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Review article

Stochasticity of anticancer mechanisms underlying clinical effectiveness of vorinostat

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ABSTRACT

The Food and Drug Administration (FDA) has approved vorinostat, also called Zolinza®, for its effectiveness in fighting cancer. This drug is a suberoyl-anilide hydroxamic acid belonging to the class of histone deacetylase inhibitors (HDACis). Its HDAC inhibitory potential allows it to accumulate acetylated histones. This, in turn, can restore normal gene expression in cancer cells and activate multiple signaling pathways. Experiments have proven that vorinostat induces histone acetylation and cytotoxicity in many cancer cell lines, increases the level of p21 cell cycle proteins, and enhances pro-apoptotic factors while decreasing anti-apoptotic factors. Additionally, it regulates the immune response by up-regulating programmed death-ligand 1 (PD-L1) and interferon gamma receptor 1 (IFN-γR1) expression, and can impact proteasome and/or aggresome degradation, endoplasmic reticulum function, cell cycle arrest, apoptosis, tumor microenvironment remodeling, and angiogenesis inhibition. In this study, we sought to elucidate the precise molecular mechanism by which Vorinostat inhibits HDACs. A deeper understanding of these mechanisms could improve our understanding of cancer cell abnormalities and provide new therapeutic possibilities for cancer treatment.

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1. Introduction

Cancer disease is characterized by high complexity, involving a variety of risk factors that may differ between individuals. Researchers have established the contribution of hormonal, genetic, environmental, nutritional, and epigenetic factors to the development of cancer disease [1]. Epigenetic modifications (histone acetylation and deacetylation) can potentially affect gene expression without causing any physical changes in DNA structure [2,3]. Deacetylation can cause chromatin to loosen up and an active transcriptional state [4,5]. Acetylation is usually linked to gene repression.

Histone deacetylation that doesn't work right can cause genes, including oncogenes that help tumors grow, to be expressed without control [6]. Studies have shown that human cancers exhibit a state of loose and uncompacted chromatin, linked to tumor transformation [7,8]. Histone deacetylases (HDACs) facilitate histone deacetylation by transferring an acetyl group to histone proteins, thereby inducing a relaxed chromatin state that activates gene expression. Inhibiting HDACs can limit the growth of cancerous cells and is a promising approach for cancer chemotherapy [6]. As early as the 1960s, researchers developed HDAC inhibitors (HDACis) to combat colorectal cancer (CRC). Natural substances have also been developed as HDACis, but most have failed to reach clinical use [9–11].

Vorinostat, or suberoyl-anilide hydroxamic acid (SAHA) (Fig. 1), has been shown to be effective in treating specific cancer forms [12]. In October 2006, the FDA approved this drug, which belongs to a class of drugs called HDACis, to treat cutaneous T-cell lymphoma (CTCL) in patients who do not respond to other treatments. It was the first HDACi approved for this purpose. Vorinostat is taken orally, usually once a day, and is available in capsule form. Its common side effects include vomiting, nausea, fatigue, diarrhea, and decreased appetite. More serious side effects, such as low platelet counts or liver problems, may also occur.

As with all medications, it is important to follow the prescribing information and consult a healthcare professional to determine if vorinostat is an appropriate cancer treatment option. Vorinostat acts by blocking the activity of HDAC enzymes, implicated in regulating gene expression. In fact, vorinostat inhibits HDAC, which can alter the expression of genes responsible for cell differentiation and growth, ultimately leading to cancer cell death. Vorinostat induces clinical efficacy in the management of human cancers through several mechanisms [13]. Its major anti-cancer mechanism involves inducing apoptosis, a controlled cell death, in tumor cells. HDACis, such as vorinostat, increase histone acetylation, leading to the transcriptional activation of genes involved in regulating apoptosis [14]. This results in pro-apoptotic gene activation and anti-apoptotic gene suppression, causing cancer cell death. In addition to inducing apoptosis, vorinostat can also disrupt cancer cell growth and proliferation. HDAC inhibition can lead to the accumulation of acetylated proteins, which can interfere with critical cellular processes involved in cell cycle regulation, angiogenesis, and DNA repair [15]. This can ultimately result in the suppression of cancer cell growth and angiogenesis, preventing the development of new blood vessels essential for cancer metastasis and growth. Vorinostat and other HDACis exhibit their anti-cancer impact by modulating immune cells through another crucial mechanism. By increasing the expression of immune-related genes and intensifying the activity of immune cells such as T cells, natural killer cells, and dendritic cells, HDACis may enhance immune surveillance and the eradication of cancer cells [16].

Vorinostat, a HDACi, has been shown to be an effective treatment for human cancers by acting through multiple mechanisms, such as inducing cancer cell death, inhibiting their growth, and improving the immune response. This review aims to clarify the molecular pathways implicated in the action of vorinostat and reevaluate its potential as a potent anti-tumor agent while addressing its potential limitations and known or anticipated challenges in clinical trials, with discussion of future directions associated with vorinostat treatment.

2. HDAC and cancer

Various factors influence the multifaceted process of cancer development, making it a complex disease. Environmental factors such as exposure to carcinogens, lifestyle factors like smoking or a diet high in processed foods, and hereditary factors such as cancer family history or genetic mutations can all increase the risk of developing cancer. Current understanding of cancer etiology has expanded the previous perception of cancer as solely a consequence of genetic mutations to include abnormalities in epigenetic regulation [17–19].

Cancer development is often attributed to the initial epigenetic changes that disrupt normal tissue homeostasis and lead to genetic instability, causing mutations in tumor-suppressor genes. Epigenetic mechanisms often silence tumor suppressor genes (TSGs) in various cancers at the pre-invasive stage, regardless of their genetic mutation status. These mechanisms involve histone modifications, DNA methylation, and the deregulation of non-coding RNAs and their interactions with nucleic acids or proteins. Four types of regulators, namely remodelers, readers, erasers, and writers, dynamically control epigenetic regulation. Various forms of cancer have detected mutations and dysregulation of the genes responsible for controlling these regulators [17–19].

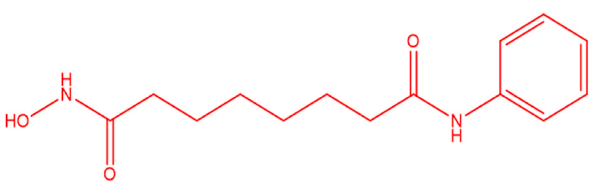

Fig. 1. Chemical structure of vorinostat.

Table 1
Cellular and Molecular anticancer mechanisms of vorinostat.

Tumour models	Methods	Anticancer mechanisms	References
Melanoma cells (A375, A549, and MeWo)	Western blot analysis Immunofluorescent staining for γ-H2AX	Induced acetylation of histones Induced apoptosis Enhanced radiosensitivity of tumor cells Prolonged γ-H2AX Foci expression	[14]
Pancreatic cancer cells (HPAC, HPAF-II, BxPC-3, Capan-2, PANC-1, AsPC-1)	MTT assay Annexin-V-FITC Chromatin immunoprecipitation assay qRT-PCR	Inhibited cell growth dose dependently Induced apoptosis and G_2 cell cycle arrest Increased expression of $p21^{WAF1}$, *C/EBPα*, *RARα*, and *E-cadherin* Decreased cyclin B1 expression	[42]
Human colon cancer cell lines HCT116 and HT29	Vorinostat (0.5 and 3 μmol/L) Bortezomib (0.05 and 0.1 μmol/L) Sulforhodamine B method Flow cytometry analysis Immunoblotting	Vorinostat + Bortezomib synergistically decreased colorectal cancer (CRC) cell proliferation Vorinostat + Bortezomib synergistically increased apoptosis Increased the pro-apoptotic protein BIM	[15]
CRC cells (LoVo, SW620, HT29, and LS174T)	Flow cytometry analysis	Vorinostat + 5-Fluorouracil or Raltitrexed induced a synergistic antiproliferative interaction with cell cycle perturbations and major S-phase arrest	[43]
CRC cells (LoVo, SW620, HT29, and LS174T) (*in vitro*) SW620 cells in a xenograft mouse model (*in vivo*)	Western blot analysis Immunohistochemistry	Up-regulated thymidine phosphorylase (TP) and down-regulated thymidylate synthase (TS) in tumor cells (*in vitro* and *in vivo*) Vorinostat + Capecitabine induced a synergistic antiproliferative activity and augmented apoptotic cell death (*in vitro*) Vorinostat + Capecitabine increased apoptosis, inhibited tumour growth, and prolonged survival (*in vivo*)	[44]
H526 mutant p53 and H209 wild-type p53 cell lines	Flow cytometry analysis Annexin V-FITC staining	Vorinostat + Topotecan/Camptothecin induced a potent synergistic cytotoxic effect in both cells Vorinostat + Topotecan/Camptothecin increased S-phase cell cycle arrest alongside apoptosis	[16]
KB and Hep-2 cell lines and epithelial CAL27 cells	Western blot analysis Immunoprecipitation RT-PCR Annexin-V-FITC staining Flow cytometry analysis Wound-healing assay	Down-regulated the signaling and expression of all receptors (ErbB2, EGFR, and ErbB3) in CAL27 cells Reversed the mesenchymal phenotype of KB and Hep-2 cells	[45]
HuCC-T1 cholangiocarcinoma cells	Trypan blue exclusion assay Annexin V/PI binding assay Western blot analysis TUNEL assay Wound healing assay	Induced apoptosis + Suppressed invasion, migration, and angiogenesis of tumor cells Vorinostat + Epigallocatechin-3-Gallate (EGCG) showed synergistic growth inhibitory potential in tumor cells Vorinostat + EGCG increased caspase-3 and -7 activity and Bax/Bcl-2 expression ratio Vorinostat + EGCG decreased the expression poly (ADP-ribose) polymerase (PARP)	[46]
HuCC-T1 cholangiocarcinoma cells (*in vitro*) HuCC-T1 cells in a xenograft mouse model (*in vivo*)	Vorinostat-incorporated nanoparticles (vorinostat-NPs) Immunocytochemistry Immunohistochemistry Quantification of image intensity	Increased acetylation of histone-H3 Suppressed or expressed histone deacetylase Improved anticancer activity compared to vorinostat (*in vivo*) Suppressed HDAC expression (*in vivo*)	[47]
Multiple myeloma (MM) cell lines, RPMI 8226 and U266 (*in vitro*) Immunoglobulin (Ig) Gj-producing MM implanted into homozygous CB-17 SCID mice (*in vivo*)	Vorinostat (0.01–30 μm) (*in vitro*) MTS assay ELISA assay	Enhanced the anti-MM effect of Bortezomib and Melphalan (*in vivo* and *in vitro*) Vorinostat (100 mg/kg) + Melphalan (3 mg/kg) inhibited significantly the tumor growth (*in vivo*)	[48]

(*continued on next page*)

Table 1 (*continued*)

Tumour models	Methods	Anticancer mechanisms	References
	Vorinostat (30, 60, or 100 mg/kg daily for 5 consecutive days per week, 100 or 300 mg/kg daily for 2 days per week) (*in vivo*) Measurement of tumor growth		
Human leukemia (Molt-4), lymphoma (Raji), MM (RPMI 8226), mouse tumor (TC-1), human cervical carcinoma (HeLa), and hepatocellular carcinoma (HepG2) cell lines HeLa, HepG2, or TC-1 cells in a xenograft mouse model (*in vivo*)	cDNA microarray analysis qRT-PCR Western blot analysis	Suppressed activation of AKT pathways Vorinostat + AA98 significantly inhibited angiogenesis (*in vitro*) Vorinostat + AA98 inhibited metastasis and tumor growth (*in vivo*)	[49]
Human uterine sarcoma cell MES-SA (*in vitro*) MES-SA cells in a xenograft mouse model (*in vivo*)	Western blot analysis Clonogenic assay Electron microscopy 50 mg/kg/day (*in vivo*)	At a low dosage (3 μM), suppressed cell growth Decreased cell survival after prolonged treatment Increased $p21^{WAF1}$ expression and apoptosis Reduced tumor growth (>50 %) (*in vivo*)	[50]
U251, DU145, SF539, U87, and PC3 cells	Immunoprecipitation Immunoblot Analysis Clonogenic Survival	UPR activation by PERK phosphorylation contributes to the antitumor activity of vorinostat	[51]
Renal cell cancers (RCC) (*in vitro*) 786-O and Caki-1 cells in a xenograft mouse model (*in vivo*)	MTT assay Clonogenic survival assays Immunoblotting Immunohistochemistry TUNEL assay	Enhanced the anticancer effect of temsirolimus in a panel of RCC cell lines (*in vivo* and *in vitro*) Vorinostat + Temsirolimus induced modest decrease in survivin levels Vorinostat + Temsirolimus induced a strong reduction in angiogenesis	[52]
Neuroblastoma cell lines NB-1691 (*in vitro*) $NB1691^{luc}$ cells in a xenograft mouse model (*in vivo*)	Clonogenic survival assay Bioluminescence imaging Western blot analyses Flow cytometry analysis	Vorinostat + RT decreased cell viability and induced additive effects (*in vitro*) Vorinostat + RT decreased tumor volumes (*in vivo*) Reduced DNA repair enzyme Ku-86	[53]
Human lung cancer cell lines (A549 and NCI H460) (*in vitro*) A549 cells in a xenograft mouse model (*in vivo*)	Vorinostat (0.1–10 μM) (*in vitro*) Vorinostat (0.1–5 μM) (*in vivo*) Luciferase assay	Increased CAR expression (*in vitro*) Enhanced the expression of TRAIL	[54]
Human endometrioid (Type I, Ishikawa) Uterine serous papillary (Type II, USPC-2) endometrial cancer cell lines	Western immunoblots Transfections and luciferase assays MTT assay PI staining Wound-healing assays	Increased IGF-IR phosphorylation Up-regulated pTEN and p21 expression Reduced p53 and cyclin D1 levels in Ishikawa cells Produced acetylation of histone H3 Down-regulated total AKT, pTEN, and cyclin D1 in USPC-2 cell expression Induced apoptosis in both cell lines	[55]
8 human mantle cell lymphoma (MCL) cell lines	Flow cytometry analysis Chromatin immunoprecipitation assay	Inhibited total HDAC activity leading to selective toxicity toward tumor cells Activated mitochondrial apoptosis Induced H4 hyperacetylation on promoter regions	[56]
Human mammary adenocarcinoma cell line ER(−) (MDA-MB-231) and ER(+) (MCF7) (*in vitro*) MCF7 and MDA-MB-231 tumor cells in a xenograft mouse model (*in vivo*)	Annexin V-PE/7-ADD staining method Flow cytometry analysis RT-PCR	Modulated a member of the tumor necrosis factor (TNF) superfamily (CD137) in breast cancer cells Vorinostat + soluble CD137 receptor induced a synergistic cytotoxic effect correlated with up-regulation of the CD137 receptor/ligand system	[57]
Prostate cancer cells (DU145, PC3, and LNCaP)	MSP assay Chromatin immunoprecipitation assay 1X Annexin V binding buffer qRT-PCR Whole genome expression profiling Immunoblotting	Vorinostat + Genistein affected certain pathways (Wnt, TNF, G_2/M DNA damage checkpoint,.) Vorinostat + Genistein induced cell death with an effect on early-stage cancer	[58]
Human epidermoid carcinoma A431 cells (*in vitro*) A431 cells in a xenograft mouse model (*in vivo*)	Dose = 2 μM (*in vitro*) Dose = 100 mg/kg b.w., i.p. (*in vivo*) Immunohistochemical staining	Reduced expression of HDAC1, 2, 3, and 7 in A431 cells (*in vitro*) Reduced tumor growth (*in vivo*) Induced apoptosis	[59]

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Table 1 (*continued*)

Tumour models	Methods	Anticancer mechanisms	References
	TUNEL assay	Inhibited mTOR signaling + reduced cell survival AKT and extracellular-signal regulated kinase (ERK) signaling pathways	
	MTT assay		
Endometrial cancer cells (Shikawa 3-H-12 and AN3CA) (*in vitro*)	MTT assay	Induced cell growth arrest and apoptosis	[60]
	Immunehistochemical analysis	Induced the activation of caspase-8 and -9	
HEC-1A cells in a xenograft mouse model (*in vivo*)	Western blot analysis	Decreased FLIP mRNA and protein levels	
	RT-PCR		
Human $CD14^+$ monocyte-derived dendritic cells and mouse immature dendritic cells (*in vitro*)	FITC-dextran	Inhibited cell differentiation and maturation	[61]
	qRT-PCR analysis	Inhibited dendritic cell-directed Th1- (Type 1T helper)	
Multiple sclerosis, experimental autoimmune encephalomyelitis (*in vivo*)	Immunohistochemistry	Inhibited expression of costimulatory molecules of dendritic cells (*in vivo*)	
Pediatric leukemia, medulloblastoma, and neuroblastoma cell lines	MTT assay	Inhibited cell survival	[62]
	Immunoblotting	Vorinostat + MLN8237 induced additive cytotoxicity	
18 urothelial cancer cell lines (UCC)	Flow cytometry analysis	Induced G_2/M arrest	[63]
	Immunocytochemistry	Increased sub-G_1 fraction	
		Up-regulated p21 and down-regulated TS	
Luminal and basal A subtype breast cancer cell lines (*in vitro*)	MTS assay	Sensitized Hs578T and TNBC MDA-MB-231 cells to AFP464	[64]
MDA-MB-231 cells in a xenograft mouse model (*in vivo*)	Chou-Talalay method	Enhanced AFP464 anticancer effect (*in vivo*)	
	Western blot analysis qRT-PCR		
	Immunofluorescence		
	Immunohistochemical staining		
Primary PyMT cells derived from mammary tumors of female PyMT (±) mice	MTT assay	Potentiated synthetic triterpenoid ability	[65]
	Immunoblot analysis	Vorinostat (250 mg/kg) + Synthetic triterpenoid (50 mg/kg) delayed tumor initial formation	
	ELISA assay		
Human CRC cell lines (HCT116, HT29, LoVo, and RKO) (*in vitro*)	MTS assay	IC_{50}: 1.2–2.8 μmol/L for Vorinostat	[66]
	Growth inhibition assay	Induced significant growth inhibition at a >24 h exposure	
HCT116 cells in a xenograft mouse model (*in vivo*)	Western blot analysis	Reduced colony formation at a >24 h exposure	
		Increased acetyl-H3 and down-regulated TS (*in vivo*)	
MOLM-13 acute myeloid leukemia (AML) cells	MTS assay	Vorinostat + BPR1J-340 synergistically induced apoptosis	[67]
	Annexin V-FITC staining		
Liver cancer-derived cell lines, HuH7 and Hep3B	qRT-PCR	Inhibited HIF-1α expression	[68]
	Immunoblotting	Regulated HIF-1α translation	
	ImmunoFluorescence		
Human mantle cell lymphoma (MCL) cells (Jeko-1, SP53, Mino, and Granta 519)	CCK-8 assay	Vorinostat + STAT3 inhibitor WP1066 resulted in a mutually reinforcing effect, leading to both a reduction in growth and an increase in apoptosis in MCL cell lines	[69]
	Annexin V/7-AAD assay	Vorinostat + WP1066 suppressed the continuous activation of STAT3 and adjusted the mRNA expression levels of genes related to both promoting and inhibiting apoptosis	
Diffuse large B cell lymphoma (DLBCL)	Vorinostat (0.5–5 μM)	Vorinostat + Bendamustine increased the acetylation of histones and caused double-strand breaks in DNA	[70]
	Bendamustine (5–100 μM)		
	Annexin-V-FITC qRT-PCR analysis		
Small cell lung cancer (SCLC) cells (H209 and H146) (*in vitro*)	MTS assay	Vorinostat + Cisplatin resulted in the inhibition of cell growth, induction of apoptosis, and promotion of cell cycle arrest	[71]
H209 cells in a xenograft mouse model (*in vivo*)	Caspase-3/CPP32 colorimetric assay	Vorinostat + Cisplatin elevated the acetylation levels of both histone H3 and α-tubulin	
	Flow cytometry analysis	Vorinostat + Cisplatin significantly inhibited tumor growth (20.5 %) (*in vivo*)	
Rituximab–chemotherapy-sensitive (RSCL) and rituximab–chemotherapy-resistant cell lines (RRCL) and primary tumor cells isolated from relapsed/refractory B cell lymphoma patients	Vorinostat (0–8 μM) (*in vitro*)	Induced cell death in both RRCL and primary tumor cells in a manner that depended on the dosage administered	[72]
	Vorinostat (0, 0.5, and 1 μM) (*in vivo*)	Increased p21 and histone H3 acetylation by inducing G_1 cell cycle arrest	

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Table 1 (*continued*)

Tumour models	Methods	Anticancer mechanisms	References
	Preston blue reduction assay	Treatment triggered apoptosis in RSCL cell lines, but it did not have the same effect on RRCL	
Murine melanoma cells (B16F10) Human breast cancer cells (MDA MB 231) (*in vitro*) B16F10 cells in a xenograft mouse model (*in vivo*)	Vorinostat encapsulated in PEG-PLGA copolymeric micelles	Treatment demonstrated better cellular internalization, increased cytotoxicity, and greater induction of apoptosis when compared to administering the drug alone	[73]
	Fluorescence microscopy	Presented a high percent cell killing (54.9 %) compared to free drug in MDA MB 231 cell line	
	Annexin V and PI	Reduced tumor volume by 1.78 times compared to animals treated with free drug (*in vivo*)	
HCT-116 cells, MCF-7 and drug-resistant MCF- 7/ADR cells	Drug release test	Vorinostat-Paclitaxel co-prodrugs exhibited potent activity, leading to both cytotoxicity and cell cycle arrest	[74]
	SRB assay Western blot analysis		
Human non-SCLC (NSCLC) cell line PC9 and gefitinib-resistant PC9 (PC9GR) cells	Hoechst 33258 staining	Vorinostat + Gefitinib induced cell death in parental PC9 cells by activating apoptosis to a greater extent than Gefitinib alone	[75]
	Assessment of reactive oxygen species (ROS) production	Vorinostat + Gefitinib, in both PC9 and PC9GR cells, resulted in the cleavage of heat shock protein 90 (HSP90) and a decrease of HSP90 client protein level Vorinostat + Gefitinib triggered the activation of caspase through the production of ROS	
Human squamous cancer cell lines (KB, Hep2, and FaDu) (*in vitro*) GFP/luc-transfected Cal27 cells in a xenograft mouse model (*in vivo*)	Western blot analysis	Vorinostat + 5-Fluorouracil/Cisplatin produced powerful synergistic effects that inhibited cell growth and induced apoptosis	[76]
	RT-PCR	Reversed the phosphorylation of EGFR and its translocation to the nucleus, which had been induced by 5-Fluorouracil/Cisplatin	
	Annexin V-FITC	Resulted in higher uptake of platinum and increased levels of DNA that had been platinated	
	Phosphoprotein detection assay Immunofluorescence assay		
Canine urothelial carcinoma cells (Sora, TCCUB, and Love) (*in vitro*) Sora cells in a xenograft mouse model (*in vivo*)	Immunohistochemistry Vorinostat (150 mg/kg/day i.p) (*in vivo*)	Inhibited cell growth Caused cell cycle arrest in the G_0/G_1 phase Mediated histone H3 acetylation, p-Rb dephosphorylation, and p21 up-regulation Inhibited tumor growth (*in vivo*)	[77]
Human female breast adenocarcinoma cell line SKBR3	Three-color imaging flow cytometry analysis	Vorinostat enhanced trastuzumab-mediated antibody-dependent cell-mediated phagocytosis (ADCP) and trastuzumab-independent cytotoxicity	[78]
	Flow cytometry analysis	Vorinostat induced an immunogenic cell death	
NSCLC	Deformable liposomal co-delivery system encapsulating Vorinostat + Simvastatin MTT assay TUNEL staining	Induced an enhanced intratumor infiltration ability Inhibited tumor growth (*in vivo*) Suppressed angiogenesis, and thus remodel the tumor microenvironment	[79]
Human cervical cancer cell lines (SiHa and HeLa) and NK-92 cell line (*in vitro*) Cervical cancer cells in a xenograft mouse model (*in vivo*)	Vorinostat (2.5, 5, and 10 μM) for 12 and 24 h (*in vitro*) Vorinostat (0.54 mg/kg) daily (*in vivo*) CCK-8 assay qRT-PCR	Inhibited cervical cancer cells growth (*in vivo* and *in vitro*) Inhibited the invasion and migration (*in vitro*) Induced MICA expression on the cervical cancer cell surface through the PI3K/Akt pathway (*in vitro*)	[80]

γ-H2AX: a specific protein called H2AX phosphorylated at a specific site (serine 139). The symbol "γ" denotes the phosphorylation of the protein. **Annexin-V:** a protein that binds to phosphatidylserine. **MTT:** methylthiazolyldiphenyl-tetrazolium bromide. **FITC:** fluorescein isothiocyanate, a fluorescent dye used to label proteins or other molecules for fluorescence-based assays. **qRT:** quantitative reverse transcription. **PCR:** polymerase chain reaction. ***p21WAF1*:** This refers to a protein called "p21WAF1", involved in regulating the cell cycle and inhibiting cell division. ***C/EBPα*:** This stands for "CCAAT/enhancer-binding protein alpha," a transcription factor that plays a role in regulating gene expression. ***RARα*:** "Retinoic Acid Receptor Alpha," a nuclear receptor protein that binds to retinoic acid. ***E-cadherin*:** "Epithelial-cadherin," a type of cell adhesion molecule that is crucial for maintaining the integrity and cohesion of epithelial tissues. **Cyclin B1:** a protein called "Cyclin B1," which is a regulatory protein involved in the cell cycle. **CRC:** colorectal cancer. **BIM:** a protein called "BIM," which is a pro-apoptotic member of the Bcl-2 protein family. **TP:** thymidine phosphorylase. **TS:** thymidylate synthase. **Annexin V:** a protein that has a high affinity for phosphatidylserine. **PI:** propidium iodide. **TUNEL assay:**

Terminal deoxynucleotidyl transferase dUTP nick end labeling assay. **EGCG:** epigallocatechin-3-gallate. **Bax:** a pro-apoptotic protein that promotes apoptosis. **Bcl-2:** an anti-apoptotic protein that inhibits cell death. **PARP:** poly (ADP-ribose) polymerase. **MM:** multiple myeloma. **Ig:** immunoglobulin. **MTS:** 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium. **ELISA:** enzyme-linked immunosorbent assay. **AKT:** known as Protein Kinase B (PKB), is a serine/threonine kinase involved in various cellular processes. **UPR:** unfolded protein response. **PERK:** protein kinase r-like endoplasmic reticulum kinase. **RCC:** renal cell cancers. **Ku-86:** a specific DNA repair enzyme that is part of a protein complex known as the Ku heterodimer. **CAR:** chimeric antigen receptor. **TRAIL:** tumor necrosis factor-related apoptosis-inducing ligand. **IGF-IR:** Insulin-Like Growth Factor-1 Receptor. **MCL:** mantle cell lymphoma. **pTEN:** Phosphatase and Tensin Homolog. **p21:** also known as p21WAF1 or CDKN1A. **p53:** a tumor suppressor protein. **Cyclin D1:** a protein involved in the regulation of the cell cycle. **PE:** Phycoerythrin, is a fluorescent dye commonly used as a label for annexin V in this staining method. **7-ADD:** 7-Aminoactinomycin D, is a fluorescent dye. **TNF:** tumor necrosis factor. **CD137:** also known as 4-1BB, is a member of the TNF superfamily of cell surface receptors. **MSP assay:** methylation-specific PCR assay. **mTOR:** mammalian target of rapamycin. **ERK:** extracellular-signal regulated kinase. **FLIP:** FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein. **Th1:** Th1 cells, or Type 1 T helper cells, are a subset of T helper cells that play a central role in cellular immunity. **UCC:** urothelial cancer cell lines. **AML:** acute myeloid leukemia. **HIF-1α:** hypoxia-inducible factor 1-alpha. **MCL:** mantle cell lymphoma. **STAT3:** signal transducer and activator of transcription 3. **DLBCL:** diffuse large B cell lymphoma. **SCLC:** small cell lung cancer. **CPP32:** another name for Caspase-3. **RSCL:** rituximab–chemotherapy-sensitive. **RRCL:** rituximab–chemotherapy-resistant cell lines. **PEG-PLGA:** polyethylene glycol-poly(lactic-*co*-glycolic acid). **SRB assay:** sulforhodamine B assay. **NSCLC:** human non-SCLC. **Hoechst 33258:** a synthetic fluorescent dye that binds specifically to DNA molecules. **ROS:** reactive oxygen species. **HSP90:** heat shock protein 90. **EGFR:** epidermal growth factor receptor. **ADCP:** antibody-dependent cell-mediated phagocytosis. **PI3K:** phosphoinositide 3-kinase.

Histone modifications play an important role in regulating the active and inactive states of chromatin, which are responsible for determining gene expression [20]. Epigenetic abnormalities in cancer cells have been associated with specific histone modifications (deregulation, methylation, and acetylation) [21,22]. Cancer cells are often characterized by the absence of methylation and acetylation of particular residues in the core histones H3 and H4 [21,22]. Several enzymes are involved in these histone modifications, including E3-ubiquitin and kinases, HDAC, histone acetyltransferase (HAT), histone methyltransferase (HMT), and histone demethylase (HDMT) [23].

The active and open conformation of chromatin is associated with histone acetylation, while the inactive and condensed form is associated with deacetylation [24]. Histone acetylation regulation is dynamic and is governed by HAT and HDAC enzymes. HAT enzymes add acetyl groups to histones, leading to a more relaxed chromatin structure by disrupting the electrostatic interaction between histones and DNA [25]. This alteration affects gene assembly and transcriptional activity. Various cancers show irregularities in histone acetylation levels [26].

Abnormal function of HDAC proteins can lead to inappropriate deacetylation and inhibition of TSGs. Additionally, the process of gene transcription can be modulated by HDACs through the deacetylation of DNMTs, HATs, and other HDACs, which are all involved in various epigenetic processes [27]. In hematological malignancies, HDACs were first observed to form inappropriate complexes, leading to the recognition of the importance of HDACs in cancer development [28,29].

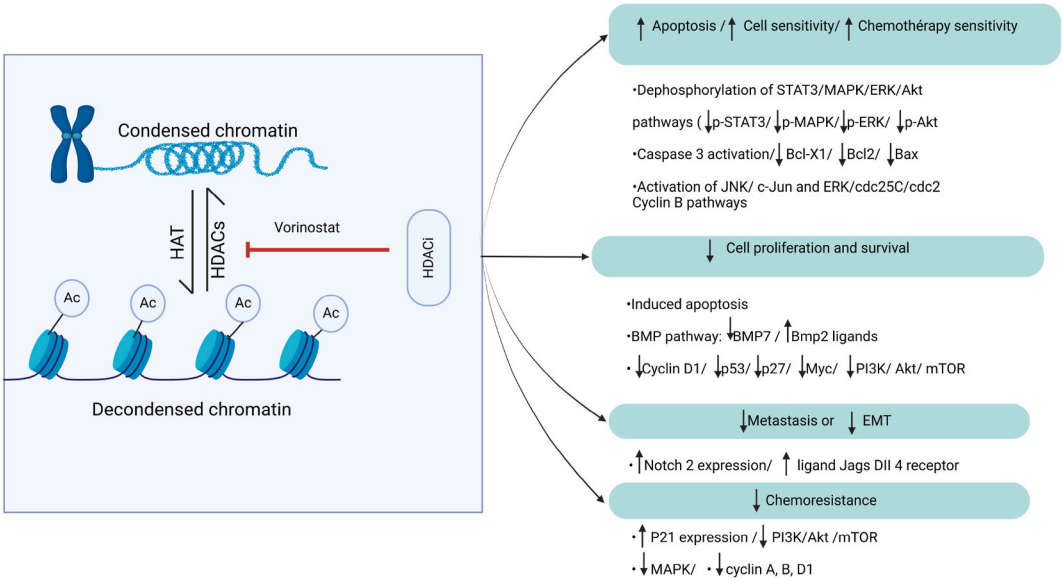

Fig. 2. Molecular targets are activated and/or inhibited indirectly through HDAC inhibition by vorinostat. *The inhibition of HDAC by Vorinostat induces several changes in different mechanisms related to anticancer effects. These mechanisms include: 1) an increase of apoptosis, cell sensitivity, and chemotherapy sensitivity, 2) a decrease of cell proliferation and survival, 3) a decrease of metastasis and EMT, 4) a decrease of chemoresistance.* **Abbreviations:** JNK: Jun N-terminal kinase; BMP: Bone morphogenetic protein; PI3k: phosphatidylinositol-3 kinase; mTOR: mammalian/mechanistic target of rapamycin; EMT: Epithelial–mesenchymal transition; HDAC: Histone deacetylase; HAT: Histone Acetyltransferases; STAT: signal transducer and activator of transcription; Mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; Akt: protein kinase B.

To date, very few mutations have been observed in tumors modifying the expression and/or activity of HDACs. However, irregularities in HDAC activity have been linked to carcinogenesis and abnormal gene expression. Multiple investigations have indicated that HDAC1 is overexpressed in various carcinomas, such as prostate, colon, gastric, and breast cancers [30–33], whereas HDAC2 is overexpressed in gastric, cervical, and colorectal cancers [34–36]. Overexpression of HDAC3, HDAC2, and HDAC1 is related to a poor prognosis for patients with ovarian and gastric cancers. In contrast, HDAC6 was found to be highly expressed in breast cancer specimens [37]. Additionally, overexpression of HDAC8 has been observed in neuroblastomas, while gastric cancers have been found to exhibit low levels of HDAC4 [38,39].

The development of several human cancers, especially leukemias, due to a defect in stem cell differentiation is attributed to the overexpression or abnormal functioning of HDACs. HDACs are involved in cancer development through various mechanisms, such as apoptosis and cell cycle arrest, differentiation, DNA damage, metastasis, angiogenesis, and autophagy [40,41].

3. Cellular and molecular mechanisms of vorinostat

The clinical application of vorinostat as an HDACi in human cancers has been highly successful. This clinical efficacy appears to be linked to the underlying mechanisms of HDAC inhibition. Understanding these mechanisms could enhance our understanding of tumor cell behavior and aid in the clinical use of vorinostat. The upcoming sections will illustrate the chronology of molecular events through a historical scenario.

This drug was the first HDACi approved in October 2006 b y the FDA to treat CTCL. Since this year, several investigations have been conducted *in vitro* and *in vivo* to better elucidate the mechanisms underlying the anti-cancer potential of this type of drug and to improve the sensitivity of human tumor cells to the therapeutic strategies already developed to kill these cells with the induction of DNA damage and/or apoptosis. Indeed, Table 1 summarises all *in vivo* and *in vitro* investigations of the anticancer mechanisms involved in HDAC inhibition by vorinostat.

In 2006, Munshi et al. [14] assessed the interaction of this drug with radiation in the treatment of human melanoma cells (A375, A549, and MeWo) and the underlying mechanisms. Indeed, vorinostat increased histone H4 acetylation, enhanced the radio-response of the three tumor cells, and increased their susceptibility to radiation-mediated apoptosis. Indeed, Western blot analysis detected decreased repair gene (*Rad50*, *Ku80*, and *Ku70*) expression in A375 cells. In addition, combined vorinostat and radiation prolonged the expression of γ-H2AX, a DNA repair protein (Fig. 2).

In contrast, evaluation of the anticancer effect of vorinostat alone (*in vitro*) on six pancreatic cancer cells showed dose-dependent growth inhibition of all cells tested, induction of cell cycle arrest at G_2 phase and apoptosis, as well as increased expression of tumor suppressor genes (*C/EBPα*, *E-cadherin*, *RARα*, and $p21^{WAF1}$) [42]. In addition, vorinostat was observed to induce histone H3 acetylation while simultaneously suppressing the expression of growth-associated genes such as cyclin B1, cyclin D1, and c-myc (Fig. 2).

The inefficacy of FDA-approved cancer treatments has made it necessary to develop molecularly targeted therapies in combination. In the case of colon cancer, vorinostat in combination with a proteasome inhibitor, bortezomib, was evaluated *in vitro* on two CRC cell lines (HT29 and HCT116) [15]. After this combination, a reduction in cell proliferation was noted with increased apoptosis, confirmed by cleaved poly(ADP-ribose) polymerase (PARP) and caspase-3/7 activity, as well as an elevation in the number of cells arrested in the G_2/M phase as well as the pro-apoptotic protein BIM.

On other CRC cell lines (mut- and wt-p53), Di Gennaro et al. [43] assessed *in vitro* the effect of vorinostat alone or combined with two chemotherapy drugs widely used in the treatment of this cancer, 5-fluorouracil (5-Fu) or raltitrexed (RTX). Therefore, vorinostat overcame the problem of resistance to both chemotherapeutics with the production of a synergistic effect associated with cell cycle disruptions and S-phase arrest. Moreover, this HDACi enhanced the effects of 5-Fu and RTX *via* down-modulation of thymidylate synthase (TS), an enzyme involved in cell growth and DNA replication that is the target of 5-Fu. In wt- and mut-p53 cells, vorinostat up- and down-regulated p53, respectively.

A year later, another study by the same research team using the same cells (*in vitro*) and an animal model (*in vitro*) confirmed the synergistic effect of the combination therapy [44]. Indeed, the combination of vorinostat with capecitabine, an anticancer drug used for colon cancer therapy, increased apoptotic cell death and anti-proliferative effect (*in vitro*) and inhibited tumor growth (*in vivo*), with prolonged survival in treated mice compared to the group receiving single-drug treatment. Additionally, vorinostat down-regulated TS in cancer cells and up-regulated thymidine phosphorylase (TP) (*in vivo* and *in vitro*). Overall, combination therapy with vorinostat and other chemotherapeutic agents can be a colossal option in the clinical treatment of CRC.

To analyze the individual impact of vorinostat on cytokine profiles (cell signaling molecules) and regulated genes in various tumor types. Zhang et al. [81] recently performed an *in vitro* study. Results showed a significant increase in the secretion of cytokines promoting anti-tumor immunity in glioblastoma (GBM) and colorectal cancer (CRC).

On the other hand, since no promising chemotherapeutic strategy was suggested for relapsed small cell lung cancer (SCLC), Bruzzese et al. [16] combined the effect of an HDACi (vorinostat) with a topoisomerase-I inhibitor (topotecan), which is the only chemotherapy drug registered for the management of this type of cancer. In SCLC cells, vorinostat alone induced a potential cytotoxic effect and potentiated topotecan-induced DNA damage, whereas the combination of these drugs increased the production of reactive oxygen species (ROS), induced apoptosis, and cell cycle arrest in S phase.

The same authors evaluated the effect of another combination between vorinostat and an epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), gefitinib, against squamous cell carcinoma of the head and neck (SCCHN), including gefitinib-resistant cells (KB and Hep-2) and epithelial CAL27 cells [45]. They found that this combination induces apoptosis as well as inhibition of SCCHN cell invasion, migration, and proliferation. This synergy has been attributed to the reversal of epithelial-mesenchymal transition (EMT) as well as modulation of ErbB receptors by vorinostat. Additionally, down-regulation of the expression of all three

epithelial CAL27 cell receptors (ErbB2, ErbB3, and EGFR) was recorded with vorinostat. In gefitinib-resistant cells, the HDACi reversed the mesenchymal phenotype by down-regulating ErbB2, EGFR, and vimentin and inducing both ErbB3 and E-cadherin. In CAL27 cells, vorinostat has been found to induce ErbB2 ubiquitination, leading to subsequent proteasome degradation.

Regarding natural substances, numerous preclinical investigations have shown that epigallocatechin-3-gallate (EGCG), the major catechin found in green tea, exhibits a variety of beneficial effects, including anticancer properties. With the aim of involving this molecule in a new chemotherapy strategy and overcoming the problem of resistance to conventional chemotherapeutic agents, Kwak and collaborators conducted a study as part of the *in vitro* evaluation of the impact of the combination of this natural substance with vorinostat on HuCC-T1 human cholangiocarcinoma cells [46]. This combination synergistically induced apoptosis and inhibited tumor cell growth while decreasing migration, matrix metalloproteinase (MMP) expression, and invasion of tumor cells with decreased PARP expression and increased caspase-3 and -7 activity as well as *Bax/Bcl-2* expression compared to treatment with each component alone (Fig. 3).

The same authors performed a second study with the objective of evaluating the anticancer potential of nanoparticles incorporated into vorinostat (vorinostat-NPs); *in vitro*, against the same tumor cells (HuCC-T1 cells) and *in vivo* against mice carrying these cells [47]. The vorinostat-NPs were prepared using a biodegradable copolymer. *In vitro*, these vorinostat-NPs showed anti-tumor effects similar to those of vorinostat alone in terms of inhibition of HDAC expression, apoptosis, and tumor growth. However, *in vivo*, they exerted an improved anticancer effect compared to vorinostat, with suppression of HDAC expression in cancerous tissue and an increase in that of acetylated histone H3, whereas nanoparticles alone showed no effect. This suggests that these nanoparticles can serve as a vehicle in HDAC-targeted chemotherapy in cholangiocarcinoma.

On the other hand, the effect of this HDACi on multiple myeloma (MM) has been investigated (*in vivo* and *in vitro*) as well as its impact on the antimyeloma activity of two chemotherapeutics, melphalan and bortezomib, used in MM treatment [48]. Indeed, the anti-MM effect of these two agents was enhanced by vorinostat (*in vivo* and *in vitro*). Moreover, the concurrent administration of vorinostat (100 mg/kg) with melphalan (3 mg/kg) exhibited a pronounced suppression of tumor growth (*in vivo*), surpassing the effects observed with the individual administration of each drug.

This type of malignant cells (RPMI 8226 MM cells) was among others that were used in another study to determine the protective responses initiated by HDACi and their role in enhancing the anticancer activity of HDACi [49]. CD146 is a molecule widely expressed in many endothelial cells and tumors. In this study, the targeting of this molecule by an anti-CD146 antibody (AA98) enhanced the killing effect of vorinostat in cancerous cells by blocking the activation of AKT pathways. Moreover, the combination of this HDACi and AA98 inhibited *in vitro* angiogenesis, as well as metastasis and *in vivo* tumor growth. Altogether, we can propose a novel strategy combining vorinostat and targeting CD146 to kill tumor cells more efficiently.

Currently, it is evident that the main target of HDACs involves histone acetylation, however, other (non-histone) substrates may be involved, namely Hsp90, which may be responsible for the anticancer effect of HDACs. Glucose-regulated protein 78 (GRP78), belonging to the Hsp70 family of heat shock proteins, has been shown to be implicated in several tumor cell processes such as immune evasion, cell proliferation, metastasis, apoptosis resistance, and angiogenesis [82]. Indeed, Kahali et al. [51] showed that GRP78 is vorinostat biological target and that activation of the unfolded protein response (UPR) *via* phosphorylation of protein-like endoplasmic

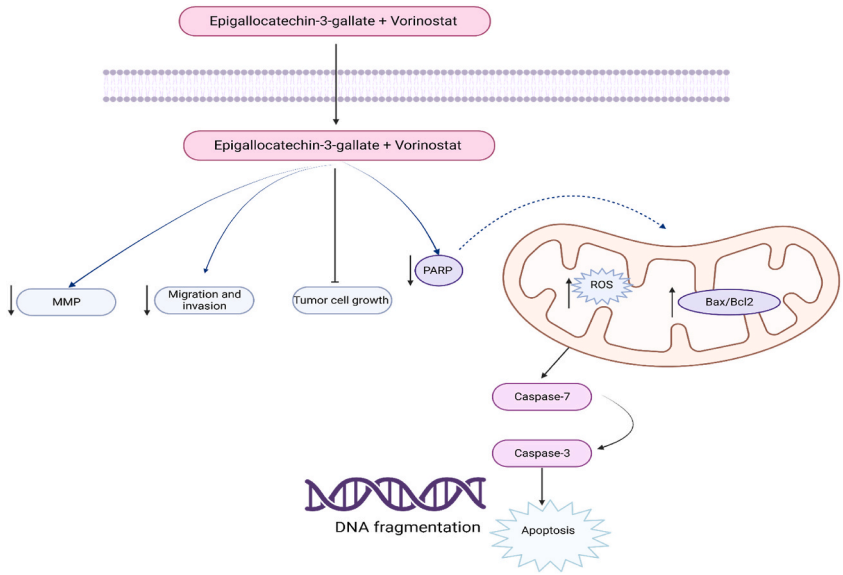

Fig. 3. Anticancer actions of vorinostat combined with epigallocatechin-3-gallate. *Vorinostat combined with EGCG can decrease MMP, migration and invasion, and PARP expression, and inhibits tumor cell growth. Both molecules induce indirectly an increase ROS production and Bax/Bcl2 ratio in mitochondria which induce the expression of caspase-7, and caspase-3 and therefore and apoptotic action of cancer cell.* **Abbreviations:** MMP: matrix metalloproteinase; ROS: reactive oxygen species; PARP: poly-ADP ribose polymerase; Bcl-2: B-cell lymphoma protein 2; Bax: Bcl-2-associated X.

reticulum kinase (PERK) contributes to its anticancer potential.

Furthermore, in nine renal cell carcinoma (RCC) cell lines (*in vitro*) and two animal models of RCC (*in vivo*), treatment with vorinostat enhanced the anti-tumor effect of temsirolimus, a drug used in the inhibition of mammalian target of rapamycin (mTOR) pathway [52]. While the combination of both drugs potentially decreased survivin levels and therefore induced apoptosis and reduced angiogenesis.

In the same year, an Australian research team was interested in the treatment of uterine sarcoma given the absence of approved chemotherapy protocols [50]. In fact, on human uterine sarcoma cell lines (MES-SA), *in vitro* treatment with vorinostat (3 μM) effectively inhibited cell growth, decreased cell survival, and blocked colony-forming ability. In addition, the expression of HDAC enzymes (class I and II) and $p21^{WAF1}$ was influenced by vorinostat. A 21-day treatment with this molecule at a dose of 50 mg/kg/day in mice injected with MES-SA cells reduced tumor growth (>50 %) by activating apoptosis.

As already proven in the investigation conducted by Munshi et al. [14], the interaction of vorinostat with radiotherapy gave promising results regarding the treatment of human melanoma by radio-sensitizing cancer cells. In 2011, this interaction was used in neuroblastoma treatment (*in vivo* and *in vitro*) [53]. In neuroblastoma cell lines NB-1691, vorinostat decreased cell viability with additive effects with radiotherapy and down-regulated expression of DNA repair enzyme, Ku-86. While the administration of this molecule to mice with metastatic neuroblastoma under radiation, reduced tumor volumes compared to each monotherapy. Furthermore, the antineoplastic activities of radiation were potentiated, which was strongly attributed to the reduction in Ku-86 expression.

Another therapeutic option was proposed in lung cancer cell treatment, combining vorinostat and adenovirus (ad)-TRAIL, known for its anticancer potential by inducing apoptosis [54]. The results showed that this combination exerts synergistic anti-cancer effects by inducing increased apoptosis, degrading Bcl-2 (anti-apoptotic molecule), and suppressing NF-κB activation. In addition, vorinostat alone increased the expression of TRAIL from ad-TRAIL *via* its transduction enhancement by increasing transcription of the adenoviral transgene and enhancing expression of the Coxackie adenoviral receptor (CAR). Concerning lung cancer, currently, the most advanced treatment is based on the use of immunotherapeutic inhibitors, in particular monoclonal antibodies which specifically target PD1 and its ligand, PD-L1 [83]. This treatment has demonstrated remarkable clinical effectiveness by blocking anti-apoptotic signals, which may lead to inhibition of tumor growth and improved patient survival [84]. However, despite the progress made, challenges remain to optimize therapeutic outcomes. A promising approach is to combine these immunotherapeutic inhibitors with other therapeutic agents to enhance their effectiveness. In this context, the use of HDACis such as vorinostat could play a crucial role. Consequently, in combination with anti-PD1/PD-L1 monoclonal antibodies, vorinostat could potentiate the apoptotic action of antibodies, thus leading to increased suppression of cancer cell proliferation and better therapeutic response in patients with of lung cancer. Furthermore, it is important to note that cancer cells and immune cells interact *via* IFN-γ, which is involved in promoting tumor cell apoptosis [85]. However, this apoptotic action is often counteracted by anti-apoptotic signals, such as PD1 and its ligand PD-L1, which are overexpressed in tumor cells, thereby promoting immune evasion and tumor progression [86]. The combination of HDAC inhibition with immunotherapeutic inhibitors therefore represents a promising approach to improve the treatment of lung cancer by enhancing the apoptotic activity of anti-PD1/PD-L1 monoclonal antibodies. This strategy could potentially overcome tumor resistance mechanisms and improve clinical outcomes for patients with this disease.

In the evaluation of the anticancer potential of vorinostat against human endometrial cancer, Sarfstein et al. [55] treated cells of this cancer (endometrioid and uterine serous papillary) with vorinostat and/or insulin-like growth factor (IGF)-I. After *in vitro* treatment with vorinostat, the authors recorded an increase in phosphorylation of the IGF-I receptor (IGF-IR), a production of histone H3 acetylation, a diminution in cyclin D1 and p53 levels, an up-regulation of pTEN expression, a decrease in colony-forming capability, and an induction of apoptosis in both cell lines.

Current therapeutic modalities have shown poor efficacy against mantle cell lymphoma (MCL), a B-cell cancer, prompting Xargay-Torrent et al. [56]to uncover the molecular mechanism involved in vorinostat-induced apoptosis. In fact, in 8 MCL cell lines, vorinostat treatment induced cell death *via* mitochondrial apoptosis activation associated with histone H4 hyperacetylation and transcriptional activation of some proapoptotic BH3-only genes (*NOXA*, *BMF*, and *BIM*). Up-regulation of BH3-only protein transcription was identified as one of the anti-tumor molecular mechanisms of vorinostat implicated in MCL treatment.

Moreover, MCL therapy was reinforced with another study performed after 4 years using another approach [69]. Indeed, Lu et al. [69] assessed *in vitro* the anti-tumor effect of the combination of vorinostat with WP1066, an inhibitor of signal transducer and activation of transcription 3 (STAT3), on MCL cells. In fact, the activation of the STAT3 pathway is observed in various subtypes of MCL tumors. The results showed that this combination synergistically inhibits cell growth as well as STAT3 activation, modulates pro- and anti-apoptotic gene expression, and induces apoptosis.

From the previous results, it was deduced that among the cytotoxic mechanisms of action of vorinostat is the induction of apoptotic genes. This has been verified *in vitro* on human mammary adenocarcinoma cells (MCF7 and MDA-MB-231) and *in vivo* on mice xenografted with these cell lines [57]. Depending on the cell line type, vorinostat activated different apoptotic genes and promoted CD137 receptor/ligand system expression. On MDA-MB-231 cells, the combination of this HDACi with the soluble CD137 receptor showed a synergistic cytotoxic effect. This study is the first to propose the combination of vorinostat with the CD137 receptor, belonging to the TNF superfamily, as a new anticancer approach.

Concerning prostate cancer (PC), it represents the most abundant type of cancer in men, often in the elderly [87]. The use of natural substances has taken a considerable place in cancer therapy. Indeed, genistein, a soy isoflavone, has shown promising results regarding gene expression/survival and cell proliferation, as well as demethylation of hypermethylated DNA, making it a major candidate in many new therapeutic approaches against PC. The combination of this isoflavone with vorinostat in an *in vitro* treatment against three PC cells induced cell death, particularly at an early stage, and affected several genes and pathways namely androgen signaling pathways, G_2/M cell cycle arrest, TNF, and Wnt [58]. Additionally, as noted above, Hsp90 plays a crucial role in maintaining the

conformation and stability of proteins [82], including the AR (androgen receptor), under stressful cellular conditions. Its activity is essential to ensure that client proteins, such as AR, retain their functional structure, which is particularly relevant in the context of PCs where AR is often overexpressed and implicated in tumor progression [88]. However, recent studies have demonstrated that acetylation of Hsp90, notably through epigenetic mechanisms, can disrupt its chaperone function [89]. Acetylation changes the electrical charge and structure of Hsp90, which affects its ability to interact with its co-chaperones and, therefore, stabilize client proteins such as AR. This disruption may lead to dysregulation of the AR signaling pathway and contribute to tumor progression. Interestingly, destabilization of Hsp90 due to its acetylation can have a direct impact on the structure and function of AR in PC cells [90]. Due to its dependence on Hsp90 for its functional conformation, AR may undergo increased degradation or altered activity in the presence of acetylated Hsp90. This may lead to decreased transcriptional regulation of AR target genes involved in the growth and survival of PC cells.

In order to test the potential of vorinostat on the growth of skin neoplasms [59], treated A431 human epidermoid carcinoma cells *in vitro* with a dose of 2 μM of this HDACi as well as mice xenografted with these cells *in vivo* with a dose of 100 mg/kg bw. *In vitro*, vorinostat decreased the expression of HDAC1, 2, 3 and 7 and augmented the acetylation of histones H3 and p53, whereas *in vivo* it reduced tumor growth and down-regulated the expression of cyclins A, D1, D2, and E. Interestingly, this substance induced apoptosis, which was attributed to inhibition of the mTOR signaling pathway associated with decreased activation of AKT pathways and extracellular-signal regulated kinase (ERK). As we indicated in a very recent study, targeting the mTOR pathway may constitute a promising therapeutic avenue against cancer, since it is involved in several tumor processes, namely cancer cell proliferation, cell cycle, and apoptosis [91].

In contrast, evaluation of the anticancer activity of vorinostat against endometrial cancer showed multiple beneficial effects *in vitro,* such as induction of apoptosis, growth arrest, and clonogenic growth loss of endometrial cancer cells, as well as activation of caspase-8 and -9 [60]. In addition, inhibition of caspase-8 decreased clonogenic capacity and tumor growth *in vivo*. More interestingly, the combination of both treatments (vorinostat with caspase-8 inhibition) accentuated the effects observed with the two separate treatments (*in vivo* and *in vitro*). This study provides a new, effective therapeutic strategy combining HDACi and caspase-8 targeting endometrial cancer.

Despite the widespread clinical use of vorinostat since 2006 as an anti-tumor drug, the mechanisms underlying its effects on inflammatory diseases of the central nervous system (CNS) remain unclear. For this reason, Ge et al. [61] tested the *in vitro* effect of vorinostat on human dendritic cells (DCs) derived from $CD14^+$ monocytes and immature mouse DCs as well as *in vivo* on a model of experimental autoimmune encephalomyelitis (EAE). Results showed inhibition of human DCs derived from $CD14^+$ monocytes and decreased CNS demyelination and inflammation (*in vitro*) with enhancement of EAE *in vivo*, indicative of the immunosuppressive and anti-inflammatory properties of the vorinostat.

In order to avoid chemoresistance in children suffering from pediatric malignancies, the development of new anti-tumor agents has become a necessity. Muscal et al. [62]suggested combining vorinostat with a selective Aurora A kinase inhibitor, MLN8237, to achieve enhanced anticancer effects against pediatric neuroblastoma, medulloblastoma, and leukemia cells. Therefore, in a dose-dependent manner, vorinostat inhibited the survival of all tumor cells, whereas its combination with MLN8237 induced an additive cytotoxic effect on the three cancer cells with enhancement of the effect of MLN8237.

In the same year, Niegisch et al. [63] noted several anticancer effects of this HDACi alone on urothelial cancer cell lines (UCC), namely down-regulation of HDAC-4, -5 and -7 mRNA expression and up-regulation of HDAC-2 and -8 mRNA expression, with an increase in the sub-G_1 fraction, an induction of cell-cycle arrest at G_2/M, and a down-regulation of TS.

Another therapeutic model was suggested in the study conducted by Stark et al. [64], combining vorinostat with an anticancer agent that also acts as an aryl hydrocarbon receptor (AhR) ligand, aminoflavone (AF), in the treatment of mesenchymal-like triple--negative breast cancer (TNBC), Hs578T and MDA-MB-231. An AF pro-drug, AFP464, was used for this combination. These cells were resistant to AFP464, whereas vorinostat treatment sensitized them *in vitro* with potentiation of the anticancer effect of AFP464 *in vivo*. Reactivation of estrogen receptor α (ERα) was behind the sensitizing effect of this HDACi.

The development of new anti-tumor drugs and new therapeutic combinations is increasingly in demand given the strong emergence of cancers and the high mortality rates associated with them. Tran et al. [65] have suggested *in vivo* treatment of estrogen receptor (ER)-negative breast cancer by combining vorinostat with synthetic triterpenoids, CDDO-Me and CDDO-Ea, two classes of cancer treatment and prevention drugs [92]. As a result, vorinostat with both triterpenoids synergistically inhibited nitric oxide (NO) synthesis in macrophage cells, RAW 264.7, and in mouse peritoneal macrophages. In fact, triterpenoids are multifunctional agents targeting multiple cells, especially macrophages [93] and also act as anti-inflammatory agents [92,94]. In addition, tumor formation was delayed *in vivo* by synthetic triterpenoids potentiated by the effect of vorinostat. At a dose of 250 mg/kg, this HDACi delayed tumor development by 2 weeks and inhibited (alone or combined with CDDO-Me) tumor-related macrophage infiltration in mouse mammary glands.

As previously demonstrated in the study conducted by Di Gennaro et al. [43], vorinostat combined with two anticancer drugs showed promising anticancer activity against CRC *in vitro*. In the same context, Wilson et al. [66] evaluated the anti-tumor efficacy (*in vivo* and *in vitro*) of two HDACis, vorinostat and panobinostat, against CRC. After 3 days of treatment, panobinostat and vorinostat showed important anti-proliferative effects, with IC_{50}s of 5.1–17.5 nmol/L and 1.2–2.8 μmol/L, respectively. In addition, both HDACis reduced colony formation. Interestingly, panobinostat increased acetyl-H3 and down-regulated TS (*in vivo*). Despite the remarkable beneficial effects of treating CRC cells with these HDACis, cells showed high survival potential at HDACi doses and at treatment durations exceeding those achieved in the clinic. This encourages researchers to improve the therapeutic effect of these HDACis alone or combined against solid tumors.

Indeed, a year later, Lin et al. [67] combined vorinostat with BPR1J-340, an FLT3 inhibitor with strong antitumor effects, in acute

myeloid leukemia (AML) therapy. In MOLM-13 AML cells, they found that the association of these two agents induces apoptosis *via* Mcl-1 down-regulation.

Hypoxic conditions trigger the development of adaptive mechanisms in cancer cells, allowing them to survive. One of the key players in this hypoxic response is the transcription factor (HIF-1), which promotes several survival pathways, including angiogenesis. Regulation of a component of the HIF-1 heterodimer, HIF-1α, occurs primarily at translation, and its expression is implicated in tumor survival, making its targeting a promising strategy for cancer therapy. HDACis have been shown to inhibit angiogenesis *via* HIF-1α degradation [95], however, the underlying molecular mechanisms need to be further characterized. Indeed, in liver cancer cell lines, Hutt et al. [68] showed that vorinostat inhibits HIF-1α expression *via* inhibition of translation, independent of proteasome degradation, autophagy, and p53.

On the other hand, an *in vitro* combination between vorinostat and bendamustine, an anticancer drug used in chronic myeloid leukemia (CML) therapy, has been suggested to treat diffuse large B-cell lymphoma (DLBCL) more effectively [70]. Interestingly, a synergistic cytotoxic effect was recorded with an improvement in DNA double-strand breaks and histone acetylation.

Another combination associating vorinostat with cisplatin was investigated (*in vitro* and *in vivo*) in SCLC therapy [71]. Indeed, this *in vitro* association reduced cell viability, induced apoptosis, promoted cell cycle arrest, reduced TS expression, and inhibited cell growth. It also showed elevated levels of α-tubulin and histone H3 acetylation compared to vorinostat alone, while this association *in vivo* inhibited tumor growth by 20.5 %. These beneficial effects were linked to the ability of this combination to relax chromatin structure and therefore improve cisplatin accessibility and cytotoxicity. With the aim of confirming the synergistic anticancer effect of this combination, as well as verifying its safety and effectiveness, a more recent study was carried out through experiments on three prostate cancer cell lines (PC-3, C4–2B, and DU-145) and on animal models [96]. The results showed that the combination of vorinostat and cisplatin has an increased cytotoxic effect by causing more DNA damage to cancer cells. Interestingly, vorinostat reduced the expression of some proteins involved in DNA repair, as well as enzymes involved in glutathione (GSH) biosynthesis and an enzyme (GSTP1) that facilitates the binding of GSH to cisplatin. Additionally, the level of GSH inside cells decreased with increasing vorinostat concentrations, as did the concentration of intracellular platinum (Pt) elements.

Another study carried out in the same year evaluated the combined use of vorinostat with decitabine, a DNA methylation inhibitor (DNMTi), in the treatment of hepatocellular carcinoma (HCC) [97]. Indeed, the association of these two epigenetic modulators presented a synergistic effect, with inhibition of cell proliferation and induction of apoptosis compared to each drug administered alone. Moreover, the combination induced autophagy as an early event, but this induction waned after three days. This suggests that this epigenetic modulation had anticancer activity by coordinating the crosstalk between autophagy and apoptosis, thereby promoting the cell death of HCC cancer cells.

In monotherapy, *in vitro* treatment with vorinostat of primary tumor cells and rituximab-chemotherapy resistant cell lines (RRCL) induced cell death and increased histone H3 acetylation, inducing G_1-phase cell cycle arrest [72]. This treatment also induced apoptosis in rituximab-chemotherapy-sensitive cell lines (RSCL) but not in RRCL; suggesting the involvement of alternative pathways in the induction of RRCL death by vorinostat, especially irreversible cell cycle arrest.

Considering the low absorption and solubility of vorinostat, consequently decreasing its therapeutic potential, the development of novel methods improving its biopharmaceutical properties has increased in the last few years. Among these methods is nano-formulation; encapsulation in PEG-PLGA copolymer micelles, which were used by Rompicharla et al. [73] against murine melanoma (B16F10) (*in vivo* and *in vitro*) and human breast cancer (MDA MB 231) (*in vitro*) cell lines. Consequently, vorinostat-loaded PEG-PLGA (Vorinostat-PEG-PLGA) micelles exhibited superior apoptotic activity and enhanced cytotoxic effects compared to free vorinostat. After 24 h MDA MB 231 cell treatment with Vorinostat-PEG-PLGA micelles, the percentage of cell destruction was approximately 55 % compared to 36 % for the free agent. Similarly (*in vivo*), a reduction in tumor volume in animals bearing B16F10 tumor cells was recorded following treatment with Vorinostat-PEG-PLGA micelles, showing a 1.78 fold tumor suppression compared to the group treated with a free drug. From these findings, we can see that this nano-formulation can constitute an effective therapeutic option in the case of solid tumors.

In the same context, and to overcome the problem of drug resistance, a Chinese research team combined vorinostat with paclitaxel (PTX) to synthesize co-prodrugs conjugated by succinic acid (***1a***) and glycine (***1b***), respectively [74]. In fact, PTX is a molecule used in cancer chemotherapy, especially breast cancer; however, its clinical use was limited due to its low selectivity and high resistance. The results of this investigation showed that both co-prodrugs exhibit high cytotoxicity with the induction of cell cycle arrest. Additionally, using the thin film technique, ***1b*** was converted into nanomicelles to serve as a carrier. Indeed, ***1b*** nanomicelles released the drug in a prolonged way, which reduced the resistance to PTX, with a cytotoxic effect comparable to or even better than that of PTX. These findings add to those of the previous study and suggest that vorinostat-PTX co-prodrug nanomicelles are an effective therapy.

Furthermore, the anti-tumor activity of the association of vorinostat with gefitinib, which has already been investigated in the study performed by Bruzzese et al. [45], was re-evaluated in another study in human non-SCLC (NSCLC) cells with the aim of reversing EGFR-TKI resistance in this form of lung cancer [75]. In PC9 gefitinib-resistant (PC9GR) cancer cells and parental PC9 cells, this combination promoted cell death *via* apoptosis activation more effectively than gefitinib alone and induced Hsp90 cleavage with reduced levels of its clients (AKT, MET, and EGFR).

To further promote these antitumor responses against NSCLC, Tu et al. [79] proposed a new therapeutic strategy combining this HDACi with simvastatin, a novel drug against breast cancer [98], by co-delivering a system of deformable liposomes (D-Lipo); with the aim of remodeling the tumor microenvironment (TME), implicated with its main constituents, the tumor-associated macrophages (TAM), in the development of NSCLC. This application inhibited tumor growth *in vivo* and enhanced the potential for intratumoral infiltration *in vitro*, through anti-angiogenesis, repolarization of TAM (from the M2 to M1 phenotype), and consequently TME remodeling. This decreased the amounts of regulatory T cells (Tregs) and M2 pro-tumor macrophages and increased those of cytotoxic

$CD8^+$ T cells and M1 anti-tumor macrophages.

In another context, the 5-fluorouracil/cisplatin (CDDP) regimen is among the most commonly used therapeutic strategies with good anti-tumor responses. However, it is often linked to treatment resistance and high toxicity. To improve the effectiveness of this association, Piro et al. [76] combined it with vorinostat in squamous cell cancer models. *In vitro* results of this combination showed synergistic pro-apoptotic and anti-proliferative effects on DNA damage and cell cycle disruption. These outcomes were confirmed *in vivo*. In addition, EGFR nuclear translocation and phosphorylation produced by this regimen were reversed by vorinostat mechanistically, subsequently altering cyclin D1 and TS. The authors of this study attributed these effects to the induction of lysosomal-mediated EGFR degradation and platinum uptake *via* up-regulation of the expression of the copper transporter CTR1. Indeed, this transporter is involved in the absorption of all drugs containing platinum [99]. These are the first outcomes on the inhibitory potency of vorinostat towards two mechanisms of CDDP resistance, namely overexpression of the copper transporter CTR1 and nuclear translocation of EGFR, as well as on the mechanism of the combination between CDDP- and HDACi-based chemotherapeutic treatments. This study provides better justification for exploring this interaction in the clinic to avoid the obstacles of resistance to chemotherapeutic agents and dose-limiting toxicities.

On the other hand, chemotherapy for urothelial carcinoma, the most common type of bladder cancer (90 %), has so far shown low efficacy given the occurrence of recurrences and metastases, hence the need to develop more effective systematic therapies. The same is true for animals, especially dogs, treatment for this type of cancer is under development. Indeed, Eto et al. [77] evaluated (*in vitro* and *in vivo*) the anticancer activity of vorinostat on several cell lines of canine urothelial carcinoma (CUC). They noted inhibition of tumor cell growth (*in vitro* and *in vivo*), induction of G_0/G_1 cell-cycle arrest, up-regulation of p21, and acetylation of histone H3.

The antitumor mechanisms of HDACis have been shown to include immunostimulatory effects. However, their impact on the trastuzumab (TRA)-mediated anticancer immune response is not yet well elucidated. In fact, TRA is a monoclonal antibody (mAb) that has improved the treatment of patients with HER2-overexpressing cancers. Among the mechanisms of action of this mAb are antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis (ADCP). This impact was investigated for vorinostat on the human breast adenocarcinoma cell line SKBR3 [78]. Indeed, it enhanced TRA-independent cytotoxicity and TRA-mediated ADCP, down-regulated the anti-apoptotic protein MCL-1, and induced immunogenic cell death. From these data, it can be understood that among the major anticancer mechanisms of vorinostat are increased TRA-independent cytotoxicity and TRA-mediated phagocytosis.

Still in the context of properly characterizing the mechanisms of action of vorinostat against solid tumors, in particular the molecular mechanisms, a recent study evaluated the tumor-inhibitory capacity of this HDACi against cervical cancer *in vitro* and *in vivo*, focusing on the capacity of NK-92 cells to lyse tumor cells [80]. After a 24-h treatment with vorinostat, the authors recorded an inhibition of the invasion, migration, and proliferation of cancer cells, an induction of apoptosis with cell cycle arrest at phase S, an improvement of the NK cell response, and an up-regulation of MICA expression (*in vivo* and *in vitro*) that was associated with the PI3K/Akt signaling pathway. Furthermore, in order to deepen our understanding of the molecular mechanism of action of this HDACi in the treatment of cervical cancer, Pan et al. [100] implemented an innovative approach. They combined parallel reaction monitoring (PRM) technology with iTRAQ-based proteomics to precisely identify potential targets of vorinostat. The study identified 254 proteins differentially expressed in cervical cancer cells treated with vorinostat, which acted by reversing the epithelial-mesenchymal transition (EMT) by specifically targeting UBE2C and controlling cell cervical cancer proliferation via the ubiquitination pathway.

On the other hand, it has been pointed out that autophagy may be responsible for resistance to HDACis [101], a frequent problem in the management of solid tumors. Thus, the synergistic association of HDACis with autophagy inhibitors appears to be a promising therapeutic strategy aimed at increasing the effectiveness of antitumor treatment. To this end, Chen et al. (2023) [102] explored the use of zirconium dioxide (ZrO_2) nanoshells for co-delivery of these two drug classes, vorinostat and chloroquine, as autophagy inhibitors, despite their differences in physicochemical properties. This developed co-delivery system was capable of encapsulating both types of drugs at once, allowing their release in a controlled manner. Indeed, ZrO_2 nanoshells successfully encapsulated both drugs with well-controlled release. The autophagy inhibitory potential of the ZrO_2 nanoshells improved the efficacy of individual drugs (vorinostat and chloroquine) as well as their combination. Furthermore, this co-delivery system, in mice bearing 4T1 tumors, showed better autophagy inhibition and higher hyperacetylation compared to individually administered drugs, resulting in superior anticancer activity. Overall, this approach could potentially improve the effectiveness of solid tumor treatment by targeting two different resistance mechanisms.

Given that vorinostat was the first HDACi to receive FDA approval for the treatment of CTCL, it is imperative to develop a thorough understanding of the precise molecular mechanism by which this drug exerts its action against this lymphoma pathology. Therefore, a recent study was undertaken to evaluate its effect on cells involved in a common form of CTCL, mycosis fungoides (MF), characterized by the accumulation of malignant $CD4^+$ T lymphocytes in the skin [103]. In response to vorinostat treatment, a reduction in the expression of certain proteins in MF cells was recorded in both HH and Myla cell lines. However, it is important to note that the effect of vorinostat on other proteins and pathways varied between these two cell lines. Indeed, it induced an increase in the expression of certain cytokines in Myla cells, while it reduced them in HH cells, thus resulting in distinct modulations of the immune signaling pathways in each of these cell lines. These results suggest that vorinostat exerts divergent effects on MF cell lines, possibly due to intrinsic phenotypic variations, even though these lines both originate from MF patients.

To expand the understanding of the epigenetic effects of vorinostat in clinical oncology, Maksimova et al. [104] explored its impact on histone methylation, an important aspect of epigenetic regulation of the genome, focusing on several enzymes involved in this process. The results showed that in addition to inhibiting HDAC1, vorinostat also reduced the activity of several HMTs, including SUV39H1, EZH2, SUV420H1, and SUV39H2, as well as the expression of other enzymes involved in histone methylation, such as DOT1L and SUV420H2. This study suggests that vorinostat, in addition to its well-known role in inhibiting HDACs, impacts histone methylation by regulating several enzymes involved in this process. Understanding this epigenetic mechanism of action may

contribute to the more precise use of vorinostat in the treatment of tumors with an altered epigenetic profile. However, further research is needed to further explore the clinical implications of these findings.

As conclusion, the inhibitory effects of vorinostat induces various cellular, sub-cellular, and molecular changes which lead to a stochasticity and versality of molecular events leading indirectly to cancerous cell death and/or the arrest of cell cycle. These events involve the modulation of transcriptional networks mediated by chromatin changes after HDAC inhibiting (Fig. 4).

4. Anticancer clinical investigations of vorinostat

Vorinostat's potential in treating various cancers in preclinical studies has made evaluating its anticancer activity in clinical trials a crucial topic in oncology. Indeed, rigorous clinical trials are necessary to determine its efficacy and safety in this setting. These studies will aid in identifying the role of this HDACi in cancer therapy and identifying the patient populations that can benefit the most. Table 2 summarises clinical investigations carried out on Vorinostat as an anticancer treatment alone or in combination with other anticancer drugs.

Since its formal FDA approval in October 2006 as the first HDACi approved to treat CTCL [123], vorinostat's potential as an anti-cancer drug was assessed in several clinical investigations for various types of cancer. The preclinical data mentioned earlier reveals that vorinostat's anti-cancer properties are attributed to various mechanisms, whether following a single treatment, combined molecular targeted therapy, or combined radiation therapy. Indeed, a clinical study conducted in 2007 aimed to identify the recommended phase II doses (RP2Ds) of this HDACi when administered alone or combined with PTX and carboplatin in advanced solid tumor (AST) patients [105]. It should be noted that paclitaxel and carboplatin are commonly combined in chemotherapy to treat various types of cancer (lung, ovarian, and breast cancers). This combination disrupts cell division processes, leading to cancer cell death [124,125]. Both drugs have complementary mechanisms of action and can enhance each other's effectiveness when used together. The study findings showed that vorinostat is safe and tolerated at certain doses (300 mg bd or 400 mg qd), and when combined with other chemotherapeutics exerts encouraging antitumor activity in patients with previously untreated NSCLC. Anemia, thrombocytopenia, neuropathy, fatigue, diarrhea, and nausea were the prevalent toxicities observed. Consequently, the combined use of vorinostat, paclitaxel, and carboplatin constitutes a new approach to treating solid tumors.

The encouraging results of this study justify conducting phase II trials specifically targeting the disease. This was achieved in the same year in a phase II study investigating the potential of vorinostat in relapsed NSCLC patients who had failed to respond to more than one prior cytotoxic treatment [106]. A total of 14 patients were recruited, and although no objective anticancer response was noted in the first twelve evaluable patients, seven patients had stable disease (SD). Vorinostat was well tolerated, and the compliance rate was over 95 %. The study concludes that further investigations should be directed towards therapies combining vorinostat with other chemotherapeutics.

In their phase II clinical study, Blumenschein et al. [107] further assessed the efficacy and safety of vorinostat (400 mg p.o.) for the management of recurrent and/or metastatic SCCHN in twelve patients who did not tolerate or respond to conventional chemotherapy. Generally, the treatment was well tolerated, but some patients experienced drug-related side effects. Although no confirmed complete or partial response was reported, three cases of SD were observed. Based on these clinical data, vorinostat was not significantly effective as a single agent in this group of patients. However, its association with other treatments may be warranted, as demonstrated in preclinical studies. The findings of this study corroborate those obtained by Bradley et al. [108] following the same experimental

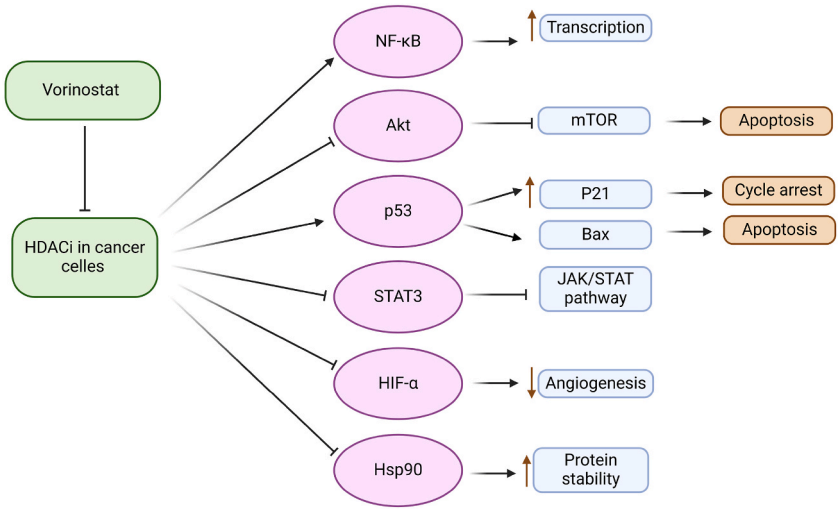

Fig. 4. Phenotype consequences of HDAC inhibiting by vorinostat in cancer cells. *The inhibitory effect of HDAC by vorinostat can induce the activation and/or the inhibition of different transcriptional factors like NF-κB, Akt, P53, STAT3, HIF-α, and Hsp90. Gene activation depending on these transcriptional factors can lead to protective phenotypes against cancer cells.* **Abbreviations:** STAT3: signal transducer and activator of transcription 3; *NF-κB*: Mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; Akt: protein kinase B.

Table 2
Clinical investigations of anticancer efficiencies vorinostat (clinic).

Methods	Key results	Mechanisms	Tumor phases	Ref.
Previously untreated NSCLC patients Orally administered vorinostat was prescribed either once daily for a duration of 14 days or twice daily for a week, with a three-week interval between doses Once every 3 weeks, Carboplatin and paclitaxel (PTX) were intravenously administered	Well tolerated combination Encouraging anticancer activity	Not determined	Phase I trial	[105]
Relapsed NSCLC patients 400 mg orally per day, with a cycle of 21 days	Well tolerated treatment >80 % of patients completing cycle 1 Compliance = 95.8 % During the treatment, vascular events were encountered by 4 out of 14 patients	Not determined	Phase II trial	[106]
Recurrent and/or metastatic head and neck cancer patients 400 mg of vorinostat taken orally on a daily basis	Well tolerated treatment A less effective activity compared to the tumor response observed in heavily pre-treated patients	Not determined	Phase II trial	[107]
Patients with metastatic castration-resistant prostate cancer (CRPC) who have received prior chemotherapy 400 mg of vorinostat taken orally on a daily basis	Significant toxicities limiting the evaluation of treatment efficacy Median time to progression = 2.8 months Median overall survival (mOS) = 11.7 months	Not determined	Phase II trial	[108]
Solid tumor patients consisting of 18 Japanese individuals The treatment period consists of 14 days, followed by a 7-day break, and the dosage prescribed was either 200 mg twice a day or 500 mg once a day	Well tolerated treatments Thrombocytopenia, anorexia, and fatigue Maximum-tolerated dose (MTD) not reached	Not determined	Phase I trial	[109]
Differentiated thyroid cancer (DTC) (16) and medullary thyroid cancer (MTC) (3) patients 200 mg of oral vorinostat twice daily for a duration of 2 weeks, followed by a week-long break, constituting a cycle of 3 weeks	Deep vein thrombosis, bruises, pneumonia, ataxia, dehydration, and fatigue Ineffective treatment Median duration of therapy = 17 weeks for DTC patients Median duration of therapy = 25 weeks for MTC patients	Not determined	Phase II trial	[110]
Patients with gastrointestinal (GI) carcinoma Oral administration of vorinostat, once a day, 3 h before each radiotherapy (RT) fraction (100, 200, 300, and 400 mg)	Fatigue and GI events (all patients) MTD (vorinostat + palliative RT) = 300 mg once a day	Induced biological activity	Phase I trial	[111]
Patients with non-Hodgkin's lymphomas (NHL) or advanced solid tumors (ASTs) Sequential and concurrent schedules Combined therapy: Vorinostat + Decitabine	For sequential schedule: MTD = 10 mg/m^2/day on days 1–5 (Decitabine) MTD = 200 mg three times a day on days 6–12 (Vorinostat) For concurrent schedule: MTD = 10 mg/m^2/day on days 1–5 (Decitabine) MTD = 200 mg twice a day on days 3–9 (Vorinostat) DLTs: constitutional, myelosuppression, and GI symptoms Disease stabilization in 11 of 38 evaluable patients (29 %)	Not determined	Phase I trial	[112]
Patients with hormone therapy-resistant (HTR) breast cancer Vorinostat (400 mg/day) for 3 of 4 weeks + Tamoxifen (20 mg/day)	Objective response rate (ORR) = 19 % Clinical benefit rate = 40 % Median response duration = 10.3 months Well tolerated combination	Demonstrated promising efficacy in overcoming hormone resistance	Phase II trial	[113]
Patients with cutaneous T-cell lymphoma (CTCLs) In part I: Vorinostat (200, 300, and 400 mg daily) Bexarotene (150, 225, and 300 mg/m^2) In part II: Vorinostat (once-daily at 400 mg)	In part I: MTD (vorinostat) = 200 mg/day MTD (bexarotene) = 300 mg/m^2/day In part II: The MTD was not reached	Not determined	Phase I trial	[114]

(*continued on next page*)

Table 2 (*continued*)

Methods	Key results	Mechanisms	Tumor phases	Ref.
Bexarotene (once-daily at 150 mg) Refractory metastatic colorectal cancer (CRC) patients Vorinostat at 800 or 1400 mg/day once a day × 3, every 14 days 5-Fu administered as a bolus and then by a 46-h infusion on days 2 and 3 of vorinostat	On the low-dose arm: Median progression-free survival (mPFS) = 2.4 months Median OS = 6.5 months Well tolerated treatments	No difference in vorinostat pharmacokinetics	Phase II trial	[115]
Patients with melanoma, pancreatic and lung cancer Vorinostat (300 mg daily) for 16 days + increasing doses of marizomib weekly, in 28-day cycles	Stable disease (SD) in 61 % patients Decreased tumor measurements in 39 % patients RP2D = 300 mg Highly synergistic antitumor activity Well tolerated treatment	Not determined	Phase I trial	[116]
Children with leukemia, lymphoma or relapsed solid tumor Vorinostat supplied as capsules (100 mg)	Pulmonary embolism, hyperglycemia, deep vein thrombosis, and electrolyte disturbances	Not determined	Phase I/II trial	[117]
GI tumor patients 300 mg twice daily for three days followed by a four-day break before starting the next cycle (1st groupe) 400 mg once daily for 21 consecutive days per cycle (2nd group)	First group: No patients experienced DLTs Five patients-maintained SD for more than 8 weeks Second group: Two patients had DLTs of thrombocytopenia Two patients-maintained SD for more than 8 weeks	Not determined	Phase I trial	[118]
55 patients with clinical stage IIA-IIIC breast cancer 12 weekly doses of PTX (80 mg/m^2) + vorinostat (200–300 mg po) on days 1–3 of each PTX dose	Increased acetylation of Hsp90 and α-tubulin Reduced expression of Hsp90 client proteins and HDAC6 in the primary tumor	Not determined	A phase I/II study	[119]
Patients with ASTs [renal cell carcinoma (RCC), CRC] 400 mg vorinostat (day 1–21) + Hydroxychloroquine (HCQ) daily (day 2–21 of a 21-day cycle)	DLT = Fatigue and GI events MTD = 400 mg de vorinostat MTD = 600 mg de hydroxychloroquine Durable partial response for 1 RCC patient Prolonged SD for 2 CRC patients	Not determined	Phase I trial	[120]
Metastatic CRC patients Vorinostat (400 mg po) + HCQ (600 mg po) daily, in a 3-week cycle	Vorinostat + HCQ was well tolerated, and active 40 % of patients experienced treatment-related AEs mPFS = 2.8 months mOS = 6.7 months	Induced autophagy inhibition	Phase I trial	[121]
Patients with clear-cell RCC Vorinostat (200 mg) orally twice a day for a period of two weeks, along with Bevacizumab administered intravenously (15 mg/kg) every three weeks	Relatively well tolerated combination 6 objective responses (18 %): 1 complete response + 5 partial responses Six-month PFS = 48 % mPFS = 5.7 months mOS = 13.9 months	Not determined	Phase I/II trial	[122]

NSCLC: non-small cell lung cancer. **PTX:** paclitaxel. **CRPC:** castration-resistant prostate cancer. **mOS:** median overall survival. **MTD:** maximum-tolerated dose. **DTC:** differentiated thyroid cancer. **MTC:** medullary thyroid cancer. **MTD:** maximum-tolerated dose. **GI:** gastrointestinal. **RT:** radiotherapy. **NHL:** non-Hodgkin's lymphomas. **AST:** advanced solid tumor. **HTR:** hormone therapy resistant. **ORR:** objective response rate. **CTCL:** cutaneous T-cell lymphoma. **CRC:** colorectal cancer. **mPFS:** median progression-free survival. **SD:** stable disease. **Hsp90:** heat shock protein 90. **α-tubulin:** a protein that is a structural component of microtubules. **HCQ:** hydroxychloroquine. **RCC:** renal cell carcinoma.

framework and the same schedule. Indeed, in twenty-seven patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with conventional chemotherapy, oral administration of the vorinostat daily dose (400 mg) was associated with significant toxicities (weight loss, diarrhea, vomiting, anorexia, nausea, and fatigue) that forced discontinuation of treatment and limited the evaluation of vorinostat efficacy in this patient population; therefore, encouraging combination therapy.

In contrast, pharmacokinetics pertains to the processes by which a drug is absorbed, distributed, metabolized, and eliminated by the body, while safety focuses on evaluating the drug's safety for patient use [126]. Indeed, evaluating the pharmacokinetics and safety of vorinostat in a specific population that has failed standard therapy will be of great interest. A study by Fujiwara et al. [109] evaluated these two profiles in 18 Japanese patients with solid tumors who received increasing doses of vorinostat for two weeks, followed by a week rest, allowing the body to eliminate the drug and recover before the next dose. This approach is commonly used to evaluate a drug's pharmacokinetics and safety, as it helps determine how the body processes the drug at different doses.

Study results revealed that it was not possible to determine the maximum tolerated dose (MTD) for vorinostat, and some patients who received the highest doses (200 mg twice daily and 500 mg once daily) experienced dose-limiting toxicities (DLT) such as

anorexia, thrombocytopenia, and fatigue. However, the pharmacokinetic profile of vorinostat was similar to that of non-Japanese patients, and the drug's area under the concentration-time curve (AUC) increased proportionally with the dosage. According to the protocol of this study, it was found that vorinostat at doses of 200 mg twice daily or 500 mg once daily were well-tolerated and could serve as recommended doses for future phase II clinical trials. Although no objective anticancer activity has been recorded, the unique and promising clinical activity profile of this drug warrants further clinical development, especially in association with other anti-cancer agents.

The approach followed in this study, consisting of allowing a week of rest during treatment, was also adopted the same year in another study evaluating the anti-tumor properties of vorinostat against metastatic radioiodine-refractory thyroid carcinoma (MRRTC) [110]. Generally, the preferred treatment for most types of thyroid carcinoma is surgery followed by radioiodine therapy (radiotherapy) to destroy any remaining tumor cells [127]. However, in some cases, the cancer may become metastatic and may also become refractory to radioiodine therapy [128]. This means that the tumor cells no longer respond to radiotherapy (RT) with radioiodine, and this treatment is no longer effective. MRRTC may include different types of thyroid cancers, especially medullary thyroid cancer (MTC) and differentiated thyroid cancer (DTC). In the case of this phase II trial, 19 patients with MTC or DTC were recruited and received a single oral dose of vorinostat (200 mg twice a day). Consequently, none of the metastatic patients (MTC and DTC) presented a complete or partial response to treatment with this HDACi, while those with MTC did not show SD. Reasons leading to treatment discontinuation were disease progression as well as the occurrence of adverse effects, in particular thrombocytopenia associated with minor bruising or bleeding. According to these, the use of vorinostat alone, at the treatment schedule and dose specified in this study, did not show effectiveness in treating advanced thyroid cancer. To explore other possibilities, future studies may investigate alternative dosages or treatment regimens involving vorinostat in association with other treatments, with an emphasis on assessing progression-free survival (PFS).

In response to all of these recommendations encouraging the combination of vorinostat with other cancer therapies to achieve more promising results, Ree et al. [111] investigated its tolerability, safety, and tumor histone acetylation in combination with pelvic palliative RT for gastrointestinal (GI) carcinoma. It's important to note that pelvic palliative RT aims to manage symptoms and enhance the patient's quality of life rather than cure the cancer [129]. This study recruited eligible patients with confirmed carcinoma who were to receive 30 Gy of radiation in daily fractions of 3 Gy over 14 days. Vorinostat was po administered once a day at four dose levels ranging from 100 to 400 mg, 3 h before each RT fraction. After evaluating sixteen patients, the tolerability and safety of vorinostat/short-term pelvic palliative RT combination were confirmed. However, although grade 1 and 2 adverse events (AEs) were the most common, with fatigue and GI events predominating, some patients also experienced grade 3 AEs. Only one of the six patients treated with the 300 mg dose experienced grade 3 treatment-related AEs, indicating that the MTD of vorinostat combined with palliative RT was 300 mg a day. Furthermore, the detection of histone hyperacetylation provides evidence of the biological activity of this HDACi. Findings from this phase I study underscore the potential utility of vorinostat in conjunction with radiation therapy and support the need for additional research on this compound's potential in long-term curative pelvic RT.

On the other hand, DNMTis are a class of drugs used in oncology for the treatment of certain cancers, by inhibiting the activity of enzymes called DNA methyltransferases (DNMT) [130]. DNA methylation is an epigenetic mechanism that regulates gene expression, and an increase in DNA methylation is often associated with the suppression of tumor suppressor genes. DNMTi can reduce DNA methylation in cancer cells, which can lead to apoptosis, cell differentiation, inhibition of cell proliferation, and reactivation of tumor suppressor genes in cancer cells [131]. They can also induce an immune response against cancer cells. Therefore, the combined use of epigenetic therapies could constitute a promising therapeutic approach for cancer treatment. In order to achieve this objective, Stathis et al. [112]studied the combination of vorinostat/decitabine (HDACi/DNMTi) in a phase I trial evaluating its efficacy, pharmacokinetics, tolerability, and safety in 43 patients with non-Hodgkin's lymphoma (NHL) or ASTs and receiving this treatment at 9 distinct dose levels, with concurrent and sequential regimens investigated, which showed an RP2D of 200 mg twice daily on days 6–12 and 10 mg/m^2/day on days 1–5 for vorinostat and decitabine, respectively. Taken together, the vorinostat/decitabine combination was tolerable on both schedules studied in patients previously treated with NHL or ASTs. The combination has shown activity with prolonged stabilization in various tumor types; suggesting that HDACi/DNMTi association has potential as a treatment option for this patient population and many others.

Another type of combination has been proposed in a phase II clinical study against hormone-resistant breast cancer (BC) [113], which is a type of BC that does not respond to treatment with hormones such as tamoxifen. The latter is a drug used to treat and prevent BC by inhibiting the effects of estrogen on breast tissue, which can slow or inhibit the growth of breast tumor cells that depend on estrogen to develop [132]. In fact, 43 patients with metastatic BC who had progressed on hormone therapy were recruited for this trial. They received a daily dose of vorinostat (400 mg) for 3 of 4 weeks and a continuous dose of tamoxifen (20 mg) daily. The objective response rate (ORR) was used as the primary endpoint. Consequently, the vorinostat/tamoxifen combination was well tolerated and had encouraging potential to reverse hormonal resistance. Additionally, histone hyperacetylation and increased HDAC2 expression levels were correlated with treatment response, suggesting that these may be useful pharmacodynamic and predictive biomarkers, respectively, for the effectiveness of this combination.

In contrast, since the FDA has approved the use of vorinostat as a single agent in the treatment of CTCL, a type of NHL that affects the skin, Dummer et al. [114] performed a study with preclinical and clinical aspects, evaluating the anti-CTCL potential of this substance associated with bexarotene, a drug used to treat CTCL, by binding to specific retinoid receptors in the body, which can induce tumor cell death, stunt their growth, or inhibit their division [133]. For the preclinical aspect (*in vitro*), the study revealed that the combination of both agents resulted in a decrease in tumor cell viability and a synergistic activation of gene transcription. Subsequently, clinical confirmation of this effect was carried out by a phase I trial that aimed to determine the MTD of the combination in CTCL patients. Interestingly, the concomitant administration of these two drugs recorded an objective response in four patients with

relief of pruritus in seven, and it was concluded that combining these drugs is possible, but only if each one is given at a lower dose than what is recommended for monotherapy on their respective product labels. This study provides evidence supporting the increased activation of retinoid receptors by HDAC inhibition in CTCL treatment.

Regarding CRC, it has been previously demonstrated in preclinical investigations that vorinostat combined with 5-Fu exhibits remarkable synergistic activity, while its effect alone is able to enhance the effect of 5-Fu and overcome the problem of resistance to this agent [43]. The anti-CRC potential of this combination was subsequently verified clinically in a phase II trial by testing the efficacy of two different doses of vorinostat (800 and 1400 mg/day) in refractory CRC patients [115]. In addition, every two weeks, a combined therapy was given, comprising bolus administration of leucovorin followed by a 46-h infusion of 5-Fu and then vorinostat on days 2 and 3. Even though the fusion of vorinostat with 5-Fu stabilized the disease and produced a partial response, the limited results do not justify the routine use of this combination in the treatment of cases of chemotherapy-refractory CRC. This study highlights the importance of conducting randomized controlled trials to assess the effectiveness of combination therapies in patients with CRC, and the need to examine the potential benefits and limitations of new therapies in different types and stages of cancer.

Over time, new combinations have been studied based on the anti-tumor properties of the separate agents. Researchers sought to maximize therapeutic efficacy while minimizing unwanted side effects. Using sophisticated screening and analytical techniques, they were able to identify synergistic combinations that exhibit superior anti-tumor properties than the individual compounds. These encouraging results have paved the way for new avenues for cancer treatment, offering hope for patients with this devastating disease. In fact, proteasome inhibitors have been used in the treatment of certain types of cancer, including MM and NHL [134]. They are also being studied for the treatment of other cancers. The proteasome is a structure present in all cells that breaks down damaged or useless proteins [135]. Proteasome inhibitors interfere with this function and prevent the tumor cell from degrading certain proteins essential to its survival. These inhibitors are generally used in combination with other enhancement drugs, as has been shown *in vitro* against CRC [15]. Indeed, Millward et al. [116] combined one of these most common inhibitors, marizomib, with vorinostat in the treatment of patients with NSCLC, pancreatic carcinoma, or melanoma. The study aimed to determine the safety, pharmacodynamics/pharmacokinetics, RP2D, and preliminary antitumor effect of the vorinostat/marizomib association in 22 patients. The study revealed that the combination was well tolerated, and no increased toxicity was observed, suggesting that the vorinostat/marizomib combination may have potential as a treatment option for the three types of cancer studied.

Regarding the treatment of pediatric tumors, it depends on the tumor type, size, stage, and location, as well as the child's general health. Treatment options can vary greatly based on these factors. It was revealed above that vorinostat, as a single agent or in combined treatments, exhibits enhanced anti-cancer effects against pediatric neuroblastoma, medulloblastoma, and leukemia cells [62]. In this context, Witt et al. [117]clinically evaluated the optimal dose (OD), safety, and efficacy of this HDACi in pediatric patients with leukemia, lymphoma, or recurrent solid tumors, implementing a dose escalation approach individualized to ensure that each patient receives an OD regarding both efficacy and toxicity. The results suggested that this trial could contribute to the development of new therapeutic options for pediatric patients with these types of cancers.

Considering the results obtained from the previous trial conducted by Ree et al. [111] in the treatment of GI cancer, a subsequent phase I trial further investigated several parameters related to vorinostat monotherapy for this cancer [118]. The trial involved two vorinostat dosing regimens, and as a result, the authors showed that vorinostat was generally well tolerated in patients with GI cancer, with the most common treatment-related AEs being hyperglycemia, fatigue, nausea, and anorexia. Interestingly, vorinostat (300 mg twice a day) given for three consecutive days followed by four days off was better tolerated than a higher dose (400 mg) given once a day. This provides important insights about the potential use of this drug as a treatment option for GI cancer. However, this safety should be determined in long-term treatments.

Building on the previous encouraging potential of the vorinostat/tamoxifen combination in the treatment of BC [113], a phase I/II clinical trial evaluated the efficacy of vorinostat in combination with chemotherapy in 55 patients with clinical stage IIA-IIIC BC [119]. The study found that a treatment regimen consisting of weekly doses of PTX and TRA for 12 weeks, combined with vorinostat taken orally twice daily at 300 mg on days 1–3 of each PTX/TRA dose, followed by four cycles of high-dose doxorubicin-cyclophosphamide, was well tolerated and resulted in a breast and lymph node pathologic complete response (pCR) rate of about 50 % of patients. These data were in line with previous *in vitro* and *in vivo* studies, indicating that vorinostat augmented α-tubulin and Hsp90 acetylation and decreased HDAC6 and Hsp90 expression [136]. This study provides evidence for the potential of HDAC inhibition as a strategy to sensitize BC cells to chemotherapy and improve treatment outcomes. However, further investigations are needed to validate these findings and determine the optimal combination and dosing of HDACis and chemotherapy for different BC subtypes.

In contrast, autophagy can play a dual role in cancer. On one hand, it can help tumor cells survive and grow during periods of metabolic stress or treatment, while on the other hand, it can promote apoptosis. Therefore, autophagy inhibition can be used in cancer treatment to stimulate this apoptosis in tumor cells or to make these cells more sensitive to other treatments such as RT or chemotherapy. Preclinical and clinical studies have shown that inhibiting autophagy can improve the effectiveness of cancer treatments, particularly in patients with certain forms of treatment-resistant cancer [137]. In 2014, Mahalingam and collaborators [120] combined vorinostat with an autophagy inhibitor, hydroxychloroquine (HCQ), to treat 27 patients with ASTs. They observed some, generally mild, treatment-related AEs without significant impact on the pharmacokinetics of vorinostat. They also found that there were more notable treatment-related increases in the expression of two genes involved in the regulation of cell growth and proliferation, CTSD and CDKN1A, in tumor biopsy samples compared to peripheral blood mononuclear cells. This implies that the combination could have a greater impact on tumor cells than on healthy cells. Given the initial efficacy and safety of this association, the authors propose that further clinical investigations should be conducted to explore the inhibition of autophagy as a novel strategy for enhancing the effectiveness of HDACis in treating ASTs.

Recent studies have shown that inhibiting autophagy may improve anti-cancer immunity [138]. In particular, inhibition of

autophagy in tumor cells has been shown to promote antigen presentation and stimulate the immune response [139]. Furthermore, autophagy inhibition may increase the sensitivity of tumor cells to immune therapy [140]. These findings suggest that autophagy inhibition could be an effective therapeutic strategy to enhance anti-cancer immunity. However, it is important to note that autophagy is a complex process that can have different effects depending on the type of cancer and the stage of the disease. Therefore, more research is needed to determine the conditions under which autophagy inhibition may be beneficial in the treatment of cancer. In this sense, the same combination (vorinostat/HCQ) was adopted to treat patients with metastatic CRC (mCRC) in order to thoroughly evaluate the immune effects and clinical efficacy of this therapy [121]. As a result, in addition to the tolerability and safety of combined vorinostat/HCQ, a decrease in naïve T cells and exhausted/regulatory T cells and an improvement in anti-cancer immunity were recorded. The study also showed that the combined therapy inhibited autophagy in primary tumors, which could contribute to the antitumor effects of the treatment. It can be suggested from this study that the vorinostat/HCQ combination is a potentially effective therapy for mCRC, with enhanced antitumor immunity and autophagy inhibition as possible mechanisms of action. The findings could inform future research into the role of autophagy in cancer immunity and the development of combination therapies for mCRC.

From the different combinations investigated in this work, we deduced that the choice of combination therapy should be tailored to each individual patient based on their cancer type and general health status. In 2017, Pili and colleagues [122] tested another combination strategy combining vorinostat and bevacizumab, a vascular endothelial growth factor (VEGF) inhibitor, in the treatment of patients with metastatic renal cell carcinoma (mRCC). VEGFis are anticancer drugs targeting a protein called VEGF, which stimulates angiogenesis and therefore prevents the growth of new blood vessels in cancerous tumors, thereby reducing nutrient and oxygen supply, slowing tumor growth, and potentially improving patient response to other cancer treatments [141,142]. They are often used in combination with other cancer treatments, such as RT, chemotherapy, or immunotherapy; justifying the combination with vorinostat in this phase I/II clinical trial [122]. The trial enrolled 36 patients, with 33 being evaluable for efficacy and toxicity. Of these patients, 18 had received one prior treatment, 13 had received two prior treatments, and two were treatment-naive. During the treatment, three patients experienced grade 3 thromboembolic events and two patients experienced grade 4 thrombocytopenia, but overall, the combination treatment was well-tolerated. In sum, it will be possible to identify patients with mRCC who might benefit from combined therapy by further analysing those who respond to epigenetic therapies when combined with VEGF inhibition.

5. Discussion and limitations

Overall, our study underscores the significant potential of vorinostat as an HDAC inhibitor for tumor treatment. It is clearly demonstrated that vorinostat has a significant ability to modulate signaling pathways that regulate the cell cycle, leading to cell death. This paves the way for its potential use as a cancer preventive medication against various types of cancer. Furthermore, our mechanistic understanding of vorinostat's anticancer effect, as well as similar compounds, offers the opportunity to deepen our knowledge of the underlying cellular mechanisms, particularly those that control tumor transformation. This perspective is crucial for the development of targeted therapies and the improvement of the effectiveness of anticancer treatments.

However, it is essential to note some important limitations associated with vorinostat's use that we have identified. One of these limitations lies in the initial need to combine vorinostat with other chemotherapy drugs as part of a targeted therapeutic approach. This strategy aims to maximize therapeutic effects while minimizing undesirable side effects. Therefore, further research is needed to explore potential beneficial interactions between vorinostat and other chemotherapy agents. Additionally, it is imperative to continue studying and analyzing the molecular mechanisms underlying vorinostat's action as well as its impact on the cellular hierarchy by distinguishing between healthy and tumor cells. This in-depth research will not only improve our understanding of cancer but also guide the development of more targeted and effective therapies.

Overall, despite persistent challenges, the use of vorinostat as an HDAC inhibitor holds promising prospects for cancer treatment. Future research should focus on optimizing its use in combination with other therapies while deepening our understanding of the underlying molecular mechanisms.

However, the effective clinical development of vorinostat as an anticancer agent raises hopes for similar development for other natural cancer molecules. Indeed, other natural HDACi molecules have also been clinically validated, notably romidepsin [143]. This natural molecule induces sequential mechanisms that confer significant clinical effectiveness. In this context, other natural molecules, in particular natural epidrugs, have also shown a remarkable capacity to inhibit epigenetic pathways, including HDACs involved in various types of cancer. Trichostatin, for example, a natural molecule with multiple biological properties, including anticancer properties, has been widely studied in hundreds of preclinical studies, thus revealing its anticancer potential through different mechanisms of action [144].

Furthermore, clinical studies concerning this molecule have also highlighted its high anticancer potential. Consequently, it constitutes a candidate molecule for possible integration into the clinical field. Likewise, many natural molecules have been subjected to tests aimed at evaluating their effectiveness against various epigenetic actors, notably DNMTs and HDACs, as well as other epigenetic pathways such as chromatin remodeling pathways. Although these molecules have demonstrated promising effectiveness against different types of cancer, it is important to note that these investigations remain mainly at a preclinical stage (*in vitro* or *in vivo*). Their possible clinical application therefore requires in-depth studies in terms of toxicology, selectivity, and clinical effectiveness. With this in mind, the experience gained with vorinostat could prove valuable in guiding future investigations of other anticancer molecules and simplifying their transition to clinical application, with a view to obtaining validation as epidrugs used in the field of therapeutic oncology. Indeed, several molecules have already been the subject of investigations, notably carvone, recognized for its properties against various types of cancer [145], tomentosin, renowned for its anticancer activity mainly linked to its ability to reduce chronic inflammation [146], as well as other compounds such as quercetin, gallic acid, and many others [147–150].

6. Concluding remarks and future perspectives

Based on the findings of this investigative review, it is evident that Vorinostat, an HDAC inhibitor, possesses significant potential to modulate signaling pathways that indirectly regulate cell cycle progression, ultimately leading to cell death. Consequently, Vorinostat holds promise as a chemopreventive agent for managing various types of cancers. However, there are two noteworthy counterarguments to consider: first, Vorinostat may be more effective when used in combination with other drugs as part of targeted therapy, with potential synergistic effects warranting further investigation. Second, a deeper mechanistic understanding of the anticancer action of Vorinostat and similar HDAC inhibitors could enhance our comprehension of the cellular mechanisms involved in tumor transformation.

The exploration of these molecules, already in clinical use, has the potential to significantly advance our understanding of both normal and tumor cell biology. By deciphering the sequential events that drive the transition from normal cells to tumor cells, researchers may uncover new therapeutic targets and develop more effective cancer treatments.

Looking ahead, there are several potential future perspectives for the clinical application of Vorinostat as a chemopreventive agent. Firstly, future clinical studies should explore the synergistic effects of Vorinostat when used in combination with other drugs in targeted therapy regimens, aiming to optimize its efficacy in cancer treatment. Secondly, a comprehensive understanding of the molecular mechanisms underlying Vorinostat's anticancer effects, as well as those of other HDAC inhibitors, could provide valuable insights into tumor biology and facilitate the development of novel therapeutic strategies.

In conclusion, Vorinostat exhibits potent capabilities in modulating cellular pathways crucial for cancer progression. While its potential as a chemopreventive agent is promising, maximizing its efficacy may require combination therapy approaches. Additionally, a deeper understanding of its mechanisms of action could lead to significant advancements in cancer treatment. Therefore, further research into Vorinostat and related compounds is essential for realizing their full therapeutic potential in the fight against cancer.

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Data availability statement

No data is used in this study.

CRediT authorship contribution statement

Nasreddine El Omari: Writing – original draft, Project administration, Methodology. **Asaad Khalid:** Writing – review & editing, Supervision, Software, Resources, Project administration. **Hafiz A. Makeen:** Software, Resources, Project administration, Methodology. **Hassan A. Alhazmi:** Writing – original draft, Validation, Investigation. **Mohammed Albratty:** Writing – review & editing, Project administration. **Syam Mohan:** Writing – review & editing, Validation, Software, Resources. **Ching Siang Tan:** Writing – original draft, Visualization, Validation, Resources, Methodology. **Long Chiau Ming:** Writing – review & editing, Validation, Supervision, Resources, Project administration. **Jack Bee Chook:** Writing – original draft, Methodology, Formal analysis. **Abdelhakim Bouyahya:** Writing – original draft, Validation, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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