liu<sup>4</sup> | Dijia Xin<sup>1,2</sup> | Wen Lei<sup>1,2</sup> | Aibin Liang<sup>3</sup> | Weidong Han<sup>4</sup>  | Wenbin Qian<sup>1</sup>

<sup>1</sup>Department of Hematology, the Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang 310009, P. R. China

<sup>2</sup>Zhejiang Provincial Key Laboratory for Cancer Molecular Cell Biology, Life Sciences Institute, Zhejiang University, Hangzhou, Zhejiang 310058, P. R. China

<sup>3</sup>Department of Hematology, Tongji Hospital of Tongji University, Shanghai 200065, P. R. China

<sup>4</sup>Department of Bio-Therapeutic, the First Medical Centre, Chinese PLA General Hospital, Beijing 100853, P. R. China

## Correspondence

Wenbin Qian, Department of Hematology, the Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310009, Zhejiang P. R. China.

## Abstract

Over the last two decades, several epi-drugs, immune checkpoint inhibitors (ICIs) and adoptive cell therapies have received clinical approval for use in certain types of cancer. However, monotherapy with epi-drugs or ICIs has shown

**Abbreviations:** AE, adverse event; AITL, angioimmunoblastic T-cell lymphoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AZA, azacytidine; BC, breast cancer; BCOR, BCL6 corepressor; BET, bromodomain and extraterminal domain; CAR-T, chimeric antigen receptor T; CDA, cytidine deaminase; cHL, classic Hodgkin lymphoma; CIK, cytokine induced killer; CLL1, C-type lectin-like molecule-1; CR, complete response; CRC, colorectal cancer; CRi, CR with insufficient recovery; CSC, cancer stem cell; CTAs, cancer testis antigens; CTCL, cutaneous T-cell lymphoma; CTL, cytotoxic T lymphocyte; CTLA4, cytotoxic T-lymphocyte-associated protein 4; CXCL9, CXC-chemokine ligand 9; DAC, decitabine; DLBCL, diffuse large B cell lymphoma; DLI, donor lymphocyte infusion; DMNTi, DNA methyltransferase inhibitor; DNMT3A, DNA methyltransferase 3A; ENKTL, extranodal NK/T cell lymphoma; ENT, entinostat; epi-drugs, epigenetic drugs; ER, estrogen receptor; ERVs, endogenous retroviruses; EZH2, enhancer of zeste 2; FL, follicular lymphoma; FLT3, fms-like tyrosine kinase 3; GATA3, GATA-binding protein 3; GVHD, graft versus host disease; HDACi, histone deacetylase inhibitor; HI, hematological improvement; HLA, human leukocyte antigen; HMA, hypomethylating agent; HNSCC, head and neck squamous cell carcinoma; HR-MDS, higher-risk MDS; ICI, immune checkpoint inhibitor; IDH2, isocitrate dehydrogenase 2; IFN, interferon; IHC, immunohistochemistry; IRF, interferon regulatory factor; KIR, Killer cell immunoglobulin-like receptor; KMT2D, lysine methyltransferase 2D; LSD1, lysine-specific demethylase 1; MAGE, melanoma-associated antigen; MAGE-A3, MAGE family member A3; mCR, marrow CR; MDS, myelodysplastic syndrome; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; miRNA, microRNA; MPN, myeloproliferative neoplasm; MSI, microsatellite instability; MSS, microsatellite stability; ncRNA, non-coding RNA; NKTCL, natural killer/T cell lymphoma; NLR5, nucleotide oligomerization domain-like receptor subfamily C5; NSCLC, non-small cell lung cancer; NY-ESO-1, New York esophageal squamous cell carcinoma 1; OC, ovarian cancer; ORR, overall response rate; PD, progressive disease; PD-1, programmed cell death protein-1; PD-L1, PD-1 ligand 1; PFS, progression-free survival; piRNA, piwi-interacting RNA; PMBCL, primary mediastinal large B-cell lymphoma; PMN-MDSC, polymorphonuclear MDSC; PR, partial response; PRAME, preferentially expressed antigen in melanoma; PTCL, peripheral T-cell lymphoma; R/R, relapsed or refractory; SCT, stem cell transplantation; SD, stable disease; SGC, salivary gland cancer; siRNA, small interfering RNA; TAP1, transporter 1; T-BET, T-box protein in T cells; TCL, T-cell lymphoma; T<sub>cm</sub>, central memory T cell; TCR, T-cell receptor; TET2, ten-eleven translocation 2; Th, T helper cell; THU, tetrahydrouridine; TIL, tumor infiltrating lymphocyte; TIM3, T cell immunoglobulin mucin-3; TMB, tumor mutation load; TME, tumor microenvironment; TNBC, triple-negative breast cancer; TNFRSF4, TNF receptor superfamily member 4; Treg, regulatory T cell; WT-1, Wilms tumor 1.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Cancer Communications* published by John Wiley & Sons Australia, Ltd. on behalf of Sun Yat-sen University Cancer Center.

Email: [qianwb@zju.edu.cn](mailto:qianwb@zju.edu.cn)

Aibin Liang, Department of Hematology, Tongji Hospital of Tongji University, Shanghai 200065, P. R. China.  
E-mail: [lab7182@tongji.edu.cn](mailto:lab7182@tongji.edu.cn)

Weidong Han, Department of Bio-Therapeutic, the First Medical Centre, Chinese PLA General Hospital, Beijing 100853, P. R. China.  
E-mail: [hanwdrsw@163.com](mailto:hanwdrsw@163.com)

Yang Xu, Ping Li and Yang Liu contributed equally.

#### Funding information

the National Natural Science Foundation of China, Grant/Award Numbers: 81830006, 82170219, 81830004, 81800188; the Science Technology Department of Zhejiang Province, Grant/Award Number: 2021C03117; the Natural Science Foundation of Zhejiang Province of China, Grant/Award Number: LY21H080005

limited efficacy in most cancer patients. Epigenetic agents have been shown to regulate the crosstalk between the tumor and host immunity to alleviate immune evasion, suggesting that epi-drugs can potentially synergize with immunotherapy. In this review, we discuss recent insights into the rationales of incorporating epigenetic therapy into immunotherapy, called epi-immunotherapy, and focus on an update of current clinical trials in both hematological and solid malignancies. Furthermore, we outline the future challenges and strategies in the field of cancer epi-immunotherapy.

#### KEYWORDS

chimeric antigen receptor T cell, clinical trial, DNA methylation, Epi-drug, Epi-immunotherapy, histone acetylation, immune checkpoint, tumor microenvironment, vaccine

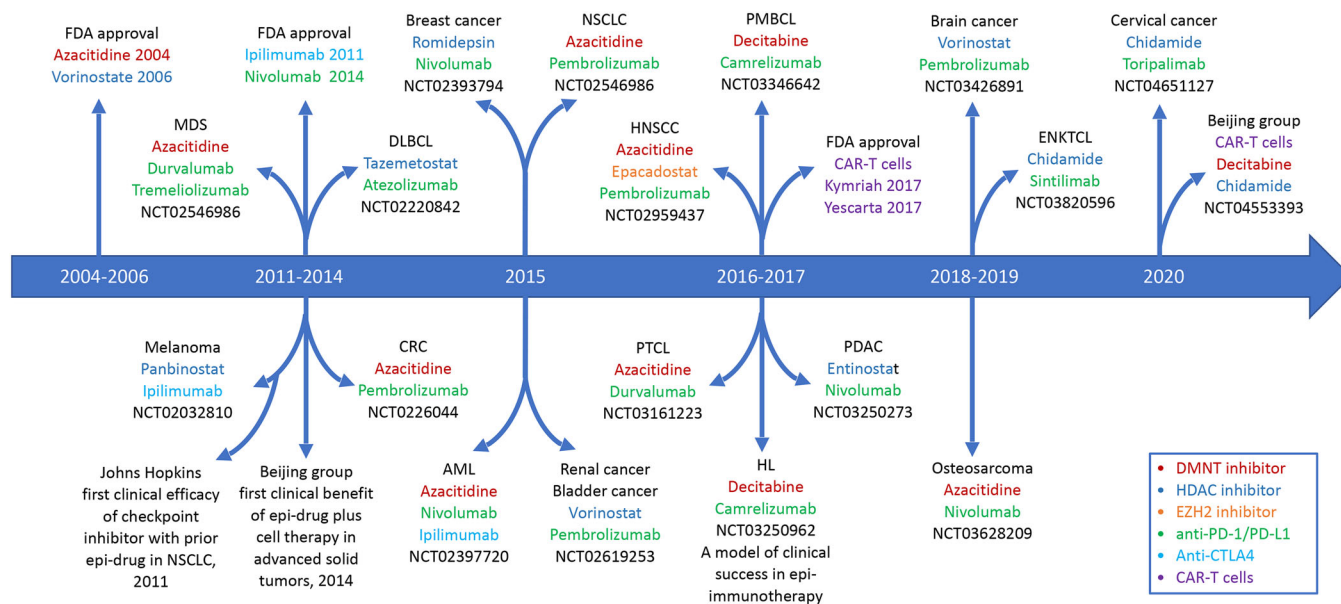
## 1 | BACKGROUND

Immunotherapy, the science of enhancing the immune system to combat cancer, has taken center stage in cancer therapeutics thanks to recent clinical successes in treating various tumors with immune checkpoint inhibitors (ICIs) and adoptive cell therapy [1, 2]. However, it is only effective in a few hematological malignancies and solid cancers, while many patients fail to achieve sustained complete response (CR) and suffer from disease relapse or experience immune-related adverse events (AEs), highlighting the needs for novel treatment strategies [3, 4]. Epigenetics refers to heritable alteration in gene expression without direct changes or mutations in the DNA sequence. Aberrant epigenetic mechanisms imposed by DNA methylation, histone modifications, chromosome remodeling and non-coding RNA play a crucial role in driving cancer initiation and progression [5]. Epigenetic drugs (epi-drugs), such as DNA methyltransferase inhibitors (DNMTis) and histone deacetylase inhibitors (HDACis), have been proven to be effective in many different types of cancer [6]. There has been growing interest in epigenetic regulation of cancer immunity [7] because epigenetic dysregulations not only are restricted to cancer cells, including cancer stem cells (CSCs) but also contribute to the dysfunction of immune cells in the tumor microenvironment (TME).

Recently, emerging strategies to enhance the anticancer potency of immunotherapies have been pursued. One of these involves incorporating epigenetic therapy into immunotherapy, called epi-immunotherapy [8]. Accumulating preclinical studies have shown that epigenetic modulation can sensitize tumors to ICIs or cell therapy, and various epi-immunotherapies for the patients with different tumor types are currently being evaluated in numerous clinical trials (Figure 1). In light of recent advances in the field of cancer epigenetics and immunology, we provide an updated, clinically oriented review of the evolving landscape of the combination of epigenetic modifiers with immunotherapy in both hematological cancers (Table 1) and solid tumors (Table 2). Although most results are reported from phase I and phase II clinical trials, unless otherwise specified, we primarily focus on the therapeutic efficacy of this strategy while omitting the toxicity or AEs because most epi-immunotherapies did not present new safety concerns in these trials.

## 2 | THE RATIONALES OF EPI-DRUGS IN COMBINATION WITH IMMUNOTHERAPY FOR CANCERS

The cancer genome is featured by global DNA hypomethylation, which results in the silence of certain tumor



**FIGURE 1** A timeline of clinical development in epi-immunotherapy highlights key milestones and selected clinical trials for hematological and solid cancers. The US FDA approved the first-generation epi-drugs, i.e., DMNT inhibitor azacitidine in 2004 and HDAC inhibitor vorinostat in 2006. Immunotherapy has gained momentum after the US FDA approval of multiple first-in-class checkpoint inhibitors, including ipilimumab and nivolumab in the early 2010s, and the approval of Kymriah, the first CAR-T product, in 2017. In 2011, a group from Johns Hopkins University first reported durable clinical responses to immune checkpoint therapy in advanced NSCLC patients who received prior epi-drug therapy [168]. In 2014, Fan et al. [213] demonstrated that low-dose decitabine in combination with CIK cell infusion led to improved survival in patients with advanced solid tumors. These findings sparked significant research interests in the potential of epi-immunotherapy. The clinical trials in solid cancer were first proposed for the treatment of melanoma and CRC in 2014, when similar trials were designed for hematological cancers such as MDS and DLBCL. Since then, a variety of trials were conducted across different cancer types, and in 2020, Fan et al. first initiated a trial of CAR-T therapy combined with epi-drugs for refractory and relapsed B cell lymphoma. The ongoing trials will pave the way for a promising future in epi-immunotherapy. Abbreviations: FDA, Food and Drug Administration; DMNT, DNA methyltransferase; HDAC, histone deacetylase; CAR-T, chimeric antigen receptor T cell; NSCLC, non-small cell lung cancer; CIK, cytokine-induced killer; CRC, colorectal cancer; MDS, myelodysplasia syndrome; DLBCL, diffuse large B cell lymphoma; PMBCL, primary mediastinal large B-cell lymphoma; ENKTCL, extranodal natural killer/T cell lymphoma; PTCL, peripheral T-cell lymphoma; HNSCC, head and neck squamous cell cancer; PDAC, pancreatic ductal adenocarcinoma

suppressor genes as well as endogenous retroviral elements. The nucleosome is formed through the wrapping of genomic DNA around histone octamers connected with linker histones and is further packed into high-order chromatin that resides within the nucleus. Chromatin remodeling complexes regulate the chromatin configuration in an ATP-dependent manner to activate or repress gene transcription. Multiple histone residues are subjected to covalent modifications, through which the accessibility of DNA to transcription factors is modulated. The non-coding RNAs (ncRNAs) represent another layer of complexity of epigenetic regulation. Short ncRNAs of <30 nucleotides in length, including microRNAs (miRNAs) and small interfering RNA (siRNAs), can bind to the 3' untranslated region and degrade target mRNA or interfere with its translation. Long ncRNAs have a wide variety of gene regulation at multiple levels, including nucleosome positioning and chromosome looping. It has been clear

that ncRNAs also function in immune regulation, which has been recently reviewed elsewhere [9, 10].

The epigenetic marks on DNA or histones, such as methylation, acetylation, phosphorylation and ubiquitination, are central nodes among a variety of epigenetic modifiers. These modifiers are commonly classified as epigenetic writers, readers and erasers, which function to add, recognize, and remove specific chromatin modifications, respectively. Targeting the epigenetic modifiers with enzymatic activities was initially explored, leading to the development of first-generation epi-drugs, i.e., DNMT or HDAC inhibitors [6]. Chromatin remodelers and ncRNAs do not directly modify DNA or histone, and their therapeutic implications for cancer are under active investigation. A number of excellent reviews have provided details on epigenetic modifiers and their regulatory roles in cancer or immunity [11–14], and we refer to these for more in-depth information on individual epigenetic mechanisms.

**TABLE 1** Clinical trials of epi-immunotherapy for hematological malignancies

Condition	Epi-immunotherapy	NCT Identifier	Phase	Status
AML	Aza + Pembro	NCT02845297	II	Active, not recruiting
	Aza + Pembro	NCT03769532	II	Recruiting
	Aza + Nivo	NCT03825367	I/II	Recruiting
	Aza + Nivo ± Ipili	NCT02397720	II	Recruiting
	Aza + Nivo	NCT04128020	I	Withdrawn
	Aza + Nivo + Relatli	NCT04913922	II	Recruiting
	Aza + Nivo	NCT03092674	II/III	Active, not recruiting
	Aza + Ave	NCT02953561	I/II	Terminated
	Aza + Ave	NCT03390296	I/II	Active, not recruiting
	Aza + Liri	NCT02399917	II	Terminated
	Aza + Lenalidomide	NCT04490707	III	Recruiting
	Dec + Pembro	NCT02996474	I/II	Completed
	Dec + Nivo	NCT03358719	I	Completed
	Dec + Cam	NCT04353479	II	Not recruiting
	Guadec + Atezo	NCT02892318	I	Completed
MDS	Aza+ Pembro	NCT03094637	II	Active, not recruiting
	Aza+ Atezo	NCT02508870	I	Completed
	Aza + Liri	NCT02599649	I/II	Terminated,
	Aza + Durva + Treme	NCT02117219	I	Completed
	Aza + Ipili	NCT02530463	II	Recruiting
	Aza + Dec + MBG453	NCT04878432	II	Recruiting
	Aza + MBG453	NCT04266301	III	Active, not recruiting
	Dec + Sparta	NCT05201066	I	Not yet recruiting
	Ent + Pembro	NCT02936752	Ib	Active,not recruiting
AML, MDS	Aza+ Durva	NCT02775903	II	Active, not recruiting
	Aza + NKR-2	NCT03612739	I	Withdrawn
	Dec + Pembro	NCT03969446	I	Recruiting
	Dec + Sparta ± MBG453	NCT03066648	Ib	Active, not recruiting
	Dec + Ave	NCT03395873	I	Terminated
	Dec + Ipili	NCT02890329	I	Recruiting
	Guadec + Atezo	NCT02935361	I/II	Active, not recruiting
PTCL/CTCL	Aza+ Durva	NCT03161223	I	Recruiting
	Romi + Pembro	NCT03278782	II	Recruiting
	Romi + Durva ± Aza	NCT03161223	I	Recruiting
	Chida+ Sintili	NCT04512534	II	Recruiting
	Chida + Sintili + Aza	NCT04052659	II	Not, yet recruiting
	Dec+ Pembro + Pralatrexate	NCT03240211	Ib	Not recruiting
	Chida+ Sintili	NCT04296786	II	Recruiting
NKTCL	Chida+ Sintili	NCT03820596	I/II	Recruiting
DLBCL	Aza+ Ave + utomilumab	NCT02951156	Ib/III	Terminated
	CXD101 + Pembro	NCT03873025	I/II	Withdrawn
	Tazemetostat + Atezolizumab	NCT02220842	Ib	Completed
PMBCL	Dec + Cam + chemo	NCT03346642	I/II	Unknown

(Continues)

TABLE 1 (Continued)

Condition	Epi-immunotherapy	NCT Identifier	Phase	Status
HL	Dec+ Cam	NCT03250962	II	Recruiting
	Dec + Cam	NCT04510610	II/III	Recruiting
	Dec + Chida + Cam	NCT04233294	I/II	Recruiting
	Dec + Chida + Cam	NCT04514081	II	Recruiting
	Ent + Pembro	NCT03179930	II	Recruiting
DLBCL, HL	Vor + Pembro	NCT03150329	I	Recruiting
B-cell lymphoma	Dec-primed Tandem 19/20 CAR-T	NCT04697940	I/II	Recruiting
	Dec-primed Tandem 19/20 CAR-T + Dec and/or Chida	NCT04553393	I/II	Recruiting
	Dec + PD-1/CD28 CD19 CAR-T	NCT04850560	I/II	Recruiting
Post-CAR-T relapsed lymphoma	Chida + Cam	NCT04337606	I/II	Recruiting

Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplasia syndrome; CMML, chronic myelomonocytic leukemia; DLBCL, diffuse large B cell lymphoma; PTCL, peripheral T cell lymphoma; CTCL, cutaneous T cell lymphoma; NKTCL, NK/T cell lymphoma; HL, Hodgkin lymphoma; PMBCL, primary mediastinal B cell lymphoma; DMNTi: Aza, Azacytuzine; Dec, Decitabine; Guadec, Guadecitabine; HDACi: Vor, Vorinostat; Ent, Entinostat; Chida, Chidamide; Romi, Romidepsin; CXD101; anti-PD-1: Pembro, Pembrolizumab; Nivo, Nivolumab; Cam, Camrelizumab; Sintili, Sintilimab; Sparta, Spartalizumab; anti-PD-L1: Durva, Durvalumab; Atezo, Atezolizumab; Ave, Avelumab; anti-CTLA4: Ipili, Ipilimumab; Treme, Tremelimumab (anti-CTLA4); CAR-T, chimeric antigen receptor T cell; **others**: Liri, Lirilumab (anti-KIR); MBG453 (anti-TIM3), Relatli, Relatlimab (anti-LAG3); utomilumab (anti-4-1BB), NKR-2 (CAR-T), chemo, chemotherapy.

As discussed below, a brief illustration of the rationales for epi-immunotherapy is shown in Figure 2.

## 2.1 | Direct antitumor effects of epi-drugs

Epi-drugs are chemical agents that alter DNA and chromatin structure and promote the disruption of transcriptional and post-transcriptional modifications [6]. To date, several epi-drugs, including DNMTis and HDACis, have received the US Food and Drug Administration (FDA) approval for a few cancers; for example, azacitidine (AZA) and decitabine (DAC) are the most common DNMTis, also known as hypomethylating agents (HMAs) [13, 15]. More recently, Tazemetostat, an H3K27 methyltransferase enhancer of zeste 2 (EZH2) inhibitor, has been indicated as front-line therapy for epithelioid sarcoma in the US [16]. Nevertheless, clinical response to epi-drugs has been mostly confined to hematological malignancies [17]. DNMTi and HDACi can induce cell cycle arrest, senescence and apoptosis in tumor cells through the re-expression of certain tumor suppressor genes silenced by DNA methylation and histone deacetylation [18–24]. On the other hand, the epigenetic alterations promote CSC self-renewal, proliferation and metastasis and confer treatment resistance [25–28]. Many preclinical studies have shown that DNMTis can inhibit the expression of stemness genes and upregulate differentiation-related genes, thereby significantly reducing the self-renewal and tumorigenesis of CSCs [29–33]. Similarly, HDACis are also

capable of controlling the CSC population [34]. Several essential genes involved in the CSC maintenance, such as  $\beta$ -catenin, Stat3 and Notch1, are targeted by HDACi, which alone or in combination can eradicate CSCs to suppress tumor growth [35–39].

However, the best clinical responses to the combined treatment with DNMTi plus programmed cell death protein-1 (PD-1) blockade were found in those patients who received low-dose DNMTi regimens in which the drug dose would not result in cytotoxicity [7]. For example, in a patient tumor-derived xenograft model of colorectal cancer (CRC), low-dose DAC can re-modulate the TME to sensitize the PD-1 blockade [40]. Moreover, Chiappinelli *et al.* [41] demonstrated that DNMTi stimulated immune signaling through the viral defense mechanisms, and low-dose AZA directly enhanced the anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) efficacy in a melanoma model. These striking synergistic effects for cancer intervention can be extended to chimeric antigen receptor T (CAR-T) cell therapy [42, 43]. More importantly, in an open-label phase II study, relapsed or refractory (R/R) classic Hodgkin lymphoma (cHL) patients without previous anti-PD-1 exposure were included, and the CR rate was significantly higher in the patients treated with low-dose DAC plus PD-1 inhibitor camrelizumab than those treated with camrelizumab alone [44]. On the other hand, the efficacy of HDACis alone in clinical trials has been largely restricted to hematological malignancies, and the clinical outcomes in a variety of solid tumors are still disappointing [24, 45]. Collectively, these findings support that

TABLE 2 Clinical trials of epi-immunotherapy for solid cancers

Condition	Epi-immunotherapy	NCT Identifier	Phase	Status
NSCLC	Aza+ Pembro	NCT02546986	II	Active, not recruiting
	Aza + Ent + Nivo	NCT01928576	II	Recruiting
	Aza + Durva	NCT02250326	II	Active, not recruiting
	Dec + Pembro	NCT03233724	I/II	Recruiting
	Dec + Nivo	NCT02664181	II	Active, not recruiting
	Guadec + Moc + Pembro	NCT03220477	I	Active, not recruiting
	Ent + Pembro	NCT02437136	Ib/II	Unknown
	Vor + Pembro	NCT02638090	II	Recruiting
	ACY241 + Nivo	NCT02635061	Ib	Active, not recruiting
	Chida + Pembro	NCT05141357	II	Recruiting
	Moc + Nivo	NCT02954991	II	Active, not recruiting
Moc+ Durva	NCT02805660	I/II	Terminated	
CRC	Aza+ Pembro	NCT02260440	II	Completed
	Aza +Romi + Pembro	NCT02512172	I	Active, not recruiting
	Guadec + Nivo	NCT03576963	I/II	Withdrawn
	Chida + Sintili	NCT04724239	II	Not recruiting
Melanoma	Aza + Pembro	NCT02816021	II	Recruiting
	Guadec + Ipili	NCT02608437	Ib	Unknown
	Ent + Pembro	NCT03765229	II	Recruiting
	Ent + Pembro	NCT02437136	Ib/II	Unknown
	Ent + Pembro	NCT02697630	II	Active, not recruiting
	Pano + Ipili	NCT02032810	I	Active, not recruiting
	Moc + Nivo + Ipili	NCT03565406	Ib	Terminated
Tino + Nivo	NCT03903458	Ib	Recruiting	
NSCLC, CRC	Aza + Pembro+ Epa	NCT02959437	I/II	Terminated
NSCLC, melanoma	Guadec + Nivo + Ipili	NCT04250246	II	Not recruiting
	Pano + Sparta	NCT03982134	I	Withdrawn
NSCLC, RC, melanoma,	Chida + Nivo	NCT02718066	I/II	Active, not recruiting
NSCLC, CRC, melanoma, HNSCC	Aza + NCB059872 + Pembro + Epa	NCT02959437	I/II	Terminated
HNSCC	Dec + Durva	NCT03019003	I/II	Recruiting
	Moc + Durva	NCT02993991	I	Withdrawn
HNSCC, SGC	Vor + Pembro	NCT02538510	II	Active, not recruiting
PDAC	Aza + Pembro	NCT03264404	II	Recruiting
PDAC, CGC	Ent+ Nivo	NCT03250273	II	Active, not recruiting
PDAC, CGC, liver cancer	Guadec + Durva	NCT03257761	I	Recruiting
Breast cancer	Dec + Pembro	NCT02957968	II	Recruiting
	Ent + Atezo	NCT03280563	I/II	Recruiting
	Vor+ Pembro	NCT02395627	II	Terminated
	Vor + Pembro	NCT04190056	II	Recruiting
	Romi + Nivo + Cisplatin	NCT02393794	I/II	Suspended
	Ent + Atezo	NCT02708680	II	Unknown
Ovarian cancer	Ent + Ave	NCT02915523	Ib/II	Unknown
	Guadec + Pembro	NCT02901899	II	Active, not recruiting
	Aza + Pembro	NCT02900560	II	Completed

(Continues)

TABLE 2 (Continued)

Condition	Epi-immunotherapy	NCT Identifier	Phase	Status
Breast, ovarian cancers	Aza + Durva	NCT02811497	II	Completed
	RO6870810 + Atezo	NCT03292172	Ib	Terminated
Cervical cancer	Chida + Tori	NCT04651127	I/II	Recruiting
	VA + Ave	NCT03357757	II	Recruiting
RC	Guadec + Durva	NCT03308396	Ib/II	Active, not recruiting
	Ent + Nivo + Ipili	NCT03552380	II	Active, not recruiting
	Ent + Atezo + Beva	NCT03024437	I	Active, not recruiting
UC	Guadec + Atezo	NCT03179943	II	Active, not recruiting
	Taze + Pembro	NCT03854474	I/II	Recruiting
	Ent + Pembro	NCT03978624	II	Recruiting
	Chida + Tisle	NCT04562311	I	Recruiting
	Aza + Pembro + Epa + INCB059872	NCT02959437	I/II	Terminated
RC, UC	Vor + Pembro	NCT02619253	Ib	Active, not recruiting
Glioblastoma	Vor+ Pembro	NCT03426891	I	Active, not recruiting
Osteosarcoma	Aza + Nivo	NCT03628209	I	Recruiting
Virus-associated cancers	VA + Ave	NCT03357757	II	Recruiting
Solid tumors, lymphoma	Dec + Pembro + radiation	NCT03445858	I	Recruiting
Advanced solid cancers	Epa+ Pembro	NCT02909452	I	Completed
	Ent + Nivo+ Ipili	NCT02453620	I	Active, not recruiting
	Guadec + Pembro	NCT02998567	I	Active, not recruiting
	Lira + Ipili	NCT03525795	I	Completed
	Ent + Pembro	NCT02909452	I	Unknown
	Ent + Nivo + Ipili	NCT02453620	I	Active, not recruiting
	Taze + Durva	NCT04705818	I	Recruiting

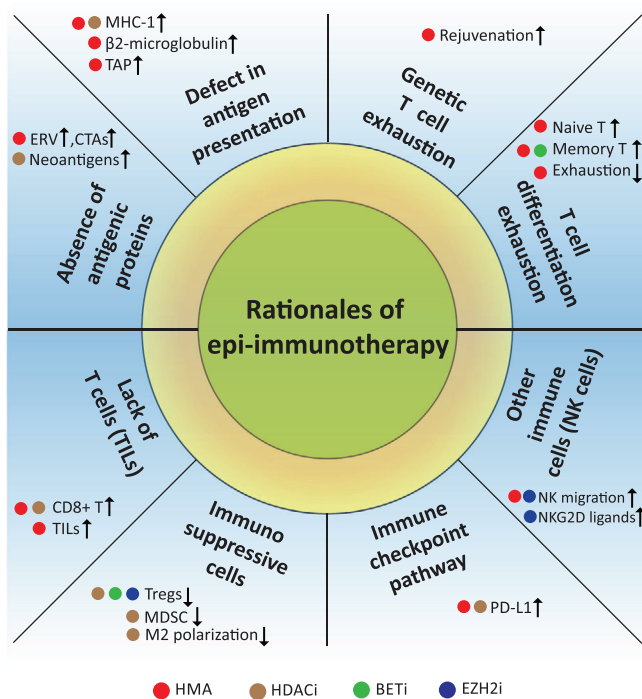
Abbreviations: NSCLC, non-small cell lung cancer; CRC, colorectal cancer; PADC, pancreatic ductal adenocarcinoma; CGC, cholangiocarcinoma; HNSCC, head and neck squamous cell cancer; SGC, salivary gland cancer; RC, renal cancer; UC, urothelial carcinoma; DMNTi: Aza, Azacytine; Dec, Decitabine; Guadec, Guadecitabine; HDACi: Vor, Vorinostat; Ent, Entinostat; Chida, Chidamide; Romi, Romidepsin; Pano, Panobinostat; Moc, Mocetinostat; Tino, Tinostamustine; VA, Valproic acid; ACY241; EZHi: Lira, Liracetostat; Taze, Tazemetostat; LSD1i: NCB059872; BETi: RO6870810; anti-PD-1: Pembro, Pembrolizumab; Nivo, Nivolumab; Cam, Camrelizumab; Sintili, Sintilimab; Sparta, Spartalizumab; Tisle, Tislelizumab; Tori, Toripalimab; anti-PD-L1: Durva, Durvalumab; Atezo, Atezolizumab; Ave, Avelumab; anti-CTLA4: Ipili, Ipilimumab; Treme, Tremelimumab; others: Liri, Lirilumab (anti-KIR), Epa, Epacadostat (anti-IDO1); Beva, Bevacizumab (anti-VEGF).

modulation of antitumor immune responses, rather than direct antitumor activity of epi-drugs, provide rationales for epi-immunotherapy.

## 2.2 | Enhanced immunogenicity of cancer cells by epi-drugs

The absence of tumor antigens and defects in the antigen-presenting machinery, which result in the lack of recognition by T cells, greatly contribute to primary and adaptive resistance to immunotherapy [46]. Epi-drugs are known to elicit viral mimicry to activate the interferon (IFN) pathway, thereby augmenting immune responses [47]. Accounting for 5%-10% of genomic DNA sequences, human endogenous retroviruses (ERVs) are remnants of germline integrations of exogenous infectious retro-

viruses during evolution [48, 49]. The cancer-testis antigens (CTAs) are not expressed in healthy tissues other than germ cells but are often abnormally expressed in tumors [50]. ERVs and CTAs, initially suppressed by cytosine methylation in cancer cells, are closely associated with antitumor cytotoxic immune response [51]. Both ERVs and CTAs are reactivated through demethylation following exposure to DMNTi, leading to a state of viral mimicry, through which the neoantigen expression increases immunogenicity and triggers an innate immune response against tumor [41, 52]. During viral mimicry response, double-strand RNA is produced and activates immunogenic pattern recognition receptors, type I and III IFNs are secreted, thus further enhancing antigen processing and presentation through transporter 1 (TAP1) and human leukocyte antigen (HLA)-class I, respectively [48, 51]. DMNTis can upregulate immunogenic CTAs such as



**FIGURE 2** Representative rationales of epi-immunotherapy. Epi-drugs including HMA (red), HDACi (brown), BETi (green) and EZH2i (blue) have been used in clinical trials for cancer epi-immunotherapy. Downward black arrows indicate inhibition or reduction, while upward black arrows indicate upregulation or increase. Details are given in the text. Abbreviations: HMA, hypomethylating agent; HDACi, histone deacetylase inhibitor; BETi, bromodomain inhibitor; EZHi, EZH2 inhibitor; ERV, endogenous retrovirus; CTA, cancer-testis antigen; Treg, regulatory T cell; MDSC, Myeloid-derived suppressor cell; TIL, tumor-infiltrating lymphocyte.

New York esophageal squamous cell carcinoma 1 (NY-ESO-1) and melanoma-associated antigen (MAGE) family member A1 (MAGE-A1) [53, 54]. Defect in antigen presentation machinery also contributes to the reduced immunogenicity. Major histocompatibility complex (MHC) class I genes are often silenced due to promoter DNA methylation in cancer, and DNMTi reverses MHC-I gene methylation and increases MHC-I expression in response to IFN [55]. Likewise,  $\beta$ 2-microglobulin and TAP1 are also increased when DNA methylation is inhibited, which is responsible for increased antigen processing and presentation [55]. DNMTi can reactivate nucleotide oligomerization domain-like receptor subfamily C5 (NLRC5), an IFN-inducible gene, to increase MHC-I gene expression [56]. HDACi also increases antigen presentation and restores HLA class-I expression in solid tumors [57]. Recently, Truong *et al.* [58] demonstrated that entinostat (ENT), a selective HDACi/3 inhibitor, enhanced immunogenicity through neoantigen editing and induced robust specific antitumor response, which was mediated by increased effector T cell infil-

tration in TME. Inhibition of the histone demethylase lysine-specific demethylase 1 (LSD1) also induces a viral mimicry response following ERVs activation [59]. Additionally, ERV-independent activation is seen in several IFN-responsive genes, such as CXC-chemokine ligand 9 (CXCL9) and CXCL10, which are directly regulated by DNA methylation and histone modifications [60, 61].

The tumor PD-L1 expression appears to associate with the efficacy of PD-1 blockade [62–64]. There is increasing evidence that epi-drugs lead to PD-L1 upregulation in preclinical cancer models [65–67]. This modulation is largely dependent on the reactivation of ERVs and the IFN pathway [68].

### 2.3 | The effects of epi-drugs on T cells

It is well known that epigenetic changes can alter the function and differentiation of T cells [7]. CD4<sup>+</sup> T helper (Th) cells predominantly secrete cytokines to stimulate cellular immunity against tumor cells [69]. Low-dose ENT, a class I HDACi, decreases Foxp3 expression in regulatory T cells (Tregs), leading to tumor suppression [70]. CG-745, another HDACi, can also inhibit Treg proliferation and modulate the TME that potentiates anti-PD-1 activity against tumors [71]. Importantly, the combination of HDACi and anti-CTLA4 further improves CD4<sup>+</sup> T-cell infiltration and effector functions [72]. In addition, inhibition of EZH2 enhances the pro-inflammatory functions of tumor-infiltrating Tregs and rewires the TME with increased effector T cells [73]. Another study found that CPI-1205, a small-molecule EZH2 inhibitor, can alter Tregs' phenotype and functions to augment anticancer responses induced by CTLA4 blockade [74]. The bromodomain and extraterminal domain (BET) bromodomain inhibitor JQ1 could synergize with PD-1 inhibitor to promote a robust anticancer response in lung cancer, which was associated with reduced tumor-infiltrating Tregs but increased T-helper type 1 (Th1) cells [75, 76].

It has been established that both intrinsic and extrinsic mechanisms, i.e., terminal differentiation, exhaustion and activation-induced cell death, contribute to T cell dysfunction, which can be rescued by epigenetic reprogramming therapy [76]. T cell exhaustion is dependent on DNMT3a-mediated de novo DNA methylation, block of which by DAC can enhance T cell rejuvenation and sensitize anti-PD-1 therapy in cancer [77]. Recently, we showed that low-dose DAC significantly improves CAR-T cell phenotype and function, which is characterized by increased non-exhausted T cells and naive, early memory T cell differentiation [42, 43]. Loo Yau *et al.* [78] also demonstrated that low-dose DAC increases CD8<sup>+</sup> T cell infiltration and their antitumor activities. Mechanistically, DAC can



selectively increase both the number and abundance of a granzyme B<sup>high</sup>, perforin<sup>high</sup> effector subpopulation. JQ1 had been shown to prevent the transition to effector memory T cells and enhance antitumor response in murine models of CAR-T therapy [79]. The effects of HDACi on T cells are complex and paradoxical, varying by isoform-selective HDAC inhibition. Laino *et al.* [80] found that low-dose pan-HDACis, but not selective HDACis, impair T cell viability. In patients who received HDAC6-selective inhibitors ACY-1215 and ACY-241, peripheral blood T cells showed increased Th2 transcription factor GATA-binding protein 3 (GATA3) and decreased Th1 transcription factor T-box protein in T cells (T-BET), shifting from exhaustion to central memory phenotype, and enhanced cytotoxicity. HDAC8, often overexpressed in cancers, suppresses the production of T cell-trafficking chemokines; downregulation of HDAC8 promotes global and enhancer acetylation of H3K27 to reactivate chemokine gene transcriptions [81]. In a liver cancer model, selective HDAC8 inhibition synergizes with anti-PD-L1 to eradicate cancer through increased CD8<sup>+</sup> T cell infiltration in the TME [81].

## 2.4 | Targeting the TME

In addition to cancer cells, the TME consists of extracellular matrix, vasculature, and stromal cells surrounding the tumor, as well as cytokines, chemokines, and exosomes. These components form a complicated immunosuppressive microenvironment [82, 83]. The epigenetic dysregulation is pivotal in the generation and maintenance of an immunosuppressive TME, resulting in immune evasion of cancer [84, 85]. As mentioned above, epi-drugs can promote maturation of functional Tregs, converting an immunosuppressive TME to an immunocompetent TME. Moreover, epigenetic changes by histone modifications also affect the differentiation and activation of the myeloid cells [86]. Myeloid-derived suppressor cells (MDSCs) include polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs) [87]. Valproic acid, a common HDACi, when combined with an anti-PD-L1 antibody, induces polarization of precursor cells toward M-MDSCs in the bone marrow through transcriptional activation of the interferon regulatory factor (IRF)1/IRF8 pathway [88]. The class I/IV HDACi mocetinostat had been demonstrated to inhibit intratumoral Treg and MDSC populations and increase intra-tumoral CD8<sup>+</sup> populations [89]. ENT impairs the immunosuppressive activity only in PMN-MDSCs but not M-MDSCs or macrophages; ricolinostat, an HDAC6 inhibitor, suppresses M-MDSC activity other than PMN-MDSC activity [90]. Interestingly, combined treatment abrogates the activities of MDSC populations and markedly inhibits tumor growth [90].

Macrophages have become promising immune effectors for cancer treatment [91]. The HDAC6 inhibitor, nexturastat A, reduces pro-tumorigenic M2 macrophages [92, 93], while class IIa HDACi improves phagocytic and immunostimulatory functions in macrophages, steering them toward an antitumor phenotype with enhanced capacity to activate cytotoxic T lymphocytes (CTLs) [94, 95]. Some HDACis can suppress M2 macrophage polarization and decrease MDSCs [71]. Although few studies have investigated the effect of HMA on the TME, recent data show that guadecitabine can down-regulate inhibitory accessory cells in the TME and reduce the leukemia-mediated expansion of MDSCs [96]. In a pancreatic ductal adenocarcinoma model, DAC treatment led to increased tumor infiltrating lymphocytes (TILs) and Chi3I3 (Ym1) upregulation, indicating an increase of M2 macrophages in the TME [97].

Epigenetic therapies also play an essential role in modulating natural killer (NK) cell and dendritic cell functions and, therefore, augment antitumor immunity [98, 99]. For example, EZH2 inhibitors can upregulate natural killer cell receptor protein 2D (NKG2D) ligands on cancer cells to enhance NK antitumor responses and induce CXCL10 re-expression, which is necessary and sufficient for NK cell migration [100–102]. Low-dose AZA significantly increases the expression of multiple killer cell immunoglobulin-like receptors in NK cells, thereby boosting NK cell-mediated recognition of leukemia cells [103].

## 3 | ADVANCES IN CLINICAL TRIALS OF EPI-IMMUNOTHERAPY FOR SOLID AND HEMATOLOGICAL CANCERS

### 3.1 | Hematological malignancies

#### 3.1.1 | Hodgkin's lymphoma

Currently, there are various FDA-approved ICIs, including antibodies against PD-1 (pembrolizumab and nivolumab), PD-L1 (atezolizumab), and CTLA4 (ipilimumab). In cHL, nivolumab or pembrolizumab alone elicits overall response rates (ORR) of 70%-85% [104–106]. However, the CR rate was only 25%-30%, suggesting that a great proportion of cHLs remain resistant to anti-PD-1 treatment [107, 108]. Nie *et al.* [44] performed a proof-of-concept study to investigate whether DAC could improve the efficacy of camrelizumab, a PD-1 antibody approved in China, in patients with R/R cHL. In this prospective, open-label, phase II trial (NCT02961101), among anti-PD-1-naïve patients, camrelizumab alone was compared with low-dose DAC (10 mg/day, days 1 to 5) plus camrelizumab. At

a median follow-up of 14.9 months, CR rates were 32% and 71% in camrelizumab alone and DAC-camrelizumab combination groups, respectively [44]. When the median follow-up extended to 34.5 months, a greater improvement in CR rate was achieved in patients receiving DAC plus camrelizumab (79% vs. 32%). Progression-free survival (PFS) was longer for those receiving DAC plus camrelizumab compared with camrelizumab alone (35.0 vs. 15.5 months). The benefit of adding DAC to camrelizumab was observed especially in patients who had relatively high tumor burdens or received  $\geq 3$  prior lines of therapies. Interestingly, the increase in circulating peripheral central memory T cells was associated with improved clinical response and PFS, suggesting a potential biomarker for epi-immunotherapy in cHL [109]. Lately, Wang *et al.* [110] updated the clinical results of DAC combined with camrelizumab in cHL patients receiving prior anti-PD-1 (NCT02961101 and NCT03250962). Of 50 patients with progressed or relapsed cHL after anti-PD-1 treatment, the combined treatment resulted in an objective response rate of 52%, CR rate of 36% and longer PFS compared with prior anti-PD-1 monotherapy. The response appears durable once CR is achieved at 24 months. The exploratory studies showed that, when the tumor progresses, the ratio of peripheral CCR7<sup>+</sup>CD45RA<sup>-</sup> central memory T cells (T<sub>cm</sub>) over total CD8<sup>+</sup> or CD4<sup>+</sup> cells decrease from the baseline level; after treatment with DAC plus camrelizumab, the T<sub>cm</sub> ratio increases and persists in patients who obtained partial response (PR) or better, but not in those with stable disease (SD) or progressive disease (PD) [110].

In preclinical studies using various tumor models, ICIs also had been demonstrated to have synergistic effects when combined with HDACis [111, 112]. In a phase II trial (NCT03179930), R/R cHL patients were given ENT, an oral class I-specific HDACi, plus pembrolizumab [113]. Of 13 evaluable patients, ORR was 92%, including 3 patients who progressed on prior anti-PD-1 therapy, suggesting that the combination of ICI and HDACi have promising clinical activity. The study also showed a reasonable safety profile. Chidamide, a novel, orally active benzamide class of selective inhibitors against HDAC 1, 2, 3 and 10, was evaluated in a clinical study in cHL patients who were resistant to or relapsed after DAC plus camrelizumab treatment [114]. Of 14 evaluable patients, 13 (93%) achieved objective response, including 6 CRs. The toxicities were acceptable without any immune-related AEs. Collectively, compared with ICI alone, the combination of DMNTi or HDACi with ICI resulted in higher CR rate in anti-PD-1-naïve patients with R/R cHL. Epigenetic agents appear to partially reverse the resistance to ICIs since a proportion of patients with prior anti-PD-1 exposure still respond well when an epi-drug is added to anti-PD-1.

### 3.1.2 | B-cell lymphoma

Unlike cHL, diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) generally do not respond well to PD-1 blocker, although EBV-positive status associates with reasonable efficacy of pembrolizumab [115, 116]. Primary mediastinal large B-cell lymphoma (PMBCL), a DLBCL subtype, is characterized by chromosome 9p24 aberrations and upregulated PD-L1 [117]. A phase Ib and phase II trials (KEYNOTE-013/KEYNOTE-170) have shown that pembrolizumab produced durable responses and acceptable toxicity in R/R PMBCL [118]. To improve the anti-lymphoma activity, several ongoing clinical studies are assessing the combination of PD-1 blockade with DAC or vorinostat in DLBCL, FL and HL (NCT03346642 and NCT03150329). A high frequency of somatic mutations in epigenetic modifier genes, including CREBBP and EZH2, are identified in B cell lymphoma [115]. The mutant EZH2 reprograms germinal center B cells to alter their interactions with follicular Th cells and follicular dendritic cells, promoting B cell transformation in FL [119, 120]. CREBBP mutant-associated program can be reversed by HDAC3 inhibitor, which induces transcription of BCL6 targets to restore immune surveillance [121]. HDAC3 inhibitor can also enable TILs to eradicate DLBCL cells in an MHC-dependent manner and has synergistic effects with PD-L1 antibody *in vivo* [121].

Recently, multiple new epi-drugs are being evaluated for cytotoxicity against B-cell lymphoma and their mechanisms of action in preclinical and clinical studies. Tazemetostat, an oral first-in-class EZH2 inhibitor, has single-agent activity against DLBCL and FL [122–124]. Interestingly, a phase Ib study (NCT02220842) was performed to assess the efficacy of tazemetostat in combination with atezolizumab on R/R DLBCL [125]. A total of 43 patients were enrolled. However, at the data cut-off, 19 (44%) had discontinued study treatment because of death ( $n = 17$ ) and withdrawal ( $n = 2$ ). Best ORR was 16%, including 2 (5%) CRs and 5 (12%) PRs. Among the 5 patients with EZH2 mutations, 3 achieved a response.

### 3.1.3 | T-cell lymphoma

PD-1 is considered a potential therapeutic target of T-cell lymphoma (TCL) because PD-1 is frequently overexpressed in angioimmunoblastic T-cell lymphoma (AITL), natural killer-/T-cell lymphoma (NKTCL), and peripheral T-cell lymphoma (PTCL) [126]. It has been shown that nivolumab results in an ORR of 40% in R/R TCL, and pembrolizumab leads to an ORR of 100% in EBV-associated NK cell lymphoma and TCL [127, 128]. Additionally,

several HDACis, including belinostat, romidepsin and chidamide, have been indicated for certain TCL subtypes, but the efficacy of single agents rarely exceeded 30% [129]. It was also well known that azacitidine is effective in follicular Th cell-derived PTCL by targeting recurrent ten-eleven translocation 2 (TET2), DNA methyltransferase 3A (DNMT3A) and isocitrate dehydrogenase 2 (IDH2) mutations [130]. Thus, the incorporation of epi-drugs into PD-1 checkpoint inhibition is worth further clinical assessment in TCL.

Recently, a phase I/II trial of pembrolizumab combined with romidepsin and pralatrexate for R/R AITL and PTCL with follicular Th cells was conducted (NCT03278782) [131]. Among 14 patients who received pembrolizumab in combination with romidepsin, the ORR was 50%, including 5 CRs and 2 PRs, which was durable with 18-month follow-up and with acceptable safety. High level of PD-L1 is predictive of good response [131]. Pembrolizumab is also being tested along with DAC and pralatrexate in a phase I clinical trial (NCT03240211) [132]. In this study, 13 patients with R/R PTCL and cutaneous T-cell lymphoma (CTCL) were enrolled, and the patients who received triplet combination treatment achieved objective responses with a duration of response (DOR) of 18 months. The preliminary data from another phase I/IIa trial (NCT03161223) showed that the epi-immunotherapy was tolerable in PTCL patients, and 3/5 evaluable patients achieved CR when treated with durvalumab, oral azacitidine, and romidepsin [132].

Extranodal NK/T cell lymphoma (ENKTL) is an aggressive Epstein-Barr virus-related lymphoma with a high incidence in Asia [115]. Abnormal PD-1/PD-L1 expression was demonstrated in both neoplastic and immune cells in the TME, which offers opportunities for applying ICIs in this lymphoma subtype [115, 133]. On the other hand, the tumor suppressor epigenetic regulators, lysine methyltransferase 2D (KMT2D) and BCL6 corepressor (BCOR), were frequently mutated in ENKTL [134]. Kwong et al. [135] first demonstrated the effectiveness of pembrolizumab in a small series of patients with R/R ENKTL. In a phase II ORIENT-4 study, sintilimab, another anti-PD-1 antibody, also showed efficacy, and ORR and disease control rate (DCR) were 67.9% (19/28) and 85.7% (24/28), respectively [136]. Recently, a single-arm, open-label, multicenter clinical trial (NCT03820596) was designed to evaluate the safety and efficacy of sintilimab combined with chidamide for R/R ENKTL, showing an impressive response in 36 evaluable patients [137]. In this study, the combination therapy yielded an ORR of 58.3% with CR in 16 (44.4%) patients. Notably, tumor PD-L1 expression could predict clinical response [137]. Furthermore, a single-arm phase II trial is conducted to test the combination of sintilimab, chidamide and azacitidine in patients with R/R PTCLs, and the results are pending [138].

### 3.1.4 | Acute myeloid leukemia and myelodysplastic syndrome

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are highly heterogeneous myeloid malignancies with complex molecular genetic abnormalities, and the prognosis remains poor for elderly or refractory patients. Yang *et al.* [139] first reported that PD-1, PD-L1/L2 and CTLA4 are overexpressed in 8%-34% of bone marrow CD34<sup>+</sup> cells from patients with MDS and AML. Currently, there is great clinical interest in immunotherapies for AML, with over 30 clinical studies evaluating ICIs for AML and MDS, which included PD-1, CTLA4, and T cell immunoglobulin mucin-3 (TIM3) blockade [140–142]. However, ICIs, especially anti-PD-1 antibodies, have limited activity in these diseases [143, 144]. Given their potential for induction of checkpoint molecules, HMAs have been combined with PD-1/PD-L1 inhibitors in several studies. In an open-label, phase II study, 70 patients with R/R AML were treated with nivolumab and AZA [145]. The ORR was 33%, including 15 (22%) CR or CR with insufficient recovery of counts (CRi), 1 PR, and 7 hematological improvement (HI). Importantly, the ORR of 58% in HMA-naïve R/R AML patients compared favorably with the historical controls treated with AZA alone [145]. Combinations of HMAs with another anti-PD-1 antibody, pembrolizumab, produced similar benefits to those observed with AZA and nivolumab in R/R AML patients [146]. Pembrolizumab-AZA combination therapy also had been investigated in a clinical trial for newly diagnosed AML (NCT02845297), in which 22 unfit, elderly patients were enrolled [147]. Seventeen patients were evaluable with CR/CRi 47% (8/17) and PR 12% (2/17). This front-line treatment resulted in a median OS of 13.1 months [147]. For high-risk MDS (HR-MDS), a phase II trial (NCT03094637) evaluated AZA plus pembrolizumab in patients with HR-MDS after failure of HMA therapy. The combination treatment of pembrolizumab and AZA was well tolerated in 17 therapy-naïve patients and showed an ORR of 80%, including 3 CR, 7 marrow CRs and 1 HI [148]. This clinical study showed that the combination therapy might have anticancer activity in certain HMA-failure patients, but no significant improvement in OS was demonstrated. Interestingly, a recent study of triple combination showed encouraging CR/CRi and OS [149]. In this study, 31 R/R AML patients were given nivolumab, AZA and ipilimumab, and the ORR was 60%, including 9 (36%) CR/CRi, 2 (8%) HI lasting over 6 months, and 4 (16%) SD.

The efficacy of PD-1 or PD-L1 blockade appears to vary between AML and MDS [150]. In a single-arm phase I study, avelumab in combination with DAC was evaluated in newly-diagnosed AML patients ineligible for intensive therapy [151]. Only 1 of 5 patient (20%) achieved CR, 3

had SD, and 1 experienced PD. In the first large randomized trial (NCT02845297), 129 AML patients of 65 years or older were randomized to receive AZA plus durvalumab or AZA alone [152]. No significant difference was found in the ORR (31.3% vs. 35.4%) or CR rate (17.2% vs. 21.5%) between 2 arms, and the median OS was 13.0 and 14.4 months, respectively. Similar results were reported in the patients with HR-MDS [152]. In a phase Ib study, the efficacy of atezolizumab, with or without AZA, was evaluated in HMA-failure and HMA-naïve MDS patients (NCT02508870) [153]. Despite little effects in HMA-failure MDS patients, atezolizumab, with or without AZA, indeed produces an ORR of 62% (CR, 14%; mCR, 19%; mCR + HI, 10%; HI, 19%) in HMA-naïve patients with median OS not reached.

However, clinical studies in AML and MDS have raised safety concerns about epi-immunotherapy. For example, in HMA-naïve patients receiving AZA and atezolizumab, frequent febrile neutropenia (29%) accounted for 3-month mortality of 29%, which caused early termination of the study (NCT02508870) [146]. In addition, SWOG 1612, a randomized phase III trial in AML and HR-MDS, also showed excessive early deaths in the AZA/nivolumab arm compared with the control arm [154]. Based on our previous studies in cHL [44, 109, 110], we suggest that low-dose HMA should be tested in combination with PD-1 or PD-L1 inhibitors in AML and MDS patients.

Lirilumab is a fully-humanized IgG4 monoclonal antibody that is designed to block the killer cell immunoglobulin-like receptor (KIR)/HLA-C interaction, thereby enhancing NK cell-mediated cytotoxicity against AML in KIR-mismatched haploidentical stem cell transplantation (SCT) [155, 156]. Previously, lirilumab in combination with AZA was evaluated in relapsed AML in a phase Ib/II study (NCT02399917) [157]. Twenty-five patients were included and achieved an ORR of 20%, including a CR/CRi of 8% (2/25) and HI 12% (3/25). The median DOR and overall OS were 2.0 months and 4.0 months, respectively. In another pilot trial (NCT02599649), 8 HR-MDS patients received a median of 4 cycles of treatment with AZA plus lirilumab [158]. Overall, 2 patients achieved CR, 4 had marrow CR, and 2 had SD.

Despite significant advances, epi-immunotherapy has shown variable efficacies. Many patients do not experience a CR, and some are non-responders, highlighting the need to improve our understanding of the clinical features and molecular events associated with response. In AML and HR-MDS, a good response is associated with HMA-naïve status, low leukemia burden, ASXL1 mutation, and high number of pre-treatment CD3<sup>+</sup> and CD8<sup>+</sup> cells evaluated by mass cytometry (CyTOF) and immunohistochemistry (IHC) [145]. Herbrich *et al.* [159] analyzed the baseline immune landscape using single-cell

CyTOF profiling of serial samples collected from R/R AML patients receiving AZA and avelumab (NCT02953561). The CD4/CD8 ratio and residual T cell profiles were found to predict the response following HMA/PD-L1 inhibition. Importantly, immune landscape studies revealed that AML cells also express other immune checkpoints, in particular, PD-L2, TNF receptor superfamily member 4 (TNFRSF4) and TIM3 could be potential targets for novel epi-immunotherapy [159]. In a recent phase II trial (NCT03066648), sabatolimab (MBG453), an anti-TIM3 antibody, plus DAC or AZA led to an ORR of 58%-70% for HR-MDS, 27%-41% for newly-diagnosed AML, and 24% for R/R AML [160, 161]. The study also showed that combining HMAs with TIM3 inhibition was safe with a relatively durable response in MDS and AML [160, 161]. Based on these data, the STIMULUS MDS-US trial will further assess the feasibility and clinical efficacy of sabatolimab in combination with an oral HMA in patients with advanced MDS [162].

## 3.2 | Solid tumors

### 3.2.1 | Breast cancer

A phase II clinical trial was designed to combine CC-486, an oral HMA, and durvalumab to treat selected immunologically cold tumors, including breast cancer (BC), resulting in marginal clinical response [163]. When combined with anti-PD-1, anti-CTLA4, or both, ENT can significantly prolong tumor-free survival in a HER2/neu transgenic BC model [164]. In ENCORE 602 trial (NCT02708680) [165], 81 patients with refractory triple-negative breast cancer (TNBC) were randomized to receive atezolizumab plus ENT or atezolizumab alone. Unfortunately, no significant differences in ORR, PFS and median OS were observed between the two arms, while more AEs were seen in the combination arm. Similarly, Terranova-Barberio *et al.* [166] presented the data from a randomized phase II trial (NCT02395627) using triple combination of vorinostat, pembrolizumab, and tamoxifen. In 34 heavily pretreated patients with estrogen receptor (ER)-positive BC, this combination treatment showed limited efficacy. However, comprehensive correlative analysis revealed that an exhausted CD8<sup>+</sup> T-cell (PD-1<sup>+</sup>/CTLA4<sup>+</sup>) immune signature and an HDACi-dependent decrease in Tregs (CD4<sup>+</sup> Foxp3<sup>+</sup>/CTLA4<sup>+</sup>) might predict response to epi-immunotherapy. Recently, another HDACi, romidepsin, was combined with cisplatin and nivolumab in a phase I/II study (NCT02393794) [167], in which 51 patients with metastatic TNBC were enrolled. Among 34 evaluable patients, the ORR was 44%, median PFS was 4.4 months, and 1-year PFS rate was 23%; the median OS was 10.3

months, and 1-year OS rate was 43%. The triple combination had safe profiles and impressive efficacy in refractory metastatic TNBC, including PD-L1-negative diseases and those with liver metastasis [167].

### 3.2.2 | Non-small cell lung cancer

Juergens et al. [168] from Johns Hopkins University first reported durable clinical responses to immune checkpoint therapy in advanced NSCLC patients who received prior epi-drug therapy, and later they suggested that AZA-induced PD-L1 upregulation may account for the beneficial effect observed in the combination of epi-drugs and anti-PD-1 [169]. Recently, a Bayesian network meta-analysis revealed that PD-1/PD-L1 inhibitors were more efficacious than control treatment in patients with solid tumors, including non-small cell lung cancer (NSCLC) [170]. However, it is unclear whether the combined use of PD-1/PD-L1 inhibitor and HMA would benefit NSCLC patients [171]. In a phase II trial (NCT02546986), 100 patients with a previous line of platinum-based therapy were assigned to receive pembrolizumab combined with either oral AZA or placebo, and PFS was not improved (median, 2.9 and 4.0 months) [172]. We recently reported an unexpectedly good outcome for 3 advanced NSCLC patients carrying unfavorable ICI biomarkers, such as low tumor mutation load, low microsatellite instability and HLA loss of heterozygosity [173]. Surprisingly, all 3 patients responded well to low-dose DAC in combination with camrelizumab, with mild AEs, suggesting that low-dose DAC sensitized PD-1/PD-L1 inhibitors in NSCLC. High expression of the enzyme cytidine deaminase (CDA) that catabolizes DAC within minutes was reported in NSCLC [174]. The combination of HMA with tetrahydrouridine (THU), a CDA inhibitor, was found to cause > 2-fold DNMT1 depletion and > 5-fold increase in TILs, as well as destruction of NSCLC in vivo. Based on these data, the PRECISE trial (NCT02664181) has been conducted to compare the efficacy of nivolumab alone or in combination with THU and DAC in patients with R/R NSCLC [175]. To investigate the effect of guadecitabine, a second-generation DNA methylation inhibitor, on solid cancers, a phase II dose-escalation trial (NCT02998567) was initiated [176]. Overall, 34 patients were enrolled, of whom 10 of 15 patients with NSCLC (13 patients were resistant/refractory to PD-1/PD-L1 targeting agents) were evaluable, with a DCR of 80% and 5 patients having DCR > 6 months.

Recent clinical trials suggest that the combination of anti-PD-1/PD-L1 and HDACi is a promising option for treating NSCLC patients. A phase Ib study (NCT02635061) assessed the clinical efficacy of ACY-241, a selective HDAC6 inhibitor, plus nivolumab on metastatic NSCLC

[177]. Eighteen patients received the treatment. Eight of 13 evaluable patients showed clinical benefit, including 1 CR, 4 PRs and 3 SDs. Immune cell profiling revealed a trend of increased infiltrating cytotoxic T and NK cells following the combined treatment [177]. Two clinical trials (NCT01928576 and NCT02437136) are evaluating the combination of pembrolizumab with HDACis such as vorinostat and ENT on anti-PD-1/PD-L1-naïve or refractory NSCLCs [178]. In a phase I/Ib trial, 33 patients were given pembrolizumab plus vorinostat [179]. Among 30 evaluable patients, 20 had SD or PR. In the subsequent open-label, phase II randomized trial (NCT02638090), patients with metastatic NSCLC were randomized to receive pembrolizumab alone (arm A) or pembrolizumab plus vorinostat (arm B) [180]. Among 47 of 49 patients evaluable for response, the ORR of patients with low pretreatment TIL count (score = 1) in arm B (66.7%) was obviously higher than that in arm A (33.3%), suggesting that the combination strategy may favor NSCLC patients with a low-TIL count.

### 3.2.3 | Metastatic melanoma

ICIs, including nivolumab, pembrolizumab and ipilimumab, have significantly improved the clinical outcomes of metastatic melanoma and are now in routine use [181–183]. However, more than half of patients experience either primary or acquired resistance [184]. To date, some preclinical studies have shown the therapeutic value of CTLA4 inhibitor combined with HMA [185]. In a phase Ib NIBIT-M4 study (NCT02608437), guadecitabine combined with ipilimumab resulted in immune-related DCR of 42% and ORR of 26% [186]. The interim results of a phase II study (NCT02816021) reported that, in anti-PD-1-naïve patients with metastatic melanoma, oral AZA plus pembrolizumab led to a PR 55%, while the anti-PD-1 pretreated patients did not show any response [187].

Epi-immunotherapies using HDACi also have been investigated in metastatic melanoma patients. The ENCORE-601 (NCT02437136), an open-label study, enrolled melanoma patients in which 70% had prior pembrolizumab treatment [188, 189]. With ENT plus pembrolizumab, 10 of 53 patients achieved CR or PR (ORR = 19%). Efficacy results in patients receiving prior PD-1 therapy were consistent with the overall population. Preliminary biomarker analysis suggests that the addition of ENT restores inflammation in the TME necessary for successful re-treatment with anti-PD-1/PD-L1 [188, 189]. An early clinical study (NCT02032810) determined the safety and efficacy of panobinostat, a pan inhibitor of class I, II, and IV HDAC, combined with ipilimumab in advanced melanoma [190]. However, the results with this

combination treatment were disappointing. Mocetinostat, an investigational class I and IV HDACi, has demonstrated anticancer activity in patients with hematological malignancies and solid tumor [191]. Preclinical studies showed that mocetinostat promotes the accumulation of central memory CD8 and CD4 T cells and inhibits Treg cell and MDSC functions [192]. In the phase Ib trial (NCT03565406), the efficacy of mocetinostat in combination with ipilimumab was evaluated in 10 patients with unresectable melanoma [193]. At a median follow-up of 16 months, the ORR was 70%, including 2 CRs and 5 PRs; 7 patients developed grade 3-4 immune-related AEs.

### 3.2.4 | Renal and bladder carcinoma

Preclinical and clinical studies have provided a rationale for PD-1, PD-L1 or CTLA4 blockade for the treatment of renal cell carcinoma [194]. Recently, several studies had evaluated the safety and efficacy of epi-immunotherapies using HMA, HDACi, and EZH2 inhibitors. In a phase Ib/II study (NCT03308396), 42 patients with advanced renal cancer were treated with durvalumab and guadecitabine [195]. At a median follow-up of 20.1 months, 66% of patients achieved clinical benefit defined as either PR or SD that lasted  $\geq 6$  months. Mechanistically, decreased Tregs and MDSCs might be associated with favorable outcomes, and increased Th17 subpopulations of T cells were associated with immune-related AEs [195]. The results from another phase Ib study showed that vorinostat and pembrolizumab combination was tolerable and active in a subset of ICI-resistant urothelial and renal carcinoma patients [196]. A phase I/II study with pembrolizumab in combination with tazemetostat is ongoing (NCT03854474).

### 3.2.5 | Epithelial ovarian cancer and cervical cancer

To date, most ICI-containing clinical trials for advanced recurrent ovarian cancer (OC) have focused on anti-PD-1/PD-L1 therapy, but the results were disappointing [197]. The combination of ICI with HMA is being explored. In a phase II trial (NCT02901899), guadecitabine plus pembrolizumab brought clinical benefit in 27% of patients with R/R OC. Epic arrays were used to measure global tumor methylation, and showed 0.05% CpG sites being differentially methylated after the treatment [198]. The combination of oral AZA and durvalumab produced a median PFS of 1.9 months and a median OS of 5 months in 28 anti-PD-1-naïve patients with platinum-resistant OC in the open-label, phase II multicohort study (NCT02811497) [163]. The biomarker studies did not detect any signifi-

cant tumor DNA hypomethylation. A phase II randomized study (NCT02915523) recruited 126 advanced OC patients who received avelumab plus either ENT or placebo but found no significant difference in ORR (6% vs. 5%) or OS (NE vs. 11.3 months) [199]. Toripalimab, a humanized PD-1 antibody, in combination with chidamide is being explored in a phase Ib/II, single-arm, multi-center study (NCT04651127), in patients with R/R metastatic cervical cancer.

### 3.2.6 | Colorectal cancer

A recent real-world analysis showed that the earlier use of ICIs resulted in better tumor response in a subset of CRC patients [200]. Since either DMNTi or HDACi combined with ICIs markedly improved treatment outcomes in CRC-bearing mice and DAC-based TME reprogramming could enhance the effect of the PD-1 blockade on CRC with microsatellite stability (MSS) [40], epi-immunotherapies also have been evaluated in a few clinical settings for CRC. Unfortunately, PD-1 blockade (pembrolizumab) combined with AZA appears to have modest activity against metastatic CRC with MSS [201].

### 3.2.7 | Head and neck squamous cell carcinoma

In a phase II trial (NCT02538510), the combination of pembrolizumab and vorinostat was evaluated for recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) and salivary gland cancer (SGC) [202]. Among 25 patients with HNSCC, 8 (32%) achieved PR, and 5 (20%) had SD; the median OS was 12.6 months, and the median PFS was 4.5 months. Four (16%) of 25 patients with SGC had PR and 14 (56%) had SD; the median OS was 14 months and median PFS was 6.9 months [202]. In addition, a phase Ib study (NCT03019003) is investigating the safety and efficacy of oral DAC (ASTX727) combined with durvalumab in recurrent or metastatic HNSCC patients who were resistant to ICI monotherapy.

## 4 | ADVANCES OF EPI-CELL THERAPY FOR SOLID AND HEMATOLOGICAL CANCERS

It has become clear that epigenetic modulation can enhance CAR-T cell persistence and function as well as trafficking within an immunosuppressive TME [203, 204]. In an attempt to decipher the mechanisms underlying a delayed exceptional response to CD19 CAR-T therapy,

Fraietta *et al.* [205] suggested that TET2-disrupted CAR-T cells can acquire a central memory phenotype through altered T cell differentiation as a result of epigenetic modulation. Recently, Weber *et al.* [206] demonstrated that transient inhibition of CAR signaling, but not immune checkpoint blockade, can restore the functionality of exhausted human CAR-T cells and argued against the concept of the “epigenetically fixed state” in CAR-T cell exhaustion. Collectively, these studies highlight epigenetic reprogramming as a potential strategy to improve CAR-T efficacy.

Although clinical trials of CAR-T therapies targeting CD33, CD123, NKG2D, C-type lectin-like molecule-1 (CLL1), CD70 and fms-like tyrosine kinase 3 (FLT3) for AML are growing, the data on myeloid malignancies remain very limited [143, 150, 207]. We demonstrated that antitumor functions of CD123-specific CAR-T cells are significantly enhanced by low-dose DAC [42]. NKR-2 are CAR-T cells targeting NKG2D ligands [208]. In a phase I clinical trial (NCT02203825), the safety and feasibility of NKR2 in patients with AML, MDS, and multiple myeloma were evaluated [208]. Moreover, EPITHINK, an open-label phase I study (NCT03612739), was designed to investigate the combination therapy of NKR2 and AZA for newly-diagnosed AML/MDS patients ineligible for intensive chemotherapy or transplantation. However, this study was withdrawn by the investigator.

Zebley *et al.* [209] performed DNA methylation profiling using serial clinical samples from patients with acute lymphoblastic leukemia (ALL) and found that post-infusion CD8 and CD19 CAR-T cells undergo DNA methylation reprogramming that drives cell differentiation toward exhaustion. It was also demonstrated that low-dose DAC-treated CD19 CAR-T cells have stronger anti-lymphoma, proliferation and cytokine-releasing capacities, which is associated with less exhaustion and maintenance of memory phenotype and effector function [43]. Consistent with this result, Li *et al.* [210] suggested that DAC-primed CD19 CAR-T cells are able to kill more lymphoma cells, and DAC followed by CAR-T cell infusion led to CR in 2 patients with refractory lymphoma. Despite high response rates in R/R B-ALL patients, CD19 CAR-T cells showed limited efficacy in R/R ALL patients with p53-mutation/deletion [211]. Interestingly, Qu *et al.* [212] reported that ALL patients with p53 alternations achieved and maintained CR after treatment with CAR-T cells and DAC, indicating that DAC application may improve the outcome of CAR-T cell-treated patients.

In addition to CAR-T therapy, the efficacy of DAC combined with other cell therapies also has been evaluated. The studies in patients with solid tumors showed that low-dose DAC combined with cytokine-induced killer (CIK) cells achieved significant clinical benefits [213, 214]. Some

clinical studies suggested that AZA and donor lymphocyte infusions (DLIs) can induce remission in post-SCT relapsed myeloid cancers [215, 216]. Sommer *et al.* [217] investigated DAC in combination with DLIs in 26 patients with relapsed AML, MDS, or MPN after allogeneic SCT. Eighteen patients received DAC-primed DLIs, while 8 were on DAC only. The rates of acute and chronic graft versus host disease were 17% and 6%, respectively. CR/CRi was observed in 15%, PR in 4%, and SD in 58% of patients. Median OS was 4.7 months. PD-L1 expression appears irrelevant to the response, implying that the efficacy of DAC plus DIL does not restrict to patients with low leukemic burden [217]. Qian *et al.* [218] reported that 2 patients with relapsed AML post allogeneic SCT achieved durable CRs after combined treatment with anti-PD-1, AZA and low-dose DLI, and no severe immune-related AEs or graft versus host disease (GVHD) developed. The anti-PD-1/HMA/DLI combination possibly enhances the graft-vs-leukemia effect of the host's and infused donor's lymphocytes [218]. By serial analysis of samples following transgenic T-cell receptor (TCR) T cell infusion, Nowicki *et al.* [219] suggested that rapid loss of surface TCR expression is caused by epigenetic silencing through DNA methylation. Thus, whether HMAs could potentiate the efficacy of TCR-T therapy warrants further study.

## 5 | ADVANCES OF EPI-VACCINES FOR SOLID AND HEMATOLOGICAL CANCERS

Unlike vaccines against infectious diseases, therapeutic cancer vaccines have not shown significant responses and clinical benefits in patients, and currently only 2 US FDA-approved vaccines with modest efficacy are indicated for prostate or bladder cancer [220]. Despite feasibility and tolerability, the combination of ICIs with vaccines has not yet been translated into survival improvement in early-phase clinical trials [221, 222]. NY-ESO-1 is a vaccine target for multiple cancers, but its limited expression is a barrier to cancer vaccine efficacy, although it is expressed at higher levels on cancer cells than on normal cells [223]. Preclinical studies demonstrated that DAC could enhance both NY-ESO-1 expression in cancer cells and NY-ESO-1-specific CTL-mediated responses *in vitro* [224]. The subsequent phase I trial (NCT00887796) showed increased NY-ESO-1 antibodies and T-cell responses, which contribute to disease stabilization or clinical PR in 6 of 10 evaluable patients with relapsed OC [224]. In a randomized phase I/IIb trial (NCT03206047), AEs and best dose of atezolizumab (anti-PD-L1) when given together with guadecitabine and CDX-1401 vaccine (a dendritic cell vaccine against NY-ESO-1) were investigated. A phase I study of guadecitabine in combination with a colon cancer

vaccine (GVAX) failed to show any significant immunologic responses in 18 patients with advanced CRC [225].

In a phase I trial enrolling 9 patients with MDS, an HLA-independent NY-ESO-1 vaccine (CDX-1401 plus poly-ICLC adjuvant), when administered in combination with standard decitabine schedules, can induce an NY-ESO-1-specific adaptive immune responses, supporting epigenetic stimulation of vaccine response in myeloid cancers [226]. On the basis of these findings, they are now initiating a second phase I study combining NY-ESO-1 vaccine or DAC with nivolumab for MDS patients (NCT03358719). However, another phase I trial was performed to evaluate the combination of AZA and a multi-peptide therapeutic vaccine targeting NY-ESO-1, MAGE-A3, preferentially expressed antigen in melanoma (PRAME), and Wilms tumor 1 (WT-1). Unfortunately, the trial was terminated due to the lack of clinical benefit despite modest immune response observed in 5 MDS patients [227]. Taken together, epigenetic interventions could theoretically improve therapeutic vaccines, but there is still a long way to achieve clinically meaningful responses [228].

## 6 | PERSPECTIVES AND CHALLENGES

Better understanding the epigenetic determinants of immune response would reveal more potential therapeutic targets. Recently, Griffin *et al.* [229] found that suppression of SET domain bifurcated 1 (SETDB1), a H3K9 lysine methyltransferase, can enhance antitumor cytotoxic T-cell responses through activation of immunostimulatory genes and presentation of MHC-I peptides as neoantigens, providing a novel epigenetic strategy to improve ICIs' efficacy. The advent of immunotherapy has significantly revolutionized cancer treatment. Despite encouraging clinical activity in multiple cancer types, especially hematological malignancies, expanding the indications of immunotherapy and overcoming treatment resistance are the major challenges. It is well known that combining epigenetic and immune therapy can overcome tumor resistance and has shown effectiveness in several cancer types. Nowadays, growing clinical trials are currently testing combinations of epi-drugs with immunotherapy, cell therapy, and cancer vaccine, most commonly DNMTi and HDACi. However, several new epi-drugs and immune therapy, such as anti-CTLA4 antibodies, are now being evaluated in the field of epi-immunotherapy. Moreover, novel triplet regimens of synergistic combinations of immunotherapy with epi-drugs are also being investigated in a variety of cancers. In addition, recent preclinical and clinical data have demonstrated that the combination of low-dose DAC or HDACi with ICIs produced compelling antitumor activity in patients with cHL and solid tumors. Based on these excit-

ing findings and because of the complexity of interplay between cancer epigenetics and cancer immunology, the dose, schedule, and combination of epi-immunotherapy should be optimized in the future clinical trials. In addition, the assessment of new regimens in preclinical models may enable rational, hypothesis-driven identification of mechanism-based epi-immunotherapies for clinical testing. Finally, relevant biomarker analysis may shed light on understanding multiple genetic and molecular factors in a longitudinal manner, thereby providing comprehensive and dynamic information regarding response to treatment, and help identify best candidates for epi-immunotherapy.

## 7 | CONCLUSIONS

It is now clear that epigenetic processes play a significant role in regulating immune response against cancer. Numerous preclinical studies have shown that different classes of epi-drugs are able to increase tumor immunogenicity, enhance immune cell functions and modulate immunosuppressive TME, providing strong rationales for cancer epi-immunotherapy. The combination of epi-drugs with ICIs or cell therapy has led to improved efficacies in several clinical trials, especially for hematological malignancies. However, this field still faces many challenges, such as poor response seen in solid cancer, treatment resistance and limited use of vaccine or cell therapy. We anticipate that the development of next-generation epi-drugs, incorporation of appropriate biomarker and optimized treatment strategy will provide further insight and opportunities for epi-immunotherapy.

### DECLARATIONS

### ACKNOWLEDGMENTS

We thank Dr. Zhaoxing Wu for technical assistance in figure preparation. This work was supported by the funds from the National Natural Science Foundation of China (No. 81830006, 82170219, 81830004, and 81800188), the Science Technology Department of Zhejiang Province (No. 2021C03117), and the Natural Science Foundation of Zhejiang Province of China (LY21H080005).

### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

### AUTHOR CONTRIBUTIONS

Wenbin Qian, Yang Xu, Weidong Han and Aibin Liang proposed the concepts; Aibin Liang and Weidong Han provided valuable suggestions; Wenbin Qian, Yang Xu, Ping Li, Yang Liu and Wen Lei drafted the manuscript; Yang Xu and Dijia Xin prepared the tables and figures; Yang Xu, Ping Li, Yang Liu, Wenbin Qian and Dijia Xin



made the revision; all authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## ORCID

Yang Xu  <https://orcid.org/0000-0003-4737-5523>

Weidong Han  <https://orcid.org/0000-0002-9817-6674>

## REFERENCES

1. Yang Y. Cancer immunotherapy: harnessing the immune system to battle cancer. *J Clin Invest*. 2015;125(9):3335–7.
2. Pan C, Liu H, Robins E, Song W, Liu D, Li Z, et al. Next-generation immuno-oncology agents: current momentum shifts in cancer immunotherapy. *J Hematol Oncol*. 2020;13(1):29.
3. Upadhaya S, Neftelino ST, Hodge JP, Oliva C, Campbell JR, Yu JX. Combinations take centre stage in PD1/PDL1 inhibitor clinical trials. *Nat Rev Drug Discov*. 2021;20(3):168–9.
4. Hegde PS, Chen DS. Top 10 challenges in cancer immunotherapy. *Immunity*. 2020;52(1):17–35.
5. Dawson MA. The cancer epigenome: concepts, challenges, and therapeutic opportunities. *Science*. 2017;355(6330):1147–52.
6. Miranda Furtado CL, Dos Santos Luciano MC, Silva Santos RD, Furtado GP, Moraes MO, Pessoa C. Epidrugs: targeting epigenetic marks in cancer treatment. *Epigenetics*. 2019;14(12):1164–76.
7. Hogg SJ, Beavis PA, Dawson MA, Johnstone RW. Targeting the epigenetic regulation of antitumour immunity. *Nat Rev Drug Discov*. 2020;19(11):776–800.
8. Li X, Mei Q, Nie J, Fu X, Han W. Decitabine: a promising epi-immunotherapeutic agent in solid tumors. *Expert Rev Clin Immunol*. 2015;11(3):363–75.
9. Atianand MK, Caffrey DR, Fitzgerald KA. Immunobiology of long noncoding RNAs. *Annu Rev Immunol*. 2017;35:177–98.
10. Stalio L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol*. 2021;22(2):96–118.
11. Barrero MJ. Epigenetic strategies to boost cancer immunotherapies. *Int J Mol Sci*. 2017;18(6):1108.
12. Aspeslagh S, Morel D, Soria JC, Postel-Vinay S. Epigenetic modifiers as new immunomodulatory therapies in solid tumours. *Ann Oncol*. 2018;29(4):812–24.
13. Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, et al. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther*. 2019;4:62.
14. Cao J, Yan Q. Cancer epigenetics, tumor immunity, and immunotherapy. *Trends Cancer*. 2020;6(7):580–92.
15. Ganesan A, Arimondo PB, Rots MG, Jeronimo C, Berdasco M. The timeline of epigenetic drug discovery: from reality to dreams. *Clin Epigenetics*. 2019;11(1):174.
16. Hoy SM. Tazemetostat: First Approval. *Drugs*. 2020;80(5):513–21.
17. Morel D, Jeffery D, Aspeslagh S, Almouzni G, Postel-Vinay S. Combining epigenetic drugs with other therapies for solid tumours - past lessons and future promise. *Nat Rev Clin Oncol*. 2020;17(2):91–107.
18. Nishioka C, Ikezoe T, Yang J, Udaka K, Yokoyama A. Simultaneous inhibition of DNA methyltransferase and histone deacetylase induces p53-independent apoptosis via down-regulation of Mcl-1 in acute myelogenous leukemia cells. *Leuk Res*. 2011;35(7):932–9.
19. Hsi LC, Xi X, Wu Y, Lippman SM. The methyltransferase inhibitor 5-aza-2-deoxycytidine induces apoptosis via induction of 15-lipoxygenase-1 in colorectal cancer cells. *Mol Cancer Ther*. 2005;4(11):1740–6.
20. Yang D, Torres CM, Bardhan K, Zimmerman M, McGaha TL, Liu K. Decitabine and vorinostat cooperate to sensitize colon carcinoma cells to Fas ligand-induced apoptosis in vitro and tumor suppression in vivo. *J Immunol*. 2012;188(9):4441–9.
21. Brodská B, Otevrelva P, Holoubek A. Decitabine-induced apoptosis is derived by Puma and Noxa induction in chronic myeloid leukemia cell line as well as in PBL and is potentiated by SAHA. *Mol Cell Biochem*. 2011;350(1-2):71–80.
22. Venturelli S, Berger A, Weiland T, Essmann F, Waibel M, Nuebling T, et al. Differential induction of apoptosis and senescence by the DNA methyltransferase inhibitors 5-azacytidine and 5-aza-2'-deoxycytidine in solid tumor cells. *Mol Cancer Ther*. 2013;12(10):2226–36.
23. Newbold A, Falkenberg KJ, Prince HM, Johnstone RW. How do tumor cells respond to HDAC inhibition? *FEBS J*. 2016;283(22):4032–46.
24. Li Y, Seto E. HDACs and HDAC inhibitors in cancer development and therapy. *Cold Spring Harb Perspect Med*. 2016;6(10):a026831.
25. Toh TB, Lim JJ, Chow EK. Epigenetics in cancer stem cells. *Mol Cancer*. 2017;16(1):29.
26. Keyvani-Ghamsari S, Khorsandi K, Rasul A, Zaman MK. Current understanding of epigenetics mechanism as a novel target in reducing cancer stem cells resistance. *Clin Epigenetics*. 2021;13(1):120.
27. Tung PY, Knoepfler PS. Epigenetic mechanisms of tumorigenicity manifesting in stem cells. *Oncogene*. 2015;34(18):2288–96.
28. Easwaran H, Tsai HC, Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. *Mol Cell*. 2014;54(5):716–27.
29. Uchida N, Hsieh MM, Platner C, Sauntharajah Y, Tisdale JF. Decitabine suspends human CD34+ cell differentiation and proliferation during lentiviral transduction. *PLoS One*. 2014;9(8):e104022.
30. Tian J, Lee SO, Liang L, Luo J, Huang CK, Li L, et al. Targeting the unique methylation pattern of androgen receptor (AR) promoter in prostate stem/progenitor cells with 5-aza-2'-deoxycytidine (5-AZA) leads to suppressed prostate tumorigenesis. *J Biol Chem*. 2012;287(47):39954–66.
31. Turcan S, Fabius AW, Borodovsky A, Pedraza A, Brennan C, Huse J, et al. Efficient induction of differentiation and growth inhibition in IDH1 mutant glioma cells by the DNMT Inhibitor Decitabine. *Oncotarget*. 2013;4(10):1729–36.

32. Pollyea DA, Stevens BM, Jones CL, Winters A, Pei S, Minhajuddin M, et al. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. *Nat Med.* 2018;24(12):1859–66.
33. Tsai HC, Li H, Van Neste L, Cai Y, Robert C, Rassool FV, et al. Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. *Cancer Cell.* 2012;21(3):430–46.
34. Barbato L, Bocchetti M, Di Biase A, Regad T. Cancer stem cells and targeting strategies. *Cells.* 2019;8(8):926.
35. Lin PC, Hsieh HY, Chu PC, Chen CS. Therapeutic opportunities of targeting histone deacetylase isoforms to eradicate cancer stem cells. *Int J Mol Sci.* 2018;19(7):1939.
36. Tung B, Ma D, Wang S, Oyinlade O, Laterra J, Ying M, et al. Kruppel-like factor 9 and histone deacetylase inhibitors synergistically induce cell death in glioblastoma stem-like cells. *BMC Cancer.* 2018;18(1):1025.
37. Was H, Krol SK, Rotili D, Mai A, Wojtas B, Kaminska B, et al. Histone deacetylase inhibitors exert anti-tumor effects on human adherent and stem-like glioma cells. *Clin Epigenetics.* 2019;11(1):11.
38. Chikamatsu K, Ishii H, Murata T, Sakakura K, Shino M, Toyoda M, et al. Alteration of cancer stem cell-like phenotype by histone deacetylase inhibitors in squamous cell carcinoma of the head and neck. *Cancer Sci.* 2013;104(11):1468–75.
39. Zhang B, Strauss AC, Chu S, Li M, Ho Y, Shiang KD, et al. Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. *Cancer Cell.* 2010;17(5):427–42.
40. Yu G, Wu Y, Wang W, Xu J, Lv X, Cao X, et al. Low-dose decitabine enhances the effect of PD-1 blockade in colorectal cancer with microsatellite stability by re-modulating the tumor microenvironment. *Cell Mol Immunol.* 2019;16(4):401–9.
41. Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsrna including endogenous retroviruses. *Cell.* 2015;162(5):974–86.
42. You L, Han Q, Zhu L, Zhu Y, Bao C, Yang C, et al. Decitabine-mediated epigenetic reprogramming enhances anti-leukemia efficacy of CD123-targeted chimeric antigen receptor T-cells. *Front Immunol.* 2020;11:1787.
43. Wang Y, Tong C, Dai H, Wu Z, Han X, Guo Y, et al. Low-dose decitabine priming endows CAR T cells with enhanced and persistent antitumor potential via epigenetic reprogramming. *Nat Commun.* 2021;12(1):409.
44. Nie J, Wang C, Liu Y, Yang Q, Mei Q, Dong L, et al. Addition of low-dose decitabine to anti-PD-1 antibody camrelizumab in relapsed/refractory classical Hodgkin lymphoma. *J Clin Oncol.* 2019;37(17):1479–89.
45. Qiu T, Zhou L, Zhu W, Wang T, Wang J, Shu Y, et al. Effects of treatment with histone deacetylase inhibitors in solid tumors: a review based on 30 clinical trials. *Future Oncol.* 2013;9(2):255–69.
46. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell.* 2017;168(4):707–23.
47. Dear AE. Epigenetic modulators and the new immunotherapies. *N Engl J Med.* 2016;374(7):684–6.
48. Kassiotis G. Endogenous retroviruses and the development of cancer. *J Immunol.* 2014;192(4):1343–9.
49. Jansz N, Faulkner GJ. Endogenous retroviruses in the origins and treatment of cancer. *Genome Biol.* 2021;22(1):147.
50. Chiappinelli KB, Zahnow CA, Ahuja N, Baylin SB. Combining epigenetic and immunotherapy to combat cancer. *Cancer Res.* 2016;76(7):1683–9.
51. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell.* 2015;160(1-2):48–61.
52. Roulois D, Loo Yau H, Singhania R, Wang Y, Danesh A, Shen SY, et al. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. *Cell.* 2015;162(5):961–73.
53. Almstedt M, Blagitko-Dorfs N, Duque-Afonso J, Karbach J, Pfeifer D, Jager E, et al. The DNA demethylating agent 5-aza-2'-deoxycytidine induces expression of NY-ESO-1 and other cancer/testis antigens in myeloid leukemia cells. *Leuk Res.* 2010;34(7):899–905.
54. Adair SJ, Hogan KT. Treatment of ovarian cancer cell lines with 5-aza-2'-deoxycytidine upregulates the expression of cancer-testis antigens and class I major histocompatibility complex-encoded molecules. *Cancer Immunol Immunother.* 2009;58(4):589–601.
55. Luo N, Nixon MJ, Gonzalez-Ericsson PI, Sanchez V, Opalenik SR, Li H, et al. DNA methyltransferase inhibition upregulates MHC-I to potentiate cytotoxic T lymphocyte responses in breast cancer. *Nat Commun.* 2018;9(1):248.
56. Yoshihama S, Roszik J, Downs I, Meissner TB, Vijayan S, Chapuy B, et al. NLR5/MHC class I transactivator is a target for immune evasion in cancer. *Proc Natl Acad Sci U S A.* 2016;113(21):5999–6004.
57. Brocks D, Schmidt CR, Daskalakis M, Jang HS, Shah NM, Li D, et al. DNMT and HDAC inhibitors induce cryptic transcription start sites encoded in long terminal repeats. *Nat Genet.* 2017;49(7):1052–60.
58. Truong AS, Zhou M, Krishnan B, Utsumi T, Manocha U, Stewart KG, et al. Entinostat induces antitumor immune responses through immune editing of tumor neoantigens. *J Clin Invest.* 2021;131(16):e138560.
59. Sheng W, LaFleur MW, Nguyen TH, Chen S, Chakravarthy A, Conway JR, et al. LSD1 ablation stimulates anti-tumor immunity and enables checkpoint blockade. *Cell.* 2018;174(3):549–63 e19.
60. 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(7577):249–53.
61. Nagarsheth N, Peng D, Kryczek I, Wu K, Li W, Zhao E, et al. PR2 epigenetically silences Th1-type chemokines to suppress effector T-cell trafficking in colon cancer. *Cancer Res.* 2016;76(2):275–82.
62. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med.* 2012;366(26):2455–65.
63. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol.* 2010;28(19):3167–75.

64. Gandini S, Massi D, Mandala M. PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: A systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2016;100:88–98.
65. Huang KC, Chiang SF, Chen WT, Chen TW, Hu CH, Yang PC, et al. Decitabine augments chemotherapy-induced PD-L1 upregulation for PD-L1 blockade in colorectal cancer. *Cancers (Basel)*. 2020;12(2):462.
66. Deng S, Hu Q, Zhang H, Yang F, Peng C, Huang C. HDAC3 inhibition upregulates PD-L1 expression in B-cell lymphomas and augments the efficacy of anti-PD-L1 therapy. *Mol Cancer Ther*. 2019;18(5):900–8.
67. Hua S, Gu M, Wang Y, Ban D, Ji H. Oxymatrine reduces expression of programmed death-ligand 1 by promoting DNA demethylation in colorectal cancer cells. *Clin Transl Oncol*. 2021;23(4):750–6.
68. Perrier A, Didelot A, Laurent-Puig P, Blons H, Garinet S. Epigenetic mechanisms of resistance to immune checkpoint inhibitors. *Biomolecules*. 2020;10(7):1061.
69. Renaude E, Kroemer M, Borg C, Peixoto P, Hervouet E, Loyon R, et al. Epigenetic reprogramming of CD4(+) helper T cells as a strategy to improve anticancer immunotherapy. *Front Immunol*. 2021;12:669992.
70. Shen L, Ciesielski M, Ramakrishnan S, Miles KM, Ellis L, Sotomayor P, et al. Class I histone deacetylase inhibitor entinostat suppresses regulatory T cells and enhances immunotherapies in renal and prostate cancer models. *PLoS One*. 2012;7(1):e30815.
71. Kim YD, Park SM, Ha HC, Lee AR, Won H, Cha H, et al. HDAC Inhibitor, CG-745, enhances the anti-cancer effect of anti-PD-1 immune checkpoint inhibitor by modulation of the immune microenvironment. *J Cancer*. 2020;11(14):4059–72.
72. 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(49):5960–70.
73. Wang D, Quiros J, Mahuron K, Pai CC, Ranzani V, Young A, et al. Targeting EZH2 reprograms intratumoral regulatory T cells to enhance cancer immunity. *Cell Rep*. 2018;23(11):3262–74.
74. Goswami S, Apostolou I, Zhang J, Skepner J, Anandhan S, Zhang X, et al. Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy. *J Clin Invest*. 2018;128(9):3813–8.
75. Adeegbe DO, Liu S, Hattersley MM, Bowden M, Zhou CW, Li S, et al. BET Bromodomain inhibition cooperates with PD-1 blockade to facilitate antitumor response in Kras-mutant non-small cell lung cancer. *Cancer Immunol Res*. 2018;6(10):1234–45.
76. Jones PA, Ohtani H, Chakravarthy A, De Carvalho DD. Epigenetic therapy in immune-oncology. *Nat Rev Cancer*. 2019;19(3):151–61.
77. 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(1):142–57 e19.
78. Loo Yau H, Bell E, Ettayebi I, de Almeida FC, Boukhaled GM, Shen SY, et al. DNA hypomethylating agents increase activation and cytolytic activity of CD8(+) T cells. *Mol Cell*. 2021;81(7):1469–83 e8.
79. Kagoya Y, Nakatsugawa M, Yamashita Y, Ochi T, Guo T, Anczurowski M, et al. BET bromodomain inhibition enhances T cell persistence and function in adoptive immunotherapy models. *J Clin Invest*. 2016;126(9):3479–94.
80. Laino AS, Betts BC, Veerapathran A, Dolgalev I, Sarnaik A, Quayle SN, et al. HDAC6 selective inhibition of melanoma patient T-cells augments anti-tumor characteristics. *J Immunother Cancer*. 2019;7(1):33.
81. Yang W, Feng Y, Zhou J, Cheung OK, Cao J, Wang J, et al. A selective HDAC8 inhibitor potentiates antitumor immunity and efficacy of immune checkpoint blockade in hepatocellular carcinoma. *Sci Transl Med*. 2021;13(588).
82. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. *Cell Death Dis*. 2015;6:e1792.
83. Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. *Mol Cancer*. 2019;18(1):10.
84. Lodewijk I, Nunes SP, Henrique R, Jeronimo C, Duenas M, Paramio JM. Tackling tumor microenvironment through epigenetic tools to improve cancer immunotherapy. *Clin Epigenetics*. 2021;13(1):63.
85. Saleh R, Toor SM, Sasidharan Nair V, Elkord E. Role of epigenetic modifications in inhibitory immune checkpoints in cancer development and progression. *Front Immunol*. 2020;11:1469.
86. 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(2):75–90.
87. Li K, Shi H, Zhang B, Ou X, Ma Q, Chen Y, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. *Signal Transduct Target Ther*. 2021;6(1):362.
88. Adeshakin AO, Yan D, Zhang M, Wang L, Adeshakin FO, Liu W, et al. Blockade of myeloid-derived suppressor cell function by valproic acid enhanced anti-PD-L1 tumor immunotherapy. *Biochem Biophys Res Commun*. 2020;522(3):604–11.
89. Briere D, Sudhakar N, Woods DM, Hallin J, Engstrom LD, Aranda R, et al. The class I/IV HDAC inhibitor mocetinostat increases tumor antigen presentation, decreases immune suppressive cell types and augments checkpoint inhibitor therapy. *Cancer Immunol Immunother*. 2018;67(3):381–92.
90. Hashimoto A, Fukumoto T, Zhang R, Gabrilovich D. Selective targeting of different populations of myeloid-derived suppressor cells by histone deacetylase inhibitors. *Cancer Immunol Immunother*. 2020;69(9):1929–36.
91. Duan Z, Luo Y. Targeting macrophages in cancer immunotherapy. *Signal Transduct Target Ther*. 2021;6(1):127.
92. Banik D, Noonepalle S, Hadley M, Palmer E, Gracia-Hernandez M, Zevallos-Delgado C, et al. HDAC6 plays a noncanonical role in the regulation of antitumor immune responses, dissemination, and invasiveness of breast cancer. *Cancer Res*. 2020;80(17):3649–62.
93. Knox T, Sahakian E, Banik D, Hadley M, Palmer E, Noonepalle S, et al. Selective HDAC6 inhibitors improve anti-PD-1 immune checkpoint blockade therapy by decreasing the anti-inflammatory phenotype of macrophages and down-regulation

- of immunosuppressive proteins in tumor cells. *Sci Rep*. 2019;9(1):6136.
94. Reichman H, Munitz A. Harnessing class II histone deacetylases in macrophages to combat breast cancer. *Cell Mol Immunol*. 2017;14(7):575–7.
  95. Guerriero JL, Sotayo A, Ponichtera HE, Castrillon JA, Pourzia AL, Schad S, et al. Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages. *Nature*. 2017;543(7645):428–32.
  96. Nahas MR, Stroopinsky D, Rosenblatt J, Cole L, Pyzer AR, Anastasiadou E, et al. Hypomethylating agent alters the immune microenvironment in acute myeloid leukaemia (AML) and enhances the immunogenicity of a dendritic cell/AML vaccine. *Br J Haematol*. 2019;185(4):679–90.
  97. Gonda TA, Fang J, Salas M, Do C, Hsu E, Zhukovskaya A, et al. A DNA hypomethylating drug alters the tumor microenvironment and improves the effectiveness of immune checkpoint inhibitors in a mouse model of pancreatic cancer. *Cancer Res*. 2020;80(21):4754–67.
  98. Merino AM, Kim H, Miller JS, Cichocki F. Unraveling exhaustion in adaptive and conventional NK cells. *J Leukoc Biol*. 2020;108(4):1361–8.
  99. Lau CM, Adams NM, Geary CD, Weizman OE, Rapp M, Pritykin Y, et al. Epigenetic control of innate and adaptive immune memory. *Nat Immunol*. 2018;19(9):963–72.
  100. Yin J, Leavenworth JW, Li Y, Luo Q, Xie H, Liu X, et al. Ezh2 regulates differentiation and function of natural killer cells through histone methyltransferase activity. *Proc Natl Acad Sci U S A*. 2015;112(52):15988–93.
  101. Bugide S, Green MR, Wajapeyee N. Inhibition of enhancer of zeste homolog 2 (EZH2) induces natural killer cell-mediated eradication of hepatocellular carcinoma cells. *Proc Natl Acad Sci U S A*. 2018;115(15): E3509–E18.
  102. Bugide S, Gupta R, Green MR, Wajapeyee N. EZH2 inhibits NK cell-mediated antitumor immunity by suppressing CXCL10 expression in an HDAC10-dependent manner. *Proc Natl Acad Sci U S A*. 2021;118(30): e2102718118.
  103. Sohlberg E, Pfefferle A, Andersson S, Baumann BC, Hellstrom-Lindberg E, Malmberg KJ. Imprint of 5-azacytidine on the natural killer cell repertoire during systemic treatment for high-risk myelodysplastic syndrome. *Oncotarget*. 2015;6(33):34178–90.
  104. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015;372(4):311–9.
  105. Younes A, Santoro A, Shipp M, Zinzani PL, Timmerman JM, Ansell S, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. *Lancet Oncol*. 2016;17(9):1283–94.
  106. Armand P, Shipp MA, Ribrag V, Michot JM, Zinzani PL, Kuruvilla J, et al. Programmed death-1 blockade with pembrolizumab in patients with classical Hodgkin lymphoma after brentuximab vedotin failure. *J Clin Oncol*. 2016;34(31):3733–9.
  107. Armand P, Engert A, Younes A, Fanale M, Santoro A, Zinzani PL, et al. Nivolumab for relapsed/refractory classic Hodgkin lymphoma after failure of autologous hematopoietic cell transplantation: extended follow-up of the multicohort single-arm phase II CheckMate 205 trial. *J Clin Oncol*. 2018;36(14):1428–39.
  108. Chen R, Zinzani PL, Lee HJ, Armand P, Johnson NA, Brice P, et al. Pembrolizumab in relapsed or refractory Hodgkin lymphoma: 2-year follow-up of KEYNOTE-087. *Blood*. 2019;134(14):1144–53.
  109. Liu Y, Wang C, Li X, Dong L, Yang Q, Chen M, et al. Improved clinical outcome in a randomized phase II study of anti-PD-1 camrelizumab plus decitabine in relapsed/refractory Hodgkin lymphoma. *J Immunother Cancer*. 2021;9(4): e002347.
  110. Wang C, Liu Y, Dong L, Li X, Yang Q, Brock MV, et al. Efficacy of decitabine plus anti-PD-1 camrelizumab in patients with Hodgkin lymphoma who progressed or relapsed after PD-1 blockade monotherapy. *Clin Cancer Res*. 2021;27(10):2782–91.
  111. Booth L, Roberts JL, Poklepovic A, Kirkwood J, Dent P. HDAC inhibitors enhance the immunotherapy response of melanoma cells. *Oncotarget*. 2017;8(47):83155–70.
  112. Wang X, Waschke BC, Woolaver RA, Chen Z, Zhang G, Piscopio AD, et al. Histone deacetylase inhibition sensitizes PD1 blockade-resistant B-cell lymphomas. *Cancer Immunol Res*. 2019;7(8):1318–31.
  113. Sermer DJ, Vardhana SA, Ames A, Biggar E, Moskowitz AJ, Batlevi CL, et al. Early data from a phase II trial investigating the combination of pembrolizumab (PEM) and entinostat (ENT) in relapsed and refractory (R/R) Hodgkin lymphoma (HL). *J Clin Oncol*. 2020;38(15\_suppl): e20018.
  114. Wang C, Nie J, Liu Y, Yang Q, Han W. Safety and efficacy of chidamide in combination with decitabine plus anti-PD-1 camrelizumab after relapse or progression on decitabine-plus-camrelizumab in classical Hodgkin lymphoma. *J Clin Oncol*. 2021;39(15\_suppl): e19515.
  115. Salik B, Smyth MJ, Nakamura K. Targeting immune checkpoints in hematological malignancies. *J Hematol Oncol*. 2020;13(1):111.
  116. Kim SJ, Hyeon J, Cho I, Ko YH, Kim WS. Comparison of efficacy of pembrolizumab between Epstein-Barr virus positive and negative relapsed or refractory non-Hodgkin lymphomas. *Cancer Res Treat*. 2019;51(2):611–22.
  117. Lees C, Keane C, Gandhi MK, Gunawardana J. Biology and therapy of primary mediastinal B-cell lymphoma: current status and future directions. *Br J Haematol*. 2019;185(1):25–41.
  118. Armand P, Rodig S, Melnichenko V, Thieblemont C, Bouabdallah K, Tumyan G, et al. Pembrolizumab in relapsed or refractory primary mediastinal large B-cell lymphoma. *J Clin Oncol*. 2019;37(34):3291–9.
  119. Beguelin W, Teater M, Meydan C, Hoehn KB, Phillip JM, Soshnev AA, et al. Mutant EZH2 induces a pre-malignant lymphoma niche by reprogramming the immune response. *Cancer Cell*. 2020;37(5):655–73 e11.
  120. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. *J Hematol Oncol*. 2020;13(1):104.
  121. Mondello P, Tadros S, Teater M, Fontan L, Chang AY, Jain N, et al. Selective inhibition of HDAC3 targets synthetic vulnerabilities and activates immune surveillance in lymphoma. *Cancer Discov*. 2020;10(3):440–59.
  122. Italiano A, Soria JC, Toulmonde M, Michot JM, Lucchesi C, Varga A, et al. Tazemetostat, an EZH2 inhibitor, in relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid

- tumours: a first-in-human, open-label, phase 1 study. *Lancet Oncol.* 2018;19(5):649–59.
123. Morschhauser F, Tilly H, Chaidos A, McKay P, Phillips T, Assouline S, et al. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. *Lancet Oncol.* 2020;21(11):1433–42.
  124. Morschhauser F, Tilly H, Chaidos A, Phillips TJ, Ribrag V, Campbell P, et al. Phase 2 multicenter study of tazemetostat, an EZH2 inhibitor, in patients with relapsed or refractory follicular lymphoma. *Blood.* 2019;134(Supplement\_1):123.
  125. Palomba ML, Cartron G, Popplewell L, Ribrag V, Westin J, Chitra S, et al. Safety and clinical activity of atezolizumab in combination with tazemetostat in relapsed or refractory diffuse large B-cell lymphoma: primary analysis of a phase 1b study. *Hematological Oncology.* 2019;37(S2):517–9.
  126. Neuwelt A, Al-Juhaiishi T, Davila E, Haverkos B. Enhancing antitumor immunity through checkpoint blockade as a therapeutic strategy in T-cell lymphomas. *Blood Adv.* 2020;4(17):4256–66.
  127. Lesokhin AM, Ansell SM, Armand P, Scott EC, Halwani A, Gutierrez M, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. *J Clin Oncol.* 2016;34(23):2698–704.
  128. Barta SK, Zain J, MacFarlane AWt, Smith SM, Ruan J, Fung HC, et al. Phase II study of the PD-1 inhibitor pembrolizumab for the treatment of relapsed or refractory mature T-cell lymphoma. *Clin Lymphoma Myeloma Leuk.* 2019;19(6):356–64 e3.
  129. Mulvey E, Ruan J. Biomarker-driven management strategies for peripheral T cell lymphoma. *J Hematol Oncol.* 2020;13(1):59.
  130. Xie C, Li X, Zeng H, Qian W. Molecular insights into pathogenesis and targeted therapy of peripheral T cell lymphoma. *Exp Hematol Oncol.* 2020;9(1):30.
  131. Iyer SP, Xu J, Becnel MR, Nair R, Steiner R, Feng L, et al. A phase II study of pembrolizumab in combination with romidepsin demonstrates durable responses in relapsed or refractory T-cell lymphoma (TCL). *Blood.* 2020;136(Supplement 1):40–1.
  132. Marchi E, Ma H, Montanari F, Sawas A, Lue JK, Deng C, et al. Abstract CT160: The integration of PD1 blockade with epigenetic therapy is highly active and safe in heavily treated patients with T-cell lymphoma. *Cancer Research.* 2020;80(16 Supplement): CT160.
  133. Hu B, Oki Y. Novel immunotherapy options for extranodal NK/T-cell lymphoma. *Front Oncol.* 2018;8:139.
  134. Dobashi A, Tsuyama N, Asaka R, Togashi Y, Ueda K, Sakata S, et al. Frequent BCOR aberrations in extranodal NK/T-cell lymphoma, nasal type. *Genes Chromosomes Cancer.* 2016;55(5):460–71.
  135. Kwong YL, Chan TSY, Tan D, Kim SJ, Poon LM, Mow B, et al. PD1 blockade with pembrolizumab is highly effective in relapsed or refractory NK/T-cell lymphoma failing l-asparaginase. *Blood.* 2017;129(17):2437–42.
  136. Li J, Tao R, Fan L, Song Y, Hu Y, Zhang W, et al. Sintilimab for relapsed/refractory (r/r) extranodal NK/T cell lymphoma (ENKTL): Extended follow-up on the multicenter, single-arm phase II trial (ORIENT-4). *J Clin Oncol.* 2020;38(15\_suppl):8050.
  137. Gao Y, Huang H, Wang X, Bai B, Zhang L, Xiao Y, et al. Anti-PD-1 antibody (Sintilimab) plus histone deacetylase inhibitor (Chidamide) for the treatment of refractory or relapsed extranodal natural killer/T cell lymphoma, nasal type (r/r-ENKTL): preliminary results from a prospective, multicenter, single-arm, phase Ib/II trial (SCENT). *Blood.* 2020;136(Supplement 1):39–40.
  138. Ying Z, Song Y, Wang X, Lin N, Xie Y, Du T, et al. A phase II study of anti-PD-1 sintilimab in combination with chidamide and azacitidine in refractory and relapsed peripheral T-cell lymphoma. *Hematological Oncology.* 2021;39(S2).
  139. Yang H, Bueso-Ramos C, DiNardo C, Estecio MR, Davanlou M, Geng QR, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia.* 2014;28(6):1280–8.
  140. Lamble AJ, Lind EF. Targeting the immune microenvironment in acute myeloid leukemia: a focus on T cell immunity. *Front Oncol.* 2018;8:213.
  141. Lamble AJ, Kosaka Y, Laderas T, Maffit A, Kaempf A, Brady LK, et al. Reversible suppression of T cell function in the bone marrow microenvironment of acute myeloid leukemia. *Proc Natl Acad Sci U S A.* 2020;117(25):14331–41.
  142. Vago L, Gojo I. Immune escape and immunotherapy of acute myeloid leukemia. *J Clin Invest.* 2020;130(4):1552–64.
  143. Liu H. Emerging agents and regimens for AML. *J Hematol Oncol.* 2021;14(1):49.
  144. Liao D, Wang M, Liao Y, Li J, Niu T. A review of efficacy and safety of checkpoint inhibitor for the treatment of acute myeloid leukemia. *Front Pharmacol.* 2019;10:609.
  145. Daver N, Garcia-Manero G, Basu S, Boddur 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 study. *Cancer Discov.* 2019;9(3):370–83.
  146. Lindblad KE, Thompson J, Gui G, Valdez J, Worthy T, Tekleab H, et al. Pembrolizumab and decitabine for refractory or relapsed acute myeloid leukemia. *Blood.* 2018;132(Supplement 1):1437.
  147. Gojo I, Stuart RK, Webster J, Blackford A, Varela JC, Morrow J, et al. Multi-center phase 2 study of pembrolizumab (Pembro) and azacitidine (AZA) in patients with relapsed/refractory acute myeloid leukemia (AML) and in newly diagnosed ( $\geq 65$  years) AML patients. *Blood.* 2019;134(Supplement\_1):832.
  148. Chien KS, Borthakur G, Naqi K, Daver N, Montalban Bravo G, Cortes JE, et al. Final results from a phase II study combining azacitidine and pembrolizumab in patients with higher-risk myelodysplastic syndrome after failure of hypomethylating agent therapy. *Blood.* 2020;136(Supplement 1):23–4.
  149. Daver NG, Garcia-Manero G, Konopleva MY, Alfayez M, Pemmaraju N, Kadia TM, et al. Azacitidine (AZA) with nivolumab (Nivo), and AZA with Nivo + ipilimumab (Ipi) in relapsed/refractory acute myeloid leukemia: a non-randomized, prospective, phase 2 study. *Blood.* 2019;134(Supplement\_1):830.
  150. Daver N, Alotaibi AS, Bucklein V, Subklewe M. T-cell-based immunotherapy of acute myeloid leukemia: current concepts and future developments. *Leukemia.* 2021;35(7):1843–63.
  151. Zheng H, Mineishi S, Claxton D, Zhu J, Zhao C, Jia B, et al. A phase I clinical trial of avelumab in combination with

- decitabine as first line treatment of unfit patients with acute myeloid leukemia. *Am J Hematol.* 2021;96(2): E46–E50.
152. Zeidan AM, Cavenagh J, Voso MT, Taussig D, Tormo M, Boss I, et al. Efficacy and safety of azacitidine (AZA) in combination with the anti-PD-L1 durvalumab (durva) for the front-line treatment of older patients (pts) with acute myeloid leukemia (AML) who are unfit for intensive chemotherapy (IC) and Pts with higher-risk myelodysplastic syndromes (HR-MDS): results from a large, international, randomized phase 2 study. *Blood.* 2019;134(Supplement\_1):829.
  153. Gerds AT, Scott BL, Greenberg PL, Khaled SK, Lin TL, Pollyea DA, et al. PD-L1 blockade with atezolizumab in higher-risk myelodysplastic syndrome: an initial safety and efficacy analysis. *Blood.* 2018;132(Supplement 1):466.
  154. Hay AE, Assouline S, Walter RB, Little RF, Moseley A, Gail SM, et al. Accrual barriers and detection of early toxicity signal in older less-fit patients treated with azacitidine and nivolumab for newly diagnosed acute myeloid leukemia (AML) or high-risk myelodysplastic syndrome (MDS) in the SWOG 1612 platform randomized phase II/III clinical trial. *Blood.* 2019;134(Supplement\_1):3905.
  155. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science.* 2002;295(5562):2097–100.
  156. Romagne F, Andre P, Spee P, Zahn S, Anfossi N, Gauthier L, et al. Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. *Blood.* 2009;114(13):2667–77.
  157. Daver NG, Garcia-Manero G, Cortes JE, Basu S, Ravandi F, Kadia TM, et al. Phase IB/II study of lirilumab with azacytidine (AZA) in relapsed AML. *J Clin Oncol.* 2017;35(15\_suppl): e18505.
  158. Yalniz FF, Daver N, Rezvani K, Kornblau S, Ohanian M, Borthakur G, et al. A pilot trial of lirilumab with or without azacitidine for patients with myelodysplastic syndrome. *Clin Lymphoma Myeloma Leuk.* 2018;18(10):658–63 e2.
  159. Herbrich S, Cavazos A, Cheung CMC, Alexander-Williams L, Short NJ, Matthews J, et al. Single-cell mass cytometry identifies mechanisms of resistance to immunotherapy in AML. *Blood.* 2019;134(Supplement\_1):1428.
  160. Garcia-Manero G, Wei AH, Porkka K, Knapper S, Traer E, Scholl S, et al. MDS-420: sabatolimab plus hypomethylating agents (HMAs) in patients with high-/very high-risk myelodysplastic syndrome (HR/vHR-MDS) and newly diagnosed acute myeloid leukemia (ND-AML): subgroup analysis of a phase 1 study. *Clinical Lymphoma, Myeloma and Leukemia.* 2021;21: S350.
  161. Brunner AM, Esteve J, Porkka K, Knapper S, Vey N, Scholl S, et al. Efficacy and safety of sabatolimab (MBG453) in combination with hypomethylating agents (HMAs) in patients with acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (HR-MDS): updated results from a phase 1b study. *Blood.* 2020;136(Supplement 1):1–2.
  162. Zeidan AM, DeZern A, Borate U, Kobata K, Sadek I, Sabo JR, et al. MDS-364: STIMULUS MDS-US trial in progress: evaluating sabatolimab in combination with hypomethylating agents (HMAs) in patients with intermediate-, high-, or very high-risk myelodysplastic syndromes (MDS). *Clinical Lymphoma Myeloma and Leukemia.* 2021;21:S348–S9.
  163. Taylor K, Loo Yau H, Chakravarthy A, Wang B, Shen SY, Ettayebi I, et al. An open-label, phase II multicohort study of an oral hypomethylating agent CC-486 and durvalumab in advanced solid tumors. *J Immunother Cancer.* 2020;8(2): e000883.
  164. Christmas BJ, Rafie CI, Hopkins AC, Scott BA, Ma HS, Cruz KA, et al. Entinostat converts immune-resistant breast and pancreatic cancers into checkpoint-responsive tumors by reprogramming tumor-infiltrating MDSCs. *Cancer Immunol Res.* 2018;6(12):1561–77.
  165. O’Shaughnessy J, Moroosse RL, Babu S, Baramidze K, Chan D, Leitner SP, et al. Results of ENCORE 602 (TRIO025), a phase II, randomized, placebo-controlled, double-blinded, multicenter study of atezolizumab with or without entinostat in patients with advanced triple-negative breast cancer (aTNBC). *J Clin Oncol.* 2020;38(15\_suppl):1014.
  166. Terranova-Barberio M, Pawlowska N, Dhawan M, Moasser M, Chien AJ, Melisko ME, et al. Exhausted T cell signature predicts immunotherapy response in ER-positive breast cancer. *Nat Commun.* 2020;11(1):3584.
  167. Sharma P, Abramson VG, O’Dea A, Nye LE, Mayer IA, Crane GJ, et al. Romidepsin (HDACi) plus cisplatin and nivolumab triplet combination in patients with metastatic triple negative breast cancer (mTNBC). *J Clin Oncol.* 2021;39(15\_suppl):1076.
  168. Juergens RA, Wrangle J, Vendetti FP, Murphy SC, Zhao M, Coleman B, et al. Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. *Cancer Discov.* 2011;1(7):598–607.
  169. Wrangle J, Wang W, Koch A, Easwaran H, Mohammad HP, Vendetti F, et al. Alterations of immune response of Non-Small Cell Lung Cancer with Azacytidine. *Oncotarget.* 2013;4(11):2067–79.
  170. Huang Q, Zheng Y, Gao Z, Yuan L, Sun Y, Chen H. Comparative efficacy and safety of PD-1/PD-L1 inhibitors for patients with solid tumors: a systematic review and bayesian network meta-analysis. *J Cancer.* 2021;12(4):1133–43.
  171. Ma L, Diao B, Huang Z, Wang B, Yu J, Meng X. The efficacy and possible mechanisms of immune checkpoint inhibitors in treating non-small cell lung cancer patients with epidermal growth factor receptor mutation. *Cancer Commun (Lond).* 2021;41(12):1314–30.
  172. Levy BP, Giaccone G, Besse B, Felip E, Garassino MC, Domine Gomez M, et al. Randomised phase 2 study of pembrolizumab plus CC-486 versus pembrolizumab plus placebo in patients with previously treated advanced non-small cell lung cancer. *Eur J Cancer.* 2019;108:120–8.
  173. Yan X, Zhao Y, Liu Y, Yang Q, Dong L, Wu Z, et al. Case report: low-dose decitabine plus anti-PD-1 inhibitor camrelizumab for previously treated advanced metastatic non-small cell lung cancer. *Front Oncol.* 2020;10:558572.
  174. Tibaldi C, Camerini A, Tiseo M, Mazzoni F, Barbieri F, Vittimberga I, et al. Cytidine deaminase enzymatic activity is a prognostic biomarker in gemcitabine/platinum-treated advanced non-small-cell lung cancer: a prospective validation study. *Br J Cancer.* 2018;119(11):1326–31.
  175. Kang K, Khunger A, Schrupp DS, Rubinstein MP, Wrangle JM, Saunthararajah Y, et al. Tetrahydrouridine/decitabine/5-

- azacytidine for non-cytotoxic epigenetic-immunotherapy of NSCLC in vivo. *J Clin Oncol.* 2018;36(15\_suppl): e24134.
176. Papadatos-Pastos D, Pal A, Akay M, Ameratunga M, Mithra S, Ang J-E, et al. Abstract CT129: HyPeR: A phase I, dose escalation and expansion trial of guadecitabine (SGI-110), a second-generation hypomethylating agent in combination with pembrolizumab (MK3475) in patients with refractory solid tumors. *Cancer Res.* 2020;80(16 Supplement): CT129.
  177. Awad MM, Bruchec YL, Lu B, Miller J, Dumitru CD, Spira AI. Phase Ib study: Selective histone deacetylase (HDAC) inhibitor ACY-241 + nivolumab (Nivo) in advanced non-small cell lung cancer (NSCLC). *J Clin Oncol.* 2019;37(15\_suppl):9029.
  178. Gandhi L, Janne PA, Opyrchal M, Ramalingam SS, Rybkin II, Hafez N, et al. Efficacy and safety of entinostat (ENT) and pembrolizumab (PEMBRO) in patients with non-small cell lung cancer (NSCLC) previously treated with anti-PD-(L)1 therapy. *J Clin Oncol.* 2018;36(15\_suppl):9036.
  179. Gray JE, Saltos A, Tanvetyanon T, Haura EB, Creelan B, Antonia SJ, et al. Phase I/Ib study of pembrolizumab plus vorinostat in advanced/metastatic non-small cell lung cancer. *Clin Cancer Res.* 2019;25(22):6623–32.
  180. Saltos AN, Tanvetyanon T, Creelan BC, Shafique MR, Antonia SJ, Haura EB, et al. Phase II randomized trial of first-line pembrolizumab and vorinostat in patients with metastatic NSCLC (mNSCLC). *J Clin Oncol.* 2020;38(15\_suppl):9567.
  181. Makela S, Kohtamaki L, Laukka M, Juteau S, Hernberg M. Limited-duration anti-PD-1 therapy for patients with metastatic melanoma. *Acta Oncol.* 2020;59(4):438–43.
  182. Pires da Silva I, Ahmed T, Reijers ILM, Weppler AM, Betof Warner A, Patrinely JR, et al. Ipilimumab alone or ipilimumab plus anti-PD-1 therapy in patients with metastatic melanoma resistant to anti-PD-(L)1 monotherapy: a multicentre, retrospective, cohort study. *Lancet Oncol.* 2021;22(6):836–47.
  183. Weiss SA, Wolchok JD, Sznol M. Immunotherapy of melanoma: facts and hopes. *Clin Cancer Res.* 2019;25(17):5191–201.
  184. Wang DY, Eroglu Z, Ozgun A, Leger PD, Zhao S, Ye F, et al. Clinical features of acquired resistance to anti-PD-1 therapy in advanced melanoma. *Cancer Immunol Res.* 2017;5(5):357–62.
  185. Covre A, Coral S, Nicolay H, Parisi G, Fazio C, Colizzi F, et al. Antitumor activity of epigenetic immunomodulation combined with CTLA-4 blockade in syngeneic mouse models. *Oncoimmunology.* 2015;4(8): e1019978.
  186. Di Giacomo AM, Covre A, Finotello F, Rieder D, Danielli R, Sigalotti L, et al. Guadecitabine plus ipilimumab in unresectable melanoma: the NIBIT-M4 clinical trial. *Clin Cancer Res.* 2019;25(24):7351–62.
  187. Burton EM, Woody T, Glitza IC, Amaria RN, Keung EZ-Y, Diab A, et al. A phase II study of oral azacytidine (CC-486) in combination with pembrolizumab (PEMBRO) in patients (pts) with metastatic melanoma (MM). *J Clin Oncol.* 2019;37(15\_suppl):9560.
  188. Agarwala SS, Moschos SJ, Johnson ML, Opyrchal M, Gabrilovich D, Danaher P, et al. Efficacy and safety of entinostat (ENT) and pembrolizumab (PEMBRO) in patients with melanoma progressing on or after a PD-1/L1 blocking antibody. *J Clin Oncol.* 2018;36(15\_suppl):9530.
  189. Sullivan RJ, Moschos SJ, Johnson ML, Opyrchal M, Ordentlich P, Brouwer S, et al. Abstract CT072: Efficacy and safety of entinostat (ENT) and pembrolizumab (PEMBRO) in patients with melanoma previously treated with anti-PD1 therapy. *Cancer Res.* 2019;79(13 Supplement): CT072.
  190. Khushalani NI, Markowitz J, Eroglu Z, Giuroiu I, Ladanova V, Reiersen P, et al. A phase I trial of panobinostat with ipilimumab in advanced melanoma. *J Clin Oncol.* 2017;35(15\_suppl):9547.
  191. Grivas P, Mortazavi A, Picus J, Hahn NM, Milowsky MI, Hart LL, et al. Mocetinostat for patients with previously treated, locally advanced/metastatic urothelial carcinoma and inactivating alterations of acetyltransferase genes. *Cancer.* 2019;125(4):533–40.
  192. Woods DM, Laino AS, Vassallo M, Weber J. Abstract PO-007: The class I/IV HDAC inhibitor mocetinostat augments antitumor immune responses in melanoma patients. *Cancer Res.* 2020;80(23 Supplement):PO-007.
  193. Weber JS, Laino AS, Vassallo M, Pavlick A, Malatyali S, Krishnarajapet S, et al. Preclinical and clinical studies of a class I/IV HDAC inhibitor, mocetinostat, in melanoma. *J Clin Oncol.* 2020;38(15\_suppl):10052.
  194. Xu W, Atkins MB, McDermott DF. Checkpoint inhibitor immunotherapy in kidney cancer. *Nat Rev Urol.* 2020;17(3):137–50.
  195. Zakharia Y, Singer EA, Ross R, Joshi M, Abern M, Garje R, et al. Phase Ib/II study of durvalumab and guadecitabine in advanced kidney cancer Big Ten Cancer Research Consortium BTCRC GU16-043. *J Clin Oncol.* 2021;39(6\_suppl): 328.
  196. Pili R, Quinn DI, Albany C, Adra N, Logan TF, Greenspan A, et al. Immunomodulation by HDAC inhibition: Results from a phase Ib study with vorinostat and pembrolizumab in metastatic urothelial, renal, and prostate carcinoma patients. *J Clin Oncol.* 2019;37(15\_suppl):2572.
  197. Indini A, Nigro O, Lengyel CG, Ghidini M, Petrillo A, Lopez S, et al. Immune-checkpoint inhibitors in platinum-resistant ovarian cancer. *Cancers (Basel).* 2021;13(7): 1663.
  198. Matei D, Pant A, Moroney JW, Fleming GF, Tanner E, Swetz WM, et al. Phase II trial of guadecitabine priming and pembrolizumab in platinum resistant recurrent ovarian cancer. *J Clin Oncol.* 2020;38(15\_suppl):6025.
  199. Cadoo KA, Meyers ML, Burger RA, Armstrong DK, Penson RT, Gordon MS, et al. A phase II randomized study of avelumab plus entinostat versus avelumab plus placebo in patients (pts) with advanced epithelial ovarian cancer (EOC). *J Clin Oncol.* 2019;37(15\_suppl):5511.
  200. Zhou C, Jiang T, Li R, Yuan Y, Xie W, Huang X, et al. Outcomes and toxicities of immune checkpoint inhibitors in colorectal cancer: a real-world retrospective analysis. *Cancer Commun (Lond).* 2021;41(9):921–4.
  201. Lee JJ, Sun W, Bahary N, Ohr J, Rhee JC, Stoller RG, et al. Phase 2 study of pembrolizumab in combination with azacytidine in subjects with metastatic colorectal cancer. *J Clin Oncol.* 2017;35(15\_suppl):3054.
  202. Rodriguez CP, Wu QV, Voutsinas J, Fromm JR, Jiang X, Pillarisetty VG, et al. A phase II trial of pembrolizumab and vorinostat in recurrent metastatic head and neck squamous cell carcinomas and salivary gland cancer. *Clin Cancer Res.* 2020;26(4):837–45.

203. Akbari B, Ghahri-Saremi N, Soltantoyeh T, Hadjati J, Ghassemi S, Mirzaei HR. Epigenetic strategies to boost CAR T cell therapy. *Mol Ther.* 2021;29(9):2640–59.
204. Zebley CC, Gottschalk S, Youngblood B. Rewriting history: Epigenetic reprogramming of CD8(+) T cell differentiation to enhance immunotherapy. *Trends Immunol.* 2020;41(8):665–75.
205. Fraietta JA, Nobles CL, Sammons MA, Lundh S, Carty SA, Reich TJ, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature.* 2018;558(7709):307–12.
206. Weber EW, Parker KR, Sotillo E, Lynn RC, Anbunathan H, Lattin J, et al. Transient rest restores functionality in exhausted CAR-T cells through epigenetic remodeling. *Science.* 2021;372(6537): eaba1786.
207. Baumeister SH, Murad J, Werner L, Daley H, Trebeden-Negre H, Gicobi JK, et al. Phase I trial of autologous CAR T cells targeting NKG2D ligands in patients with AML/MDS and multiple myeloma. *Cancer Immunol Res.* 2019;7(1):100–12.
208. Loney C, Verma B, Hendlitz A, Aftimos P, Awada A, Van Den Neste E, et al. Study protocol for THINK: a multinational open-label phase I study to assess the safety and clinical activity of multiple administrations of NKR-2 in patients with different metastatic tumour types. *BMJ Open.* 2017;7(11): e017075.
209. Zebley CC, Brown C, Mi T, Fan Y, Alli S, Boi S, et al. CD19-CAR T cells undergo exhaustion DNA methylation programming in patients with acute lymphoblastic leukemia. *Cell Rep.* 2021;37(9):110079.
210. Li S, Xue L, Wang M, Qiang P, Xu H, Zhang X, et al. Decitabine enhances cytotoxic effect of T cells with an anti-CD19 chimeric antigen receptor in treatment of lymphoma. *Onco Targets Ther.* 2019;12:5627–38.
211. Pan J, Yang JF, Deng BP, Zhao XJ, Zhang X, Lin YH, et al. High efficacy and safety of low-dose CD19-directed CAR-T cell therapy in 51 refractory or relapsed B acute lymphoblastic leukemia patients. *Leukemia.* 2017;31(12):2587–93.
212. Qu C, Song Y, Yin J, Ma Y, Kang L, Li Z, et al. Decitabine may improve CAR-T efficacy in refractory/relapsed acute leukemia patients carrying TP53 alterations. *Bone Marrow Transplant.* 2021;56(7):1710–3.
213. Fan H, Lu X, Wang X, Liu Y, Guo B, Zhang Y, et al. Low-dose decitabine-based chemoimmunotherapy for patients with refractory advanced solid tumors: a phase I/II report. *J Immunol Res.* 2014;2014:371087.
214. Chen M, Nie J, Liu Y, Li X, Zhang Y, Brock MV, et al. Phase Ib/II study of safety and efficacy of low-dose decitabine-primed chemoimmunotherapy in patients with drug-resistant relapsed/refractory alimentary tract cancer. *Int J Cancer.* 2018;143(6):1530–40.
215. Schroeder T, Czibere A, Platzbecker U, Bug G, Uharek L, Luft T, et al. Azacitidine and donor lymphocyte infusions as first salvage therapy for relapse of AML or MDS after allogeneic stem cell transplantation. *Leukemia.* 2013;27(6):1229–35.
216. Steinmann J, Bertz H, Wasch R, Marks R, Zeiser R, Bogatyreva L, et al. 5-Azacitidine and DLI can induce long-term remissions in AML patients relapsed after allograft. *Bone Marrow Transplant.* 2015;50(5):690–5.
217. Sommer S, Crujnsen M, Claus R, Bertz H, Wasch R, Marks R, et al. Decitabine in combination with donor lymphocyte infusions can induce remissions in relapsed myeloid malignancies with higher leukemic burden after allogeneic hematopoietic cell transplantation. *Leuk Res.* 2018;72:20–6.
218. Qian CS, Ma X, Wang J, Wang TJ, Bai L, Zhou HX, et al. PD1 inhibitor in combination with 5-azacytidine and low-dose DLI for the successful treatment of AML patients who relapsed after transplantation. *Bone Marrow Transplant.* 2021;56(5):1003–5.
219. Nowicki TS, Farrell C, Morselli M, Rubbi L, Campbell KM, Macabali MH, et al. Epigenetic suppression of transgenic T-cell receptor expression via gamma-retroviral vector methylation in adoptive cell transfer therapy. *Cancer Discov.* 2020;10(11):1645–53.
220. Lesch S, Gill S. The promise and perils of immunotherapy. *Blood Adv.* 2021;5(18):3709–25.
221. Wilgenhof S, Corthals J, Heirman C, van Baren N, Lucas S, Kvistborg P, et al. Phase II study of autologous monocyte-derived mRNA electroporated dendritic cells (TriMixDC-MEL) plus ipilimumab in patients with pretreated advanced melanoma. *J Clin Oncol.* 2016;34(12):1330–8.
222. Wu AA, Bever KM, Ho WJ, Fertig EJ, Niu N, Zheng L, et al. A phase II study of allogeneic GM-CSF-transfected pancreatic tumor vaccine (GVAX) with ipilimumab as maintenance treatment for metastatic pancreatic cancer. *Clin Cancer Res.* 2020;26(19):5129–39.
223. Gnjjatic S, Nishikawa H, Jungbluth AA, Gure AO, Ritter G, Jager E, et al. NY-ESO-1: review of an immunogenic tumor antigen. *Adv Cancer Res.* 2006;95:1–30.
224. Odunsi K, Matsuzaki J, James SR, Mhawech-Fauceglia P, Tsuji T, Miller A, et al. Epigenetic potentiation of NY-ESO-1 vaccine therapy in human ovarian cancer. *Cancer Immunol Res.* 2014;2(1):37–49.
225. Bever KM, Thomas DL, 2nd, Zhang J, Diaz Rivera EA, Rosner GL, Zhu Q, et al. A feasibility study of combined epigenetic and vaccine therapy in advanced colorectal cancer with pharmacodynamic endpoint. *Clin Epigenetics.* 2021;13(1):25.
226. Griffiths EA, Srivastava P, Matsuzaki J, Brumberger Z, Wang ES, Kocent J, et al. NY-ESO-1 vaccination in combination with decitabine induces antigen-specific T-lymphocyte responses in patients with myelodysplastic syndrome. *Clin Cancer Res.* 2018;24(5):1019–29.
227. Holmberg-Thyden S, Dufva IH, Gang AO, Breinholt MF, Schejbel L, Andersen MK, et al. Epigenetic therapy in combination with a multi-epitope cancer vaccine targeting shared tumor antigens for high-risk myelodysplastic syndrome - a phase I clinical trial. *Cancer Immunol Immunother.* 2022;71(2):433–44.
228. Kartikasari AER, Prakash MD, Cox M, Wilson K, Boer JC, Cauchi JA, et al. Therapeutic cancer vaccines-T cell responses and epigenetic modulation. *Front Immunol.* 2018;9:3109.
229. Griffin GK, Wu J, Iracheta-Vellve A, Patti JC, Hsu J, Davis T, et al. Epigenetic silencing by SETDB1 suppresses tumour intrinsic immunogenicity. *Nature.* 2021;595(7866):309–14.

**How to cite this article:** Xu Y, Li P, Liu Y, Xin D, Lei W, Liang A, et al. Epi-immunotherapy for cancers: rationales of epi-drugs in combination with immunotherapy and advances in clinical trials. *Cancer Commun.* 2022;1–24.  
<https://doi.org/10.1002/cac2.12313>