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Review

Anticancer clinical efficiency and stochastic mechanisms of belinostat

Nasreddine El Omari [a,j], Saad Bakrim [b,j], Asaad Khalid [c,j,*], Mohammed Albratty [d,j], Ashraf N. Abdalla [e,j], Learn-Han Lee [f,g,*], Khang Wen Goh [h], Long Chiau Ming [i,*], Abdelhakim Bouyahya [i,j,*]

[a] Laboratory of Histology, Embryology, and Cytogenetic, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat 10100, Morocco
[b] Geo-Bio-Environment Engineering and Innovation Laboratory, Molecular Engineering, Biotechnology and Innovation Team, Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Agadir 80000, Morocco
[c] Substance Abuse and Toxicology Research Center, Jazan University, P.O. Box: 114, Jazan 45142, Saudi Arabia
[d] Department of Pharmaceutical Chemistry and Pharmacognosy, College of Pharmacy, Jazan University, P.O. Box 114, 45142, Jazan, Saudi Arabia
[e] Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia
[f] Sunway Microbiomics Centre, School of Medical and Life Sciences, Sunway University, Sunway City 47500, Malaysia
[g] Novel Bacteria and Drug Discovery Research Group (NBDD), Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Malaysia
[h] Faculty of Data Science and Information Technology, INTI International University, Nilai, Malaysia
[i] School of Medical and Life Sciences, Sunway University, Sunway City 47500, Malaysia
[j] Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, Rabat 10106, Morocco

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ABSTRACT

Cancer progression is strongly affected by epigenetic events in addition to genetic modifications. One of the key elements in the epigenetic control of gene expression is histone modification through acetylation, which is regulated by the synergy between histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs are thought to offer considerable potential for the development of anticancer medications, particularly when used in conjunction with other anticancer medications and/or radiotherapy. Belinostat (Beleodaq, PXD101) is a pan-HDAC unsaturated hydroxamate inhibitor with a sulfonamide group that has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of refractory or relapsed peripheral T-cell lymphoma (PTCL) and solid malignancies or and other hematological tissues. This drug modifies histones and epigenetic pathways. Because HDAC and HAT imbalance can lead to downregulation of regulatory genes, resulting in tumorigenesis. Inhibition of HDACs by belinostat indirectly promotes anti-cancer therapeutic effect by provoking acetylated histone accumulation, re-establishing normal gene expressions in cancer cells and stimulating other routes such as the immune response, p27 signaling cascades, caspase 3 activation, nuclear protein poly (ADP-ribose) polymerase-1 (PARP-1) degradation, cyclin A (G2/M phase), cyclin E1 (G1/S phase) and other events. In addition, belinostat has already been discovered to increase $p21^{WAF1}$ in a number of cell lines (melanoma, prostate, breast, lung, colon, and ovary). This cyclin-dependent kinase inhibitor actually has a role in processes that cause cell cycle arrest and apoptosis. Belinostat's clinical effectiveness, comprising Phase I and II studies within the areas of solid and hematological cancers, has been evidenced through several investigative trials that have supported its potential to be a valuable anti-cancer drug. The purpose of this research was to provide insight on the specific molecular processes through which belinostat inhibits HDAC. The ability to investigate new therapeutic options employing targeted therapy and acquire a deeper understanding of cancer cell abnormalities may result from a better understanding of these particular routes.

* Corresponding authors at: Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, Rabat 10106, Morocco.

E-mail addresses: nasrelomari@gmail.com (N. El Omari), s.bakrim@uiz.ac.ma (S. Bakrim), akahmed@jazanu.edu.sa (A. Khalid), malbratty@jazanu.edu.sa (M. Albratty), anabdrabo@uqu.edu.sa (A.N. Abdalla), learnhanl@sunway.edu.my (L.-H. Lee), khangwen.goh@newinti.edu.my (K.W. Goh), chiaumingl@sunway.edu.my (L.C. Ming), a.bouyahya@um5r.ac.ma (A. Bouyahya).

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1. Introduction

Epigenetic modifications contribute to the expression of DNA, microRNAs and, histone modifications which alter gene transcription and cellular behavior. The prevalence of aberrant epigenetic modifications that lead to many diseases increases with age. The expression of genetic variations consequently leads to a number of disorders, most notably cancer [1,2]. Numerous hematologic malignancies, including Hodgkin's lymphoma, myelofibrosis, acute myelogenous leukemia (AML), and peripheral T-cell lymphoma (PTCL), exhibit increased levels of histone deacetylating enzymes; inhibition of these enzymes can arrest cell proliferation and induce cell apoptosis [3]. Histone deacetylases (HDACs) are a class of epigenetic enzymes that are important for epigenetics, chromatin biology, histone alterations, and transcriptional control [4]. By removing the acetyl group from histone and non-histone proteins, HDACs modulate the expression of target genes, leading to transcriptional inactivity in chromatin [5]. Additionally, they are necessary for preserving a dynamic homeostasis between protein acetylation and other post-translational modifications[6]. HDAC inhibitors (HDACis) decrease angiogenesis, promote cell differentiation and cell death in cancer cells, halt the cell cycle, and regulate the immune system function. The processes through which HDAC inhibitors exert their anticancer effects vary depending on the doses, the HDAC inhibitors, and the form of tumor, *etc*. HDACis are considered interesting candidates for anticancer drug development, especially when used in combination with other anticancer drugs or/and radiotherapy [7]. The U.S. Food and Drug Administration (FDA) has approved belinostat, an unsaturated hydroxamate (Fig. 1) pan-HDAC inhibitor with a sulfonamide component, for the treatment of refractory or relapsed PTCL and solid malignancies. This drug modifies histone and epigenetic pathways [8]. It promotes histone acetylation by exhibiting class I and II HDAC enzyme action. HDAC inhibition is achieved by binding the inhibitor specifically to the catalytic site of the enzyme [9]. Apoptosis, cell cycle arrest, and decreased proliferation of cancer cells are the results of this event. By altering the microtubule dynamics of the cell and stimulating cell death, belinostat enhances the levels of non-histone protein acetylation as well as histone acetylation. Belinostat has a favorable safety profile and does not cause bone marrow toxicity when used in combination with other chemotherapy drugs [1]. Furthermore, belinostat indirectly promotes anti-cancer therapeutic effect by provoking acetylated histone accumulation, re-establishing normal gene expressions in cancer cells and stimulating other routes such as the immune response, p27 signaling cascades, changes in expression of cell cycle regulatory proteins' survivin, caspase 3 activation, nuclear protein poly (ADP-ribose) polymerase-1 (PARP-1) degradation, cyclin A (G2/M phase), cyclin E1 (G1/S phase) and other signaling pathways [10–12].

Moreover, belinostat has already been discovered to stimulate $p21^{WAF1}$ in a number of cell lines (melanoma, prostate, breast, lung, colon, and ovary). This cyclin-dependent kinase inhibitor actually has a role in processes that cause cell cycle arrest and apoptosis [13]. This compound did not cause the differentiation of leukemic cells into granulocytic forms on its own, but when combined with all-trans retinoic acid (ATRA), it promoted HL-60 and NB4 cell differentiation [14]. Depending on the dose administered, belinostat induced active chromatin-specific acetylation of histones H3K9 and H3K16 and hyperacetylation of histone H4 [12]. On the other hand, the clinical effectiveness including phase I and II clinical trials in solid and hematological cancers of belinostat has been demonstrated by several research studies which have proven its application as a powerful anticancer drug. The purpose of this investigation was to highlight the precise molecular mechanisms involved in the inhibition of HDAC by belinostat. Understanding in more detail such specific pathways may greatly contribute to the better comprehension of cancer cell disorders and may open the opportunity to explore new therapeutic strategies through targeted therapy.

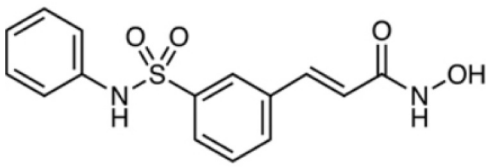

Fig. 1. Chemical structure of belinostat.

2. Research methodology

In this study, a comprehensive bibliometric search was performed to gather information on belinostat. Specifically, studies investigating the anticancer mechanisms and clinical efficacy of belinostat were consulted using a range of reputable databases, including ScienceDirect, Springer, Scopus, Google Scholar, PubMed, and others. The search was conducted using relevant keywords such as belinostat, anticancer effect of belinostat, anticancer mechanism of belinostat, clinical efficacy of belinostat, and so on, to ensure the search results were relevant and comprehensive. The data was organized into tables, then analyzed and discussed chronologically.

3. Clinical investigations

The clinical effectiveness of belinostat has been demonstrated by several research studies which have proven its application as a powerful anticancer drug. In the following section, we will discuss the clinical effectiveness of belinostat in chronological order of its development. Table 1 summarizes all studies that have clinically investigated the anticancer effects of belinostat.

Even before its approval by the FDA on July 3, 2014 for clinical treatment [28], the anticancer effect of belinostat was investigated by Gimsing et al. [15] for the treatment of subjects with advanced hematological neoplasia by determining its tolerance *via* maximum tolerated dose (MTD). To do this, the authors chose a 21-day cycle to infuse different doses of belinostat (600, 900 and 1000 mg/m^2/day) to patients. In addition, in patients with multiple myeloma, adverse effects related to renal failure have occurred, prompting more attention in the treatment of these patients with belinostat. In general, this HDACi presented an acceptable safety profile with an MTD value of 1000 mg/m^2/day, which could be recommended for phase II trials in the treatment of hematological neoplasms. This good tolerance was confirmed in the same year in a phase I study adopting the same experimental design for the treatment of forty-six advanced solid tumors (ASTs) patients [16]. Despite the recording of dose-limiting toxicities (DLTs) in some patients (nausea/vomiting, diarrhea associated with fatigue, and atrial fibrillation), the results of this study showed that belinostat treatments at different doses exhibit promising anticancer activity and dose-dependent pharmacodynamic and pharmacokinetic effects with an MTD of 1000 mg/m^2/day. Indeed, administration of this dose stabilized the disease in 50 % of treated patients with increased IL-6 levels, hyperacetylation of histone H4, and caspase-cleaved cytokeratin-18 (CK-18). From these two studies and the mechanisms of action already demonstrated, the clinical development of belinostat in phase II studies is evident, which was achieved two years later in women with platinum-resistant (PR) epithelial ovarian tumors (EOTs) and micropapillary/borderline (LMP) OTs [17]. These cancers are rarely investigated in clinical trials and are inherently resistant to chemotherapy and radiotherapy with a poor prognosis. Therefore, administration of belinostat (1000 mg/m^2/day) was well tolerated with antitumor activity without grade 4 toxicity.

To further assess the response rate of this molecule, Giaccone et al. [18] administered a dose of 1 g/m^2 to patients with thymic epithelial tumors (TETs) in a phase II study. Treatment was stopped until intolerance appeared. In addition to the good treatment tolerance in patients with thymoma, a modest antitumor effect was observed with a high

Table 1
Clinical investigations of belinostat.

Methods	Clinical phases	Key findings	References
Patients with hematologic neoplasia 30-min (i.v.) on days 1–5 of a 3 week cycle 600, 900, and 1000 mg/m^2/day	Phase I	Well tolerated treatment Maximum tolerated dose (MTD) = 1000 mg/m^2/day	[15]
Refractory AST patients 30-min (i.v.) on days 1–5 of a 3-week cycle	Phase I	Induced promising antitumor activity Exhibited dose-dependent pharmacodynamic effects Well tolerated treatment MTD = 1000 mg/m^2/day	[16]
Epithelial ovarian cancer (EOC) women Micropapillary/ borderline (LMP) ovarian tumors women 1000 mg/m^2/d (i.v.) on days 1–5 of a 3 week cycle	Phase II	Induced antitumor activity in patients with LMP Well tolerated treatment	[17]
Patients with thymic epithelial tumors (TETs), recurrent or refractory 1 g/m^2 (i.v.) on days 1–5 of a 3 week cycle	Phase II	Induced modest antitumor activity Well tolerated treatment	[18]
Patients with unresectable hepatocellular carcinoma (HCC) and chronic liver disease Administered (i.v.) once/day on days 1–5 every 3 weeks 600, 900, 1200, and 1400 mg/m^2 per day	Phase I/ II	Maximum dose did not induce MTD Progression-free survival (PFS) = 2.64 months Produced tumor stabilization Well tolerated treatment	[19]
Advanced, unresectable TET patients Belinostat (1000 and 2000 mg/m^2), cisplatin (50 mg/m^2 on day 2), doxorubicin (25 mg/m^2 on days 2 and 3), and cyclophosphamide (500 mg/m^2 on day 3)	Phase I/ II	The combination of belinostat with doxorubicin, cisplatin, and cyclophosphamide is feasible and active in TETs	[20]
Subjects with peripheral T-cell lymphoma (PTCL) Subjects with cutaneous TCL (CTCL)	Phase II	Effective and tolerated treatment 77% of patients showed treatment-related adverse events	[21]
Patients with PTCLs 1000 mg/m^2 as daily 30-minute infusions on days 1–5 of a 21-day cycle	Phase II	Produced durable and complete responses Induced manageable toxicity	[22]
Patients receiving warfarin (5 mg) and belinostat (1000 mg/m^2) orally	Phase I	No significant effect on the pharmacodynamics and pharmacokinetics of warfarin	[23]
Soft tissue sarcomas (STS) patients A 30-minute (i.v.) administration of belinostat on days 1–5 and on day 5 with doxorubicin (Dox) Cohort 1: 600 mg/m^2 belinostat and 50 mg/m^2 Dox Cohort 2: 600 mg/m^2 belinostat and 75 mg/m^2 Dox Cohort 3: 800 mg/m^2 belinostat and 75 mg/m^2 Dox Cohort 4: 1000 mg/m^2 belinostat and 75 mg/m^2 Dox	Phase I/ II	MTD = Belinostat 1000 mg/m^2/day and Dox 75 mg/m^2 Well tolerated combination Moderate response rate Median time to progression (6.0 months)	[24]
Advanced solid tumors (ASTs) patients Belinostat (1000 mg/m^2) administered on days 1–5, every 3 weeks 13-*cis*-retinoic acid (50–100 mg/m^2/d)	Phase I	Treatment with belinostat (2000 mg/m days 1–5) + 13-*cis*-retinoic acid (100 mg/m days 1–14), every 3 weeks, was tolerated	[25]
Patients with AST and various degrees of hepatic dysfunction 1000 mg/m^2/day	Phase I	Well tolerated treatment	[26]
13 patients receiving temozolomide and radiotherapy (RT) 500–750 mg/m^2 1 × / d × 5 days every 3 weeks		Induced better in-field control The median overall survival (OS) = 18.5 months for the belinostat cohort	[27]

stabilizing activity and certain adverse effects, in particular fatigue, vomiting, and nausea.

On the other hand, in patients with unresectable hepatocellular carcinoma (HCC) and chronic hepatic disease, the efficacy of belinostat was also evaluated in a phase I/II multicentre study, by determining the DLT and MTD and pharmacokinetic activity [19]. For phase I (18 patients), doses of 600, 900, 1200, and 1400 mg/m^2/day (i.v.) were given on days 1–5 every 21 days, while for phase II (42 patients), progression-free survival (PFS) was the major endpoint. Therefore, linear pharmacokinetics were obtained for doses between 600 and 1400 mg/m^2. However, even at the maximum dose, the MTD was not reached. Interestingly, tumor stabilization was noted following this treatment with belinostat, which was well tolerated.

Given the positive results already obtained against TETs in the work carried out by [18], a later phase I/II trial reassessed the effect of administering belinostat alone and combined with three anticancer agents, namely cyclophosphamide, doxorubicin, and cisplatin [20]. This assessment was performed by determining pharmacokinetics, MTD, safety, antitumor activity, biomarkers of response, and objective response rate (ORR). Authors showed that the administration of HDACi alone or in combination with other chemotherapeutics at different concentrations has immunomodulatory effects on TIM3$^+$ CD8$^+$ T cells and regulatory T cells (Treg). They further showed that this association is feasible and active in TET therapy, but without any ameliorating effect on ORR.

This endpoint was also the primary one in a phase II trial performed by Foss et al. [21] in subjects with cutaneous T-cell lymphoma (CTCL) or peripheral TCL (PTCL). All of these patients failed prior systemic therapy, while they were treated with a 1 g/m^2 (i.v.) dose of belinostat for a 21-day cycle. The results showed significant clinical activity of this agent with a good safety profile and ORR values of 14 % and 25 % for patients with CTCL and PTCL, respectively. Another phase II study confirmed this activity in the treatment of PTCL also using ORR as the primary endpoint [22]. Indeed, the same dose of belinostat (1 g/m^2/day) was administered in infusions (of 30 min) on days 1–5 every 3 weeks to subjects with relapsed/refractory PTCL. Therefore, regardless of the type or number of prior treatments, this monotherapy resulted in durable and complete responses with an acceptable toxicity profile. Based on these results, the FDA approved belinostat for relapsed/refractory PTCL therapy.

On the other hand, given the good outcomes obtained following treatments with belinostat as a single agent, its combination with other compounds will further improve its clinical efficacy in cancer therapy. This was investigated by Agarwal and colleagues in phase I clinical trial in 18 patients receiving intravenously belinostat (1000 mg/m^2) for days

1–5, every 3 weeks and orally warfarin 5 mg (days 14 and 3), a drug widely used as an anticoagulant [23]. Consequently, belinostat showed no impact on the pharmacodynamics or pharmacokinetics of warfarin. This suggests that this drug interaction (belinostat/warfarin) has no clinically significant antitumor effect. However, a combination therapy performed in the same year showed promising results in the treatment of soft tissue sarcomas (STS) patients [24]. This combination was achieved by administering belinostat and doxorubicin as a 30-min infusion (days 1–5 and day 5, respectively), in a diversified dose-escalation schedule with the objective of determining DLT and MTD in solid tumors (25 patients in phase I) as well as ORR in STS (16 patients in phase II). This combination showed some adverse events (nausea, fatigue, and alopecia), grade 3 rash (DLT), and moderate ORR with MTD values of 1000 mg/m^2/day and 75 mg/m^2 for belinostat and doxorubicin, respectively. Importantly, the PFS following this treatment was 6.0 months, which was superior to some investigations of doxorubicin monotherapy against STS [29,30]. Taken together, the belinostat/doxorubicin association was well tolerated.

Another combination was evaluated between belinostat and 13-*cis*-retinoic acid at different concentrations in a phase I trial in the treatment of patients with ASTs. Clinical reports indicate that HDACis enhance signaling of this acid in the treatment of certain solid tumors [31]. The results showed that despite the doubling of the MTD value of belinostat monotherapy, the MTD was not achieved in the combined treatment. Concerning DLTs, the combination of belinostat (1.7 g/m^2/day) with 13-*cis*-retinoic acid (0.1 g/m^2/day) caused hypoxia, dizziness, and grade 3 hypersensitivity, whereas at doses of 2 and 0.1 g/m^2/d, respectively, this combination caused a grade 3 allergic reaction. Additionally, the combination belinostat (2 g/m^2/day)/13-*cis*-retinoic acid (0.1 g/m^2/day) days 1–5 and days 1–14, respectively every 3 weeks, was well tolerated.

This high tolerance to belinostat treatment with no relationship between toxicity and belinostat treatment, as a single compound, was recorded in the work of [26] for the treatment of AST patients and different levels of liver dysfunction.

Very recently, a pilot study was carried out to evaluate for the first time the efficacy of this HDACi with radiotherapy (RT) against glioblastoma (GBM) known for its poor prognosis and high aggressiveness [27]. The choice of belinostat is based primarily on its high ability to cross the blood-brain barrier. To do this, patients with glioblastoma received a regimen of belinostat (500–750 mg/m^2/day, days 1–5, every 3 weeks) along with temozolomide and RT (weeks 0, 3, and 6). Hence, administration of belinostat with standard treatment (RT/temozolomide) was found to be harmless with a radio-sensitizing effect leading to improved anti-GBM results and strong potential to delay recurrence volumes, especially in regions receiving 60 Gy, offering this HDACi as a highly synergistic therapeutic compound for the treatment of patients with glioblastoma.

From the clinical outcomes of all the trials discussed above, it can be inferred that belinostat as a single agent or combined with other therapeutic compounds or standard treatments (RT/chemotherapy) could stabilize the disease in many patients with different tumor types and further improve the clinical efficacy of standard treatments with a largely tolerable toxicity profile.

4. Mechanisms of action underling clinical efficiency of belinostat

The anticancer potential of this HDAC inhibitor has been investigated in human bladder cancer cells (*in vitro*) (Table 2) and in an animal model of bladder cancer (*in vivo*) (Table 3) [32]. This type of cancer is a complex disease often, if not optimally managed, related to high mortality and morbidity rates. In this study, 5 days per week treatment of belinostat (100 mg/kg) in animals for 3 weeks reduced tumor weight compared to animals in the control group, which developed at least one episode, with induction of an elevated expression of p21^{WAF1}.

Table 2
In vitro investigations of Cellular and molecular anticancer mechanisms of Belinostat.

Tumor models	Methods	Anticancer mechanisms	References
5637, T24, J82, and RT4 urothelial cell lines	1–5 μM belinostat for 48 h	Inhibited bladder cancer cell growth Induced cell cycle arrest IC_{50} ranging from 1.0 to 10.0 μM	[32]
Prostate cancer cell lines (22Rv1, PC-3, LNCaP, and DU145)	Cell cycle analysis Immunoblotting	Inhibited cell growth (IC_{50} < 1.0 μM) Increased cell percentage and the rate of subG$_1$ DNA Induced G$_2$/M arrest	[33]
Human colon cancer cell line HCT116	1 and 10 μM belinostat 2-D gel electrophoresis LC-MSMS analysis	Induced hyperacetylation of the core histones (H2A, H2B, H3, and H4) Belinostat (1 mM) led to cell survival (80%) Inhibited clonogenic cell growth of HCT116 cells	[40]
Mantle cell lymphoma (MCL) (HBL-2, Jeko-1, and Granta-519) lines	1 nmol/L to 10 μmol/L Western blot analysis Flow cytometry	Exhibited concentration-dependent cytotoxicity Belinostat + Bortezomib induced potent mitochondrial membrane depolarization and apoptosis Belinostat + Bortezomib enhanced disruption of Δψm in MCL lines	[34]
Human pancreatic ductal adenocarcinoma (PDAC) cells	Immunoblot analysis Flow cytometry	Induced a significant dose-dependent decrease in cell proliferation Induced dose dependent apoptosis Enhanced the apoptotic effect of gemcitabine Augmented p21$^{Cip1/Waf1}$ expression	[35]
1 non-tumor prostate epithelial (PrE) cell and 14 PC-a cells	Apoptosis and cell cycle analysis Western blot analysis	0.5 ≤ IC_{50} ≤ 2.5 μM Increased histone H3 and H4 acetylation Induced G$_2$/M arrest Increased apoptosis percentage	[36]
Human promyelocytic leukemia NB4 and HL-60 cells	0.2–0.5–1 μmol/L Flow cytometric analysis Trypan blue exclusion test Annexin-V and PI Western blot analysis	Inhibited cell proliferation Induced cell apoptosis Belinostat + Retinoic acid accelerated cell differentiation to	[14]

(*continued on next page*)

Table 2 (*continued*)

Tumor models	Methods	Anticancer mechanisms	References
		granulocytes Induced acetylation of histone H3 and H4 Induced NB4 cell death Activated caspase 3 Reduced cyclin A2 and p27 levels	
Human renal cell carcinoma cell lines (SN12C and TK10)	MTS assay Western blot analysis Flow cytometry	Belinostat + 5-Fluorouracil inhibited the growth of renal cancer cells by blocking the induction of thymidylate synthase (TS) and the induction of ROS-induced DNA damage Belinostat + 5-Fluorouracil increased cleaved caspase-3 and 9 levels in TK-10 and SN12C cells Belinostat + 5-Fluorouracil increased the subG$_1$ population	[37]
Diffuse Large B-cell lymphoma (DLBCL) cell lines	Flow cytometry Western blot analysis Immunocytochemistry Annexin V/PI assay	Belinostat + Vincristine induced an additive cell death Belinostat + Paclitaxel induced high levels of cytotoxicity Reduced vincristine-induced SAC activation and effectively induced apoptosis Inhibited microtubule polymerization Prevented vincristine-induced formation of polyploid cells	[11]
Non-small cell lung cancer (NSCLC) cell lines, A549 and H2444	MTS assay Annexin V-APC and propidium iodide (PI) Western blot analysis	Belinostat + Seliciclib (CDK inhibitor) reduced cell proliferation (IC_{50} = 3.67 ± 0.80 μM) in p53 wild-type A549 cells Increased cell apoptosis Induced cell cycle arrest Formed cleaved caspase-3, PARP, and caspase-8 Belinostat + Seliciclib induced formation of truncated fragment (tBID) and down-regulation of anti-apoptotic Mcl-1 and XIAP	[41]

Table 2 (*continued*)

Tumor models	Methods	Anticancer mechanisms	References
Human glioblastoma cell lines LN-229 and LN-18	2 μmol/L belinostat for 48 h MTT assay Cell morphological analysis Detection of apoptosis Cell cycle analysis	Induced 70% apoptosis in LN-229 cells Induced 28% apoptosis in LN-18 cells IC_{50} = 0.21 μmol/L for LN-229 cells IC_{50} = 0.30 μmol/L for LN-18 cells Up-regulated pro-apoptotic genes in glioblastoma cells At a dose of 2 μmol/L, induced the most significant suppression of cell proliferation	[42]
Lung squamous cell carcinoma cells	Cell viability assay Western blot analysis Apoptosis assay qRT-PCR	Down-regulated the MAPK pathway Induced apoptosis Blocked SOS/MAPK activation in cisplatin-resistant cells Belinostat + Cisplatin inhibited ERK phosphorylation and exhibited strong synergistic cytotoxicity	[43]
Glucuronidation of SN-38	100 and 200 μmol/L LC-MS/MS method	Inhibited, dose-dependently, SN-38 glucuronidation Decreased the intrinsic clearance Inhibited both heterozygous and homozygous genotypes	[44]
MDA-MB-231 breast cancer cells	Annexin V-FITC qRT-PCR Western blot analysis Wound healing assay	Belinostat + 17-AAG improved viability inhibition and apoptosis rate of breast cancer cells Belinostat + 17-AAG suppressed cell migration Belinostat + 17-AAG enhanced the acetylation of HSP90 Decreased mRNA expression levels of TEA domain family proteins	[45]
Breast cancer MCF-7 cell line	XTT assay qRT-PCR	IC_{50} = 5 μM for 48 h Inhibited cell proliferation Decreased cancer cell number Down-regulated apoptosis-related gene (CASP3, CASP9 and P53) expression	[46]

Indeed, belinostat was previously found to induce p21^{WAF1} in several cell lines (melanoma, prostate, breast, lung, colon, and ovary). In fact, this cyclin-dependent kinase inhibitor is involved in events inducing cell cycle arrest and apoptosis [13].

Table 3
In vivo investigations of Cellular and molecular anticancer mechanisms of Belinostat.

Tumor models	Methods	Anticancer mechanisms	References
Ha-*ras* transgenic mice	100 mg/kg, i.p., 5 days each week for 3 weeks	Reduced mice bladder weights Decreased hematuria Induced $p21^{WAF1}$, HDAC core and cell communication genes	[32]
PC-3 cells in a xenograft mouse model	A 3-week treatment Belinostat administered (i.p.) at a dose of 20 or 40 mg/kg, twice daily, or at a dose of 40 mg/kg, three times daily	Inhibited tumor growth by up to 43% No lung metastases were recorded in animals given belinostat	[33]
HBL-2 cells in a xenograft mouse model	Belinostat administered (35 mg/kg/day) for 7 days Bortezomib administered i.p. (0.5 mg/kg) on days: 1, 4, 8, and 11	Belinostat + Bortezomib enhanced efficacy compared with either drug alone	[34]
A chimeric mouse model	Tumourigenicity study Immunohistochemistry	Reduced growth in both subcutaneous and intrapancreatic tumors Reduced xenograft tumor volumes	[35]
PC-3 and 22rv1 cells in a xenograft mouse model	Belinostat (20 and 40 mg/kg) administered (i.p.) twice daily	Slowed tumor growth of PC-3 and 22rv1 xenografts	[36]
SN12C cells in a xenograft mouse model	Treatment initiated on day 0 when tumors were about 100 mm^3	Reduced tumor volume and weight Increased g-H2AX and Ac-H3 levels	[37]
Model of Hepa 129 carcinoma implanted in immunocompetent animals	Immunoprofiling Flow cytometry IFN-γ ELISPOT assay	Improved anti-tumor therapeutic response induced by the anti-CTLA4 checkpoint inhibitor Inhibited tumor growth Belinostat + Anti-CTLA-4 induced a complete cessation in tumor growth	[38]
A murine hepatocellular carcinoma model	ELISPOT Western blot analysis Flow cytometry	Improved the antitumor activity of anti-CTLA-4 Enhanced antitumor immunity and decreased Tregs in anti-CTLA-4–treated mice Enhanced IFN-γ production by antitumor T-cells Belinostat + CTLA-4 and PD-1 induced inhibition led to complete tumor rejection Belinostat + Anti-CTLA-4 modulated PD-1 expression in Tregs and effector T-cells associated with higher effector cytokine levels	[39]

Belinostat has demonstrated promising clinical effectiveness through its direct inhibition of HDACs. However, the molecular mechanisms underlying its anticancer effectiveness are complex and involve several secondary events that are dependent on HDAC inhibition. In the following section, we discuss the major advancements regarding the molecular mechanisms behind belinostat's anticancer effectiveness. Tables 2 and 3 summarizes various research studies conducted *in vitro* and *in vivo* that investigate the potential mechanisms of belinostat for different types of cancer.

In the case of prostate cancer (PC), ranked the second most diagnosed cancer in the world and the sixth deadliest cancer for men [47], this molecule inhibited tumor growth *in vitro* ($IC_{50} < 1.0$ μM) and *in vivo* (43%) with a G_2/M arrest as well as an increase in the percent of cells and the content of subG_1 DNA [33]. It can be deduced from these results that belinostat inhibits the migration of prostate cancer cells, reduces its metastases and the expression of the oncogenic proteins associated therewith. The same findings were observed in a subsequent study using two models (*in vitro* and *in vivo*) of androgen-dependent and androgen-independent PC in addition to an increase in acetylation of histones H3 and H4 [36]. Indeed, the cell death mechanisms of this hydroxamate HDAC inhibitor were G_2/M cell cycle arrest, p21 and p27 expression, caspases-8 and −9 activity, Bcl-Xl and Bcl2 down-modulation, p53 acetylation, and survivin and Akt/pAkt reduction (Fig. 2). Thus, the authors associated this anti-tumor efficacy of belinostat with the androgen receptor (AR).

In order to study the impact of this agent in the treatment of colon cancer (CC), Beck et al. [40] adopted the proteomic approach of examining the differentially expressed proteome of a cell following drug treatment using 2D gel electrophoresis. Therefore, a dose-dependent inhibition of HCT116 CC cell line growth was observed with detection of 45 proteins, the majority of which are implicated in anti-apoptoic and apoptoic phenomena and which are associated with pro-oncogenic transcription factors such as AP1 components, p53, and JUN.

On the other hand, the effectiveness of this substance has been tested (*in vivo* and *in vitro*) on the growth of pancreatic ductal adenocarcinoma (PDAC) cells ([35]. The results showed an inhibition of cell growth in both models, an induction of tumor cell apoptosis in a dose-dependent manner, a reduction in tumor volume (*in vivo*), a reinforcement of the apoptotic effect of a chemotherapy drug (gemcitabine), and an increase in the expression of $p21^{Cip1/Waf1}$, a major protein involved in the regulation of cell cycle.

In contrast, anti-leukemic therapy based on epigenetic drugs is little investigated. This is why [14] evaluated the activity of belinostat, alone or combined with all-*trans*-retinoic acid (RA), against acute myeloid leukemia (AML). Using promyelocytic leukemia cells (NB4 and HL-60), the authors recorded cell cycle arrest in G_0/G_1 or S, PARP-1 degradation, and caspase-3 activation with changes in survivin and cyclin A2 and E1 expression. In addition, this epigenetic drug reduced the expression of HDAC1 and HDAC2 as well as that of the components of the core polycomb repressive complex 2 (PRC2), namely SUZ12 and EZH2, with a dose-dependent augmentation in histone (H3 and H4) acetylation. Additionally, the combination of belinostat with RA potentiated granulocyte differentiation and changed the expression of C/EBPε, C/EBPα, and CD11b.

Moreover, this combination therapy has been adopted in the treatment (*in vitro* and *in vivo*) of renal cell carcinoma that, despite several therapeutic strategies, represents a poor clinical outcome [37]. Indeed, in addition to the potentiation of the antitumor effect of 5-fluorouracil, the combination of these two drugs (belinostat + 5-fluorouracil) synergistically inhibited the growth of renal tumor cells by blocking the production of thymidylate synthase (TS) and reactive oxygen species (ROS) induced DNA damage. In addition, this combination reduced tumor weight and volume with increased levels of Ac-H3 and γ-H2AX (*in vivo*).

Furthermore, co-administration of belinostat with vincristine, an alkaloid used in the treatment of cancers, has been used in diffuse large B-cell lymphoma (DLBCL) therapy, an aggressive malignancy, using DLBCL cell lines [11]. In fact, early mitotic arrest of cell lines sensitive to belinostat cytotoxicity occurred before apoptosis, whereas resistant ones complete mitosis with arrest in G_1 phase. In belinostat-resistant cells, low-dose vincristine was combined with the studied HDACi to induce mitotic arrest. Thus, this association triggered a potential apoptotic effect with down-regulation of MCL-1 expression and up-regulation of BIM expression. In parallel, belinostat prevented the polyploidy responsible

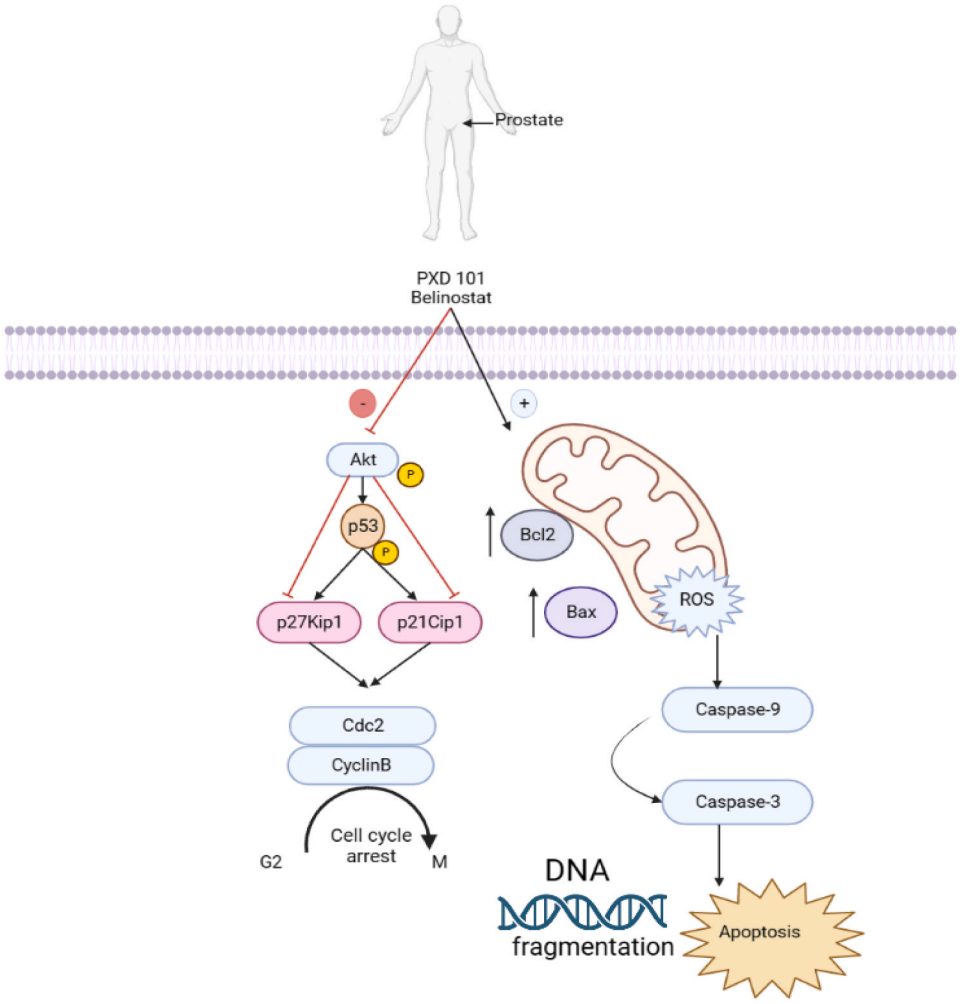

Fig. 2. Illustration of the anticancer mechanisms of belinostat (PXD 101) by enhancing apoptosis and inducing G2/M cell cycle arrest in prostate cancer cells. *G2/M cell cycle arrest, p21 and p27 expression, caspases-8 and − 9 activity, Bcl-Xl and Bcl2 down-modulation, p53 acetylation, and survivin and Akt/pAkt decrease were the cell death mechanisms of belinostat.* Abbreviations: **ROS**, reactive oxygen species; **Bax**, Bcl-2-associated X protein; **Bcl-2**, B-cell lymphoma 2; **Akt**, protein kinase B; **cdc2,** Cyclin-dependent kinase 2.

for resistance to vincristine, whereas the latter sensitized DLBCL cells to belinostat cytotoxicity.

On the other hand, lung cancer is divided into two main types; SCLC and NSCLC accounting for 85% of all cases of lung cancers [48]. In 2016, Ong et al.[41] suggested a new treatment strategy for NSCLC (*in vitro*) by combining belinostat and seliciclib (CDK inhibitor). The combination of these two drugs reduced cell proliferation (IC_{50} = 3.67 ± 0.80 μM), increased apoptosis, up-regulated BID and p53 levels, and down-regulated XIAP and Mcl-1 levels with the formation of cleaved PARP, caspase-3 and − 8.

In the same context, and to better understand the epigenetic mechanism of belinostat cytotoxicity in lung cancers, Kong et al. [43] targeted lung squamous cell carcinoma (SCC) cell lines. Consequently, this pan-HDACi sensitized SCC cells, induced apoptosis, down-regulated the MAPK pathway and up-regulated the transcription of FBXW10 and FBXO3, proteins targeting son of sevenless (SOS), which regulates the MAPK pathway for proteasome-induced degradation.

In addition, HDAC inhibition has been used against glioblastoma in order to overcome the major obstacles already found in its treatment, namely anaplasticity, high heterogeneity, and migration of its cells, justifying its poor survival prognosis [42]. It is one of the most abundant glial tumors. To our knowledge, Kusaczuk et al. [42] were the only ones who tested the impact of belinostat treatment on human glioblastoma cells, LN-18 and LN-229. They revealed that after 48 h of stimulation at a dose of 2 μmol/L of this molecule, apoptosis of 70 % and 28 % was observed in LN-229 and LN-18 cells, respectively. This effect was associated with an overexpression of certain pro-apoptotic genes (*p21*, *Chop*, *Bim*, and *Puma*) with decreased cell number in S phase, in a cell type-specific manner. Taken together, these results indicate that belinostat acts selectively with complex and variable mechanisms.

In combating cancer, combination therapy is often more effective than monotherapy [49]. While drug-drug interactions can induce serious signs of toxicities such as neutropenia and diarrhea [50,51]. Irinotecan is considered to be one of the effective anticancer agents with high toxicity potential and it is mainly transformed into SN-38, an active metabolite. Glucuronidation of this metabolite forms SN-38 glucuronide (deactivated form) using uridine diphosphate glucuronosyltransferase (UGT) enzymes. Indeed, [44] showed that belinostat (100 and 200 μmol/L) inhibits glucuronidation of SN-38 by UGT1A1, which significantly decreases V_{max} and CL_{int}.

On the other hand, the combination of HDACi with immunotherapeutic protocols may provide enhanced therapeutic benefits. In this sense, Llopiz and collaborators carried out two *in vivo* studies to investigate the therapeutic potential of belinostat combined with (anti-CTLA-4/anti-PD-1) checkpoint inhibitors in the treatment of hepatocellular carcinoma (HCC) [38,39]. In a murine HCC model, the authors recorded an improvement in the anti-cancer activity of anti-CTLA-4, a decrease in tumor volume, a decrease in regulatory T-lymphocytes, and an increase in the synthesis of IFN-γ by anticancer T-cells. Interestingly, the combined therapy completely stopped tumor growth.

Recently, some studies have evaluated the effect of belinostat combined [45] or alone [46] in breast cancer (BC) treatment. For the first study, the combination of this drug with 17-allylamino-demethoxygeldanamycin (17-AAG) against MDA-MB-231 BC cells showed an

improvement in apoptosis rates and tumor cell viability with suppression of tumor cell migration compared to groups treated with either drug alone. The anti-migration effect observed in the combined treatment was explained by the enhancement of the phosphorylation of YY1-associated protein 1 and by the decrease in the mRNA expression of TEAD family proteins. Indeed, transcription factor TEAD is involved in developmental processes and has a major role in tumorigenesis promotion [52]. The apoptotic effect recorded in this study was confirmed, in the second study, by belinostat as monotherapy against MCF-7 BC cells [46]. The results showed that this substance reduces the number of cancer cells (IC_{50} = 5 μM) and down-regulates the expression of apoptosis genes.

Taken together, these data indicate that belinostat alone or combined with other chemotherapeutics represents a promising therapeutic strategy warranting further clinical evaluation.

5. Drug development update on Belinostat

Belinostat was approved following a drug development study initiated in the late 1990s by the biotechnology company Prolifix Ltd. It is the outcome of a careful engineering strategy that first involved known natural compounds and synthetic inhibitors at the time the project was started and then expanded to include the development of structural information [53]. Belinostat, which has received marketing authorization in the USA for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL) [8], was ultimately selected and developed following a search for HDAC inhibitors [21]. Development of the Belinostat formulation focused primarily on the creation of an intravenous (IV) formulation [53]. In this sense, Spectrum Pharmaceuticals, Inc. and Onxeo SA, the two firms driving the ongoing development of belinostat, are currently conducting additional company-sponsored studies in which belinostat, in combination with other drugs, particularly epidrugs, could enhance the management of patients affected by cancer [53]. Belinostat is among the agents (vorinostat, romidepsin, tucidinostat and panobinostat) with a confirmed HDAC-mediated mechanism of action for the treatment of various indications that have been approved in 2020 [54].

An extensive spectrum of inhibitor agents being investigated focused on epigenetic modulators including belinostat and others (such as azacitidine, decitabine, vorinostat, romidepsin, panobinostat, tazemetostat, enasidenib and ivosidenib, *etc.*) and immune checkpoints (atezolizmab, avelumab, cemiplimab, durvalumb, ipilimumab, nivolumab and pembrolizmab, *etc.*).) as well as immune checkpoints (atezolizmab, avelumab, cemiplimab, durvalumb, ipilimumab, nivolumab and pembrolizmab, *etc.*) to promote anti-cancer therapy responses [55]. As demonstrated in the study conducted by Johnston PB et al., for patients with newly diagnosed PTCL, belinostat in combination with doxorubicin, vincristine, cyclophosphamide, and prednisone was well tolerated and had a high frequency of clinical responses [56].

However, drug combinations, particularly HDACis, are effective in increasing the responsiveness of FDA-approved drugs, but they often lead to increased toxicities associated with the duration of treatment. This examination reveals that the variety of indications is increasing thanks to several clinical trials and that a number of fields of preclinical research are also seeing great prospects [54].

6. Conclusions and perspectives

Here, the molecular pathways involved in belinostat's cancer treatment-related HDAC inhibition were emphasized. Belinostat, an HDAC inhibitor, is inferred to have considerable power to modify signaling pathways that indirectly mediate cell cycle regulation and result in cell death. Belinostat may therefore be employed as a chemopreventive medication in the treatment of various tumors as a result of these findings. However, we had some recommendations. Belinostat should initially be used in targeted therapy alongside other drugs used in chemotherapy while investigating any potential beneficial interactions. In addition, greater comprehension of the intracellular arrangement, notably the processes that control tumor transformation, might be rendered possible by the mechanistic understanding of the anticancer effect of these compounds, whether belinostat or other chemicals. The study of these types of compounds currently used in the clinic will help to expand our knowledge of the mechanism and the cellular hierarchy between healthy and tumor cells. Indeed, chemotherapy drugs have enabled scientists to better distinguish the function of cells by considering that the suppression of a molecular process remains a consequence of successive occurrences favoring tumor initiation and progression.

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Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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