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Review

# Molecular mechanistic pathways underlying the anticancer therapeutic efficiency of romidepsin

Nasreddine El Omari [a], Learn-Han Lee [b,*], Saad Bakrim [c], Hafiz A. Makeen [d], Hassan A. Alhazmi [e,f], Syam Mohan [e,g,h], Asaad Khalid [e,i,**], Long Chiau Ming [j,*], Abdelhakim Bouyahya [k]

[a] Laboratory of Histology, Embryology, and Cytogenetic, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat 10100, Morocco
[b] 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
[c] Geo-Bio-Environment Engineering and Innovation Laboratory, Molecular Engineering, Biotechnology and Innovation Team, Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Agadir 80000, Morocco
[d] Pharmacy Practice Research Unit, Clinical Pharmacy Department, Faculty of Pharmacy, Jazan University, Jazan, Saudi Arabia
[e] Substance Abuse and Toxicology Research Center, Jazan University, P.O. Box: 114, Jazan, Saudi Arabia
[f] Department of Pharmaceutical Chemistry and Pharmacognosy, College of Pharmacy, Jazan University, 45142 Jazan, Saudi Arabia
[g] School of Health Sciences, University of Petroleum and Energy Studies, Dehradun, Uttarakhand, India
[h] Center for Transdisciplinary Research, Department of Pharmacology, Saveetha Dental College, Saveetha In-20 stitute of Medical and Technical Science, Saveetha University, Chennai, India
[i] Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum 11111, Sudan
[j] School of Medical and Life Sciences, Sunway University, 47500 Sunway City, Malaysia
[k] Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, Rabat 10106, Morocco

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ABSTRACT

Romidepsin, also known as NSC630176, FR901228, FK-228, FR-901228, depsipeptide, or Istodax®, is a natural molecule produced by the *Chromobacterium violaceum* bacterium that has been approved for its anti-cancer effect. This compound is a selective histone deacetylase (HDAC) inhibitor, which modifies histones and epigenetic pathways. An imbalance between HDAC and histone acetyltransferase can lead to the down-regulation of regulatory genes, resulting in tumorigenesis. Inhibition of HDACs by romidepsin indirectly contributes to the anticancer therapeutic effect by causing the accumulation of acetylated histones, restoring normal gene expression in cancer cells, and promoting alternative pathways, including the immune response, p53/p21 signaling cascades, cleaved caspases, poly (ADP-ribose) polymerase (PARP), and other events. Secondary pathways mediate the therapeutic action of romidepsin by disrupting the endoplasmic reticulum and proteasome and/or aggresome, arresting the cell cycle, inducing intrinsic and extrinsic apoptosis, inhibiting angiogenesis, and modifying the tumor microenvironment. This review aimed to highlight the specific molecular mechanisms responsible for HDAC inhibition by romidepsin. A more detailed understanding of these mechanisms can significantly improve the understanding of cancer cell disorders and pave the way for new therapeutic approaches using targeted therapy.

## 1. Introduction

Epigenetics refers to the intricate interplay between the environment and genes, and is recognized as a fundamental component of developmental biology [1]. Among the various epigenetic mechanisms, histone modification and DNA methylation are critical for regulating essential cellular processes that maintain cellular plasticity and memory [2]. Dysregulation in these pathways is associated with the development of numerous diseases [3].

Histone deacetylases (HDACs) are a group of epigenetic enzymes that

* Corresponding authors.
** Corresponding author at: Substance Abuse and Toxicology Research Center, Jazan University, P.O. Box: 114, Jazan, Saudi Arabia.
*E-mail addresses:* lee.learn.han@monash.edu (L.-H. Lee), akahmed@jazanu.edu.sa (A. Khalid), longchiauming@gmail.com (L.C. Ming).

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play a crucial role in transcriptional regulation, chromatin biology, histone modifications, and epigenetics [4]. HDACs are responsible for inducing transcriptional inactivity in chromatin by removing the acetyl group from histone and non-histone proteins, thereby modulating the expression of target genes [5]. They are also essential for maintaining a dynamic balance of protein acetylation and other post-translational modifications [6].

Genetic mutations or abnormal expression of HDAC proteins have been linked to a range of human disorders, including cancer and inflammatory diseases. Aberrant HDAC activity can lead to irregular expression of oncogenes, resulting in the transformation of normal cells and promotion of cancerous cells [7]. Thus, developing HDAC inhibitors has been a crucial focus in the field, increasing our understanding of how HDACs work and their underlying mechanisms [6].

Inhibition of HDACs using chemical molecules has led to the development of anti-cancer drugs. Several drugs that inhibit HDAC activity have been characterized and designed. Romidepsin, a naturally occurring HDAC inhibitor (Fig. 1), was approved by the FDA in 2009 for the treatment of cutaneous T-cell lymphoma (CTCL) [8]. Although the European Medicines Agency (EMA) rejected it in 2012, it has since undergone clinical testing for the management of acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL).

Romidepsin, a prodrug activated internally by glutathione in cells, has a four-carbon chain between the free sulfhydryl and the cyclic depsipeptide ring, which covalently binds to the single cysteine residue found in the HDAC pocket [9]. Recent research has demonstrated that romidepsin-mediated inhibition of HDACs results in programmed cancer cell death, cycle arrest, induction of cell apoptosis, autophagy, and inhibition of cell proliferation, angiogenesis, and metastasis through multiple signaling pathways [10].

Although romidepsin specifically inhibits HDAC class 1 and class 2, it has shown promise in cancer treatment. However, the underlying mechanisms responsible for its therapeutic effects remain poorly understood. Therefore, this review aimed to shed light on the molecular mechanisms underlying the therapeutic effects of romidepsin as a potent anti-cancer drug.

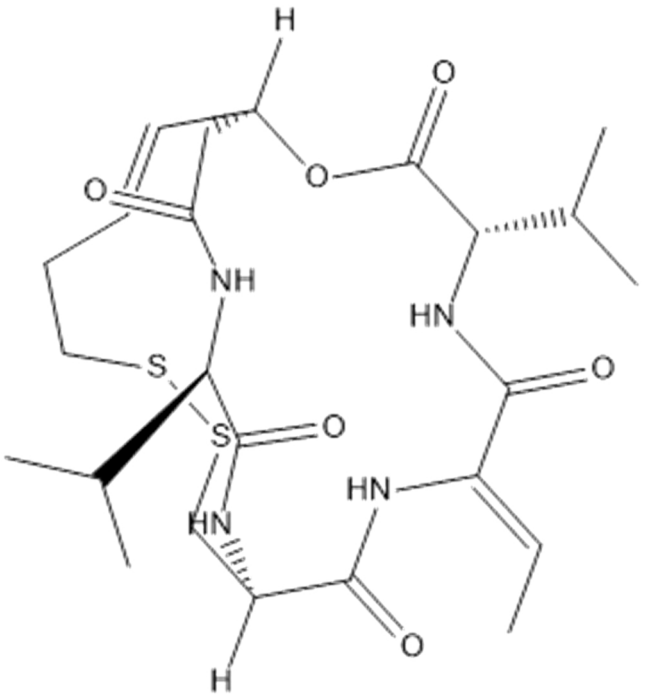


**Fig. 1.** Chemical structure of romidepsin.

## 2. Preclinical studies of romidepsin

Romidepsin is a natural substance constituting the second clinically approved Histone deacetylase inhibitors[8]. It was identified in cultures of a Gram negative bacterium from a Japanese soil sample, called *Chromobacterium violaceum* [11]. However, its synthesis is often complex with poor yield, prompting researchers to find other analogs as accessible alternatives in their synthesis.

Since its FDA approval, several investigations have evaluated the anticancer potential of romidepsin against multiple types of cancers in order to better highlight the precise mechanism of action (Table 1).

In fact, on the A549 lung cancer cell line, this molecule inhibited cell proliferation by modifying the expression of apoptotic proteins and cell cycle regulators [12]. Indeed, at doses of 25 and 50 nM, it induced apoptosis with $G_2$/M phase cell cycle arrest. In addition, it down-regulated the expression of phosphorylated pRb, cyclin B1, and Cdc2/Cdk-1, and up-regulated p21 expression (Fig. 2).

In the same year of its FDA approval, romidepsin's impact on erlotinib anti-tumor activity was evaluated on non-small cell lung cancer (NSCLC) cell lines and NCI-H1299 xenografts [13]. This form of cancer is known to be sensitive to Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) such as gefitinib or erlotinib, however, other wild types are resistant to tyrosine kinase inhibitors. As reported with vorinostat, an enhancement of the anticancer potential of gefitinib against NSCLC was recorded. More interestingly, Zhang et al. [13] showed that romidepsin enhances the anticancer activity of erlotinib by increasing cell sensitivity associated with increased apoptosis (*in vitro*) and by inhibiting NCI-H1299 xenograft tumor growth (*in vivo*). These data corroborate those recorded with vorinostat, confirming the role of therapy combining HDAC and tyrosine kinase inhibitors against NSCLC.

One year later, the anticancer activity of romidepsin was evaluated *in vitro* and *in vivo* as monotherapy against neuroblastoma (NB) cell lines [14]. After 72 h of treatment, this substance ($IC_{50}$ = 1–6.5 ng/mL) inhibited cell proliferation associated with caspase-dependent apoptosis, with a dose-dependent cytotoxic effect. It inhibited tumor growth *in vivo*. In addition, induction of gene expressions such as neurotrophin receptor p75, neurotrophic tyrosine kinase receptor type 1 (NtRK1), and cyclin-dependent kinase inhibitor 1 (p21) was observed.

The following year, Paoluzzi et al. [15] combined romidepsin with another HDAC inhibitor, belinostat, to assess the impact of this combination on the antineoplastic activity of bortezomib against MCL. Therefore, dose-dependent cytotoxicity was noted against three MCL cells. In addition, the triple therapy associating both HDACIs with bortezomib showed a high synergistic effect with mitochondrial membrane apoptosis. In parallel, these effects were related to an increase in the accumulation of acetylated α-tubulin and acetylated histone H3 and a reduction in Bcl-XL and cyclin D1. *In vivo*, supplementation of belinostat with bortezomib showed improved efficacy compared to use of either drug alone. These results suggest the combination of proteasome inhibitors with HDAC inhibitor may constitute a therapeutic option in the treatment of MCL.

Despite the enhanced effects of HDAC inhibitor when used in combination with other anticancer agents, little information is provided on the selection of the most suitable combination agent. Aspirin (acetylsalicylic acid, ASA) was previously suggested to mediate the sensitivity of ovarian cancer cells (OCCs) to HDAC inhibitor [33], with an antiproliferative effect against OCCs by selectively inhibiting cyclooxygenase-1 (COX-1) [34]. In 2010, Son et al. [16] cracked this puzzle by combining HDAC inhibitor, romidepsin, with ASA against two human OCCs; COX-1 negative (sKOV-3) and COX-1 positive (OVCaR-3). Consequently, in COX-1 positive OCCs and in contrast to COX-1 negative OCCs, ASA enhanced the growth inhibitory capacity of romidepsin. In both cell lines, this HDAC inhibitor up-regulated the cell cycle protein p21. Interestingly, the addition of ASA increased p21 expression in only COX-1-positive cells. Taken together, the chemotherapeutic effects of HDAC inhibitors may be enhanced by ASA supplementation in the

**Table 1**
*In vitro* and *in vivo* effects of romidepsin against human cancers.

| Tumour models | Methods | Anticancer mechanisms | Ref. |
|---|---|---|---|
| Lung cancer cell line A549 | MTT assay<br>Flow cytometry analysis<br>Annexin V- FITC binding assay<br>Western blot analysis<br>Caspase activity assay | Decreased expression of Cdc2/Cdk-1, cyclin B1, and phosphorylated pRb<br>Reduced cell survival ($IC_{50}$ = 50 nM)<br>Induced cell cycle arrest by targeting pRb protein<br>Induced cell cycle arrest at $G_2$/M transition<br>Induced apoptosis | [12] |
| Nine non-small cell lung cancer (NSCLC) cell lines<br>NCI-H1299 cells in a xenograft mouse model (*in vivo*) | DNA isolation and PCR<br>MTS assay<br>Apoptosis assay | Increased the sensitivity of Erlotinib synergistically in all NSCLC cell lines<br>Enhanced apoptosis<br>Romidepsin + Erlotinib inhibited cell growth (*in vivo*)<br>Romidepsin $IC_{50}$ = 1.3 – 4.9 ng/mL<br>Erlotinib $IC_{50}$ = 8.6 –115 μM | [13] |
| 7 neuroblastoma (NB) tumor cells (*in vitro*)<br>KCNR NB tumor cells in a xenograft mouse model (*in vivo*) | MTT and MTS assay<br>Immunoblot assay<br>Northern blot analysis<br>Western blot analysis<br>ELISA assay | Inhibited NB cell growth in a dose-dependent manner<br>After 72 h of treatment: $IC_{50}$ = 1 – 6.5 ng/mL<br>Induced apoptosis<br>Decreased N-myc levels and induced p21 in NB<br>Altered growth of KCNR neuroblastoma tumors (*in vivo*) | [14] |
| Mantle cell lymphoma (MCL) lines (HBL-2, Jeko-1, and Granta-519) | Flow cytometry analysis<br>Western blot analysis | Romidepsin + Belinostat exhibited concentration-dependent cytotoxicity<br>Romidepsin + Bortezomib induced potent mitochondrial membrane depolarization and apoptosis<br>Decreased cyclin D1 and Bcl-XL<br>Increased accumulation of acetylated histone H3, acetylated α-tubulin, and Noxa<br>After 24 h of treatment:<br>Romidepsin $IC_{50}$ = 10 – 100 nmol/L<br>Belinostat $IC_{50}$ = 1 – 100 μmol/L | [15] |
| Human ovarian cancer cell lines (OVCAR-3 and SKOV-3) | MTT assay<br>Flow cytometry analysis<br>Western blot analysis<br>Transient transfection and luciferase assays<br>RT-PCR | Acetylsalicylic acid enhanced the growth inhibitory effects of romidepsin in COX-1 positive ovarian cancer cells<br>Robustly induced up-regulation of the cell cycle control protein p21 in both cell lines<br>Romidepsin + Acetylsalicylic acid augmented p21 expression in the COX-1 positive cells | [16] |
| Ovarian cancer cell lines (*in vitro*)<br>SKOV-3 cells in a xenograft mouse model (*in vivo*) | Sulphorhodamine B (SRB) assays<br>Combination index method<br>Western blot analysis<br>Immunofluorescence<br>Immunohistochemistry | Enhanced the cytotoxic effects of cisplatin<br>Romidepsin + cisplatin reduced tumor weights and volumes (*in vivo*)<br>Decreased mib-1 and increased cleaved-caspase 3 expression levels | [17] |
| Ovarian cancer cells (*in vitro*)<br>Ovarian cancer cells in a xenograft mouse model (*in vivo*) | Flow cytometry analysis<br>Western blot analysis | Exerted the most potent growth inhibitory effects in the majority of cells<br>Romidepsin + 5-azacytidine enhanced the growth-inhibitory effects of cisplatin (*in vitro*)<br>Romidepsin + 5-azacytidine enhanced the growth-inhibitory effects of cisplatin (*in vivo*) | [18] |
| H9, HH, and HuT-78 TCL cell lines (*in vitro*)<br>H9 cells in a xenograft mouse model (*in vivo*) | RNA analyses<br>Flow cytometry analysis<br>Bioluminescence imaging<br>Morphology and immunohistochemistry | Romidepsin + Pralatrexate exhibited concentration-dependent synergism against a panel of TCL cell lines<br>Romidepsin + Pralatrexate showed increased efficacy compared to either drug alone (*in vivo*) | [19] |
| Human EBV-negative diffuse large B-cell lymphoma (DLBCL) cells (SU-DHL4, U2932, and OCI-LY7) and EBV-positive DLBCL cell line<br>U2932 and 85 U2932/EBV cells in a xenograft mouse model (*in vivo*) | MTT assay<br>Flow cytometry analysis<br>Western blot analysis<br>Immunofluorescence staining | Exerted strong anti-tumor activity in EBV-positive DLBCL *via* the inhibition of both LMP1 and c-myc | [20] |
| MT-1 cells in a xenograft mouse model (*in vivo*) | Annexin-V-FITC/PI<br>Western blot analysis<br>Flow cytometry analysis<br>Cell cycle analysis<br>ELISA assay | Romidepsin + Bortezomib + Compound E enhanced the antitumor efficacy as assessed by tumor size, tumor markers in the serum, and survival of the MT-1 tumor bearing mice | [21] |
| Hut-78, human cutaneous T-cell Lymphoma (CTCL)<br>Karpas-299, human anaplastic large cell lymphoma (ALCL) | Romidepsin (0.5–25 nM)<br>Lenalidomide (1–100 μM)<br>Clonogenic assays<br>Annexin V/PI assay<br>Flow cytometry analysis<br>Western blot analysis | $IC_{50}$ = 0.038 – 6.36 nM for Hut-78 cells<br>$IC_{50}$ = 0.44 – 3.87 nM for Karpas-299 cells<br>Romidepsin + Lenalidomide induced a synergistic effect in Hut-78 cells and an additive effect in Karpas-299 cells<br>Romidepsin + Lenalidomide induced apoptosis, increased ROS production, and activated caspase-8, − 9, − 3, and PARP | [22] |
| Raji, Raji-2R and Raji-4RH cells (*in vitro*)<br>Tumor cells in a xenograft mouse model (*in vivo*) | 10 ng/mL (*in vitro*)<br>Trypan blue (Thermofisher) exclusion<br>Flow cytometry analysis<br>NKG2D blocking<br>Cell cycle analysis | Inhibited both rituximab-sensitive and -resistant BL cell proliferation (*in vitro*)<br>Induced cell death in rituximab-sensitive Raji and cell cycle arrest in rituximab-resistant Raji-2R and Raji-4RH<br>Romidepsin + anti-CD20 CAR exPBNK induced cell death (*in vitro*), reduced tumor burden, and enhanced survival in humanized BL (*in vivo*) | [23] |
| Human HCC cell lines (Huh-7, HCC-LM3, SK-Hep-1, and HepG2)<br>Huh7 cells in a xenograft mouse model (*in vivo*) | CCK-8 assay<br>Flow cytometry analysis<br>Western blot analysis | Inhibited cell proliferation<br>Induced $G_2$/M cell cycle arrest<br>Promoted apoptosis<br>Suppressed tumor growth (*in vivo*) | [10] |

(*continued on next page*)

**Table 1** (*continued*)

| Tumour models | Methods | Anticancer mechanisms | Ref. |
|---|---|---|---|
| | Immunohistochemistry<br>0.5 and 1 mg/kg (*in vivo*) | | |
| Human NSCLC A549 cells and Jurkat T cells | Romidepsin (7 nM)<br>Cetaminophen (3 mM)<br>Annexin V-FITC/PI<br>Western blot analysis | Romidepsin + Acetaminophen exerted apoptosis and cytotoxicity due to caspase-3 activation<br>Romidepsin + Acetaminophen increased production of chemokines<br>$IC_{50}$ = 10 nM | [24] |
| Two Cholangiocarcinoma (CCA) cell lines: CCLP-1 and HCCC-9810 (*in vitro*)<br>CCLP-1 cells in a xenograft mouse model (*in vivo*) | CCK-8 assay<br>Annexin V/PI<br>Flow cytometry analysis<br>Western blot analysis<br>Immunohistochemistry | Inhibited the proliferation of cholangiocarcinoma cells<br>Induced $G_2$/M cell cycle arrest<br>Promoted cell apoptosis<br>Reduced cholangiocarcinoma growth (*in vivo*) | [25] |
| Human urothelial carcinoma (UC) J82, T24, SW780, and oncogenic H-Ras(V12)-expressing J82- Ras cells<br>Romidepsin + Gemcitabine + Cisplatin | MTT assay<br>Immunoblot analysis<br>Annexin-V apoptosis assay<br>Clonogenic assay<br>CDX model | Synergistically induced ROS- and ERK-Nox-dependent cell death<br>Synergistically induced death and suppressed drug resistance in SW780 cells<br>Controlled J82-Ras CDXs | [26] |
| Biliary tract cancer (BTC) cells (CCC-5, EGI-1, and TFK-1) | Western Blot analysis<br>Immunohistochemistry | Induced apoptosis in BTC cells, reduced HDAC activity and increased acetylation of histone 3 lysine 9<br>$IC_{50}$ = 3 – 15 nM | [27] |
| Human bladder cancer cells (UMUC-3, T-24, and J-82) (*in vitro*)<br>MBT-2 cells in a xenograft mouse model (*in vivo*) | Romidepsin (0.5 mg/kg)<br>Simvastatin (15 mg/kg)<br>CCK-8 assay<br>Flow cytometry analysis<br>Western blot analysis | Inhibited tumor growth (*in vitro*)<br>Romidepsin + Simvastatin induced histone acetylation by activating AMPK<br>Romidepsin + Simvastatin decreased the expression of HDACs<br>Romidepsin + Simvastatin increased PPAR-γ expression, leading to ROS production | [28] |
| HCC in a xenograft mouse model (*in vivo*) | Quantitative gene expression analysis<br>Immunohistochemistry<br>RT-qPCR | Suppressed HCC with correlation to up-and down-regulation of *Bmp2* and *Bmp7* ligands<br>Significantly elevated Bmp-inhibitor *Smurf2* and Bmp-target gene *Id3*<br>Increased expression levels of ligands *Jag1/Dll4*<br>Decreased receptor *Notch2* expression | [29] |
| RD (ERMS) and RH30 (AMRS) human cell lines<br>Mouse RH30 xenografts (*in vivo*) | Annexin V/PI assay<br>Flow cytometry analysis<br>Western blot analysis<br>Irradiation delivered by an x-6 MV photon linear accelerator<br>1.2 mg/kg (*in vivo*) | Reversibly down-regulated class I HDACs expression and activity<br>$IC_{50}$ = 1.4 ± 0.02 nM in RD<br>$IC_{50}$ = 0.6 ± 0.06 nM in RH30<br>Induced oxidative stress, DNA damage and a concomitant growth arrest<br>Romidepsin + RT reduced tumor mass (*in vivo*) | [30] |
| Mouse colon cancer cell lines CT26 and MC38 (*in vitro*)<br>CT26 cells in a xenograft mouse model (*in vivo*) | Annexin V-FITC<br>Flow cytometry analysis<br>RT-qPCR<br>Western blot analysis | Inhibited proliferation in CT26 and MC38 cells<br>Induced $G_0/G_1$ cell cycle arrest<br>Increased apoptosis in CT26 and MC38 cells<br>Increased PD-L1 expression (*in vivo* and *in vitro*)<br>Increased the percentage of $FOXP3^+$ regulatory T cells (Tregs) (*in vivo*)<br>Decreased the ratio of Th1/Th2 cells and the percentage of IFN-γ + $CD8^+$ T cells (*in vivo*) | [31] |
| Human ovarian serous cystadenocarcinoma cell, SKOV3 | Flow cytometry analysis<br>ELISA assay<br>Lactate dehydrogenase (LDH) release assay | Increased NKG2DL expression on the surface of SKOV3<br>Enhance the killing ability of NKG2D-CAR-T cells against ovarian cancer cells | [32] |

treatment of ovarian cancer *via* increased p21 expression.

In the same context, Wilson and collaborators carried out two successive studies evaluating *in vitro* and *in vivo* the effect of the combination of romidepsin with cisplatin [17] as well as with DNMTi 5-azacytidine on the effect of cisplatin [18] in the treatment of ovarian cancer.

In the first study [17], romidepsin alone enhanced the cytotoxicity of cisplatin, while their combination stimulated cell death caused by DNA damage, indicated by increased expression of p53-binding protein 1 (53BP1), DNA repair and recombination protein RAD51, and phosphorylated H2A histone family member X (pH2AX). *In vivo*, tumor volume and weight in mice were reduced by the combination of both agents with increased expression of cleaved caspase-3. In the second study [18], the combination of epigenetic agents (romidepsin plus 5-azacytidine) enhanced *in vitro* and *in vivo* the growth inhibitory capacity of cisplatin by resensitizing OCCs to this platinum analogue. This effect was attributed to pH2AX activation.

In 2015, another therapeutic option combining romidepsin and the antifolate pralatrexate was suggested against T-cell lymphomas, which are diseases with a poor prognosis [19]. Pralatrexate is used in the treatment of patients with refractory or relapsed T-cell lymphomas [35]. Indeed, this combination showed a dose-dependent synergistic effect on a panel of T-cell lymphomas cells, *in vitro* and *in vivo*, compared to each drug alone.

Additionally, in a preclinical model of T-cell lymphomas, Cosenza et al. [22] evaluated the effect of the interaction between romidepsin and an immunomodulatory agent, lenalidomide. They revealed additive and synergistic effects *in vitro* with increased production of ROS, induction of apoptosis, and activation of caspase-3, − 8, − 9, and PARP. Apoptosis was mediated by dephosphorylation of STAT3, MAPK/ERK and AKT pathways.

On the other hand, the anticancer potential of romidepsin alone was investigated against Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL), a disease with a poor clinical prognosis [20]. Therefore, *in vitro* and *in vivo* cytotoxic effects were observed against EBV-positive DLBCL cells *via* dual inhibition of c-myc and latent membrane protein-1 (LMP1) and activation of the caspase cascade.

As previously investigated in the combination of vorinostat with bortezomib in the treatment of colorectal cancer and multiple myeloma (MM), Yu et al. [21] evaluated the efficacy of a treatment combining romidepsin with this proteasome inhibitor against adult T-cell leukemia (ATL), as well as the underlying mechanism of action. Indeed, in a mouse model of human ATL, the survival of mice carrying leukemia was prolonged and tumor growth was inhibited by each agent alone, whereas the combination of romidepsin with bortezomib synergistically enhanced this anti-tumor efficacy.

Another combination of this HDAC inhibitor was tested (*in vitro* and *in vivo*) with anti-CD20 chimeric antigen receptor (CAR) expanded peripheral blood natural killer (exPBNK) against Burkitt's lymphoma (BL) sensitive and resistant to rituximab [23]. As result, romidepsin inhibited cell proliferation (*in vitro* and *in vivo*) and induced cell death and cell cycle arrest *in vitro*, with induction of expression of NKG2D (Natural Killer Group 2, Member D) ligands MICA/B. Interestingly, its combination with the anti-CD20 CAR exPBNK improved the aforementioned effects, by increasing the survival of mice carrying BL cells, reducing their tumor burden, and inducing cell death. This suggests that this HDAC inhibitor may potentiate the anti-CD20 CAR exPBNK and NK effect in the management of rituximab-sensitive and resistant BL.

In the same year, Sun et al. [10] tested the anticancer effect of romidepsin against hepatocellular carcinoma (HCC) (*in vitro* and *in vivo*) to better understand the mechanism involved. In human HCC cell lines, romidepsin induced apoptosis and $G_2$/M phase cell cycle arrest in a time- and dose-dependent manner. Apoptosis and $G_2$/M phase arrest were mediated by activation of JNK/c-Jun/caspase-3 and Erk/cdc25C/cdc2/cyclinB pathways, respectively (Fig. 2). In addition, this substance reduced tumor size in mice xenografted with HCC cells.

With the aim of better understanding the molecular mechanism of this substance in the treatment of this type of carcinoma, a recent *in vivo* study investigated its effect on the expression levels of the constituents of the Notch and Bmp signaling pathways, involved in the hepatocarcinogenesis [29]. For the Bmp pathway, a significant correlation was observed between the suppression of HCC by romidepsin and the down-regulation of *Bmp7* ligands and the up-regulation of *Bmp2* ligands, with an elevation of the target gene of Bmp *Id3* and the Bmp-inhibitor *Smurf2*. For the Notch pathway, treatment with romidepsin decreased the expression of the *Notch2* receptor and increased that of ligands *Jag1/Dll4*. The anticancer activity of this HDAC inhibitor was correlated with down- and up-regulation of the transcription factors SRY-related high-mobility-group box-9 (*Sox9*) and Kruppel-like factor-4 (*Klf4*), respectively, as well as increased expression of hairy and enhancer of split-1 (*Hes1*) target.

In view of the beneficial effects obtained from the therapy of NSCLC cells with romidepsin, Lee et al. [24] combined its effect with that of acetaminophen against the A549 NSCLC cell line. They found that this combination induces significant apoptosis and cytotoxicity *via* caspase-3 activation *in vitro*. In addition, this combination induced increased secretion of chemokines promoting the migration of activated T-cells in cancer cells, which induced important cytotoxicity in A549 cells. It can be inferred that the interaction between romidepsin and acetaminophen could induce effective anticancer effects through enhanced direct cytotoxic and immune chemotherapeutic responses.

Given the remarkable results obtained above in the two studies conducted by Wilson and colleagues [17,18] following the combination of romidepsin with cisplatin, a more recent study combined these two molecules with gemcitabine, an immunosuppressant, to effectively control the recurrence and development of urothelial carcinoma (UC) safely [26]. In different UC cells, this triple combination reduced drug resistance and synergistically induced apoptotic cell death. This combination was harmlessly verified by an *in vivo* model.

On the other hand, the effect of this HDAC inhibitor in monochemotherapy was examined against cholangiocarcinoma (CCA) in order to establish a new more effective treatment [25]. Given the advanced stages of patients diagnosed for the first time, CCA has become an extremely incurable tumor. For this reason, Li et al. [25] tested this on CCA cells *in vitro* and *in vivo*. Indeed, romidepsin alone inhibited CCA cell proliferation (*in vitro* and *in vivo*) and induced apoptosis and $G_2$/M phase cell cycle arrest. For associated mechanisms of action, $G_2$/M phase arrest was attributed to up-regulation of cyclin-dependent kinase 1

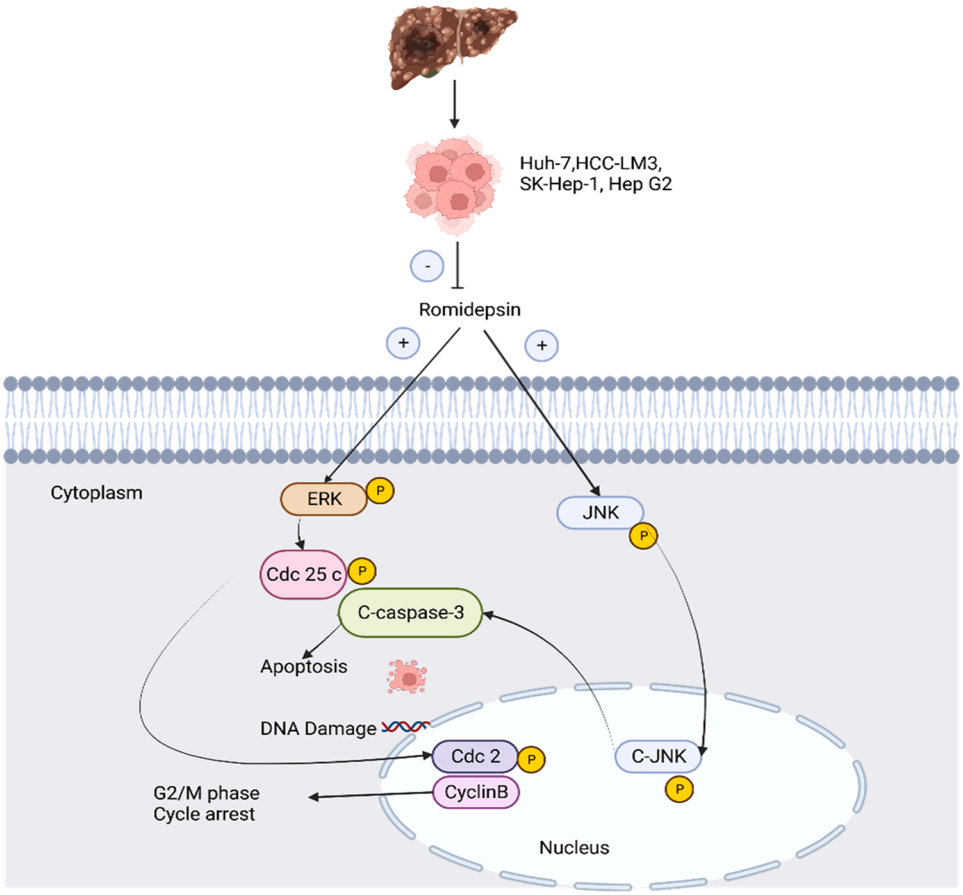


**Fig. 2.** Illustration of the mechanism associated with anticancer effects of Romidepsin by promoting apoptosis and triggering $G_2$/M cell cycle arrest in HCC cells in an *in vitro* model. Romidepsin induced apoptosis and G2/M phase arrest *via* JNK/c-Jun/caspase-3 and Erk/cdc25C/cdc2/cyclinB activation, respectively. **Abbreviations:** ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; Cdc25c, cell division Cycle 25c; DNA, deoxyribonucleic acid; HCC, hepatocellular carcinoma.

(CDK1) and down-regulation of cyclin B, while apoptosis was associated with caspase-3 activation (Fig. 2).

This efficacy in monotherapy was also obtained against biliary tract cancer (BTC), by inducing apoptosis, increasing histone 3 lysine 9 acetylation, and reducing HDAC activity in a panel of eight BTC cell lines [27].

In contrast, the impact of romidepsin on anticancer immune responses against solid cancers was studied by Shi et al. [31] using colon cancer cell lines and an *in vivo* model of this type of cancer. *In vitro* results showed that this molecule increases apoptosis, induces $G_0/G_1$ cell cycle arrest, and inhibits cell proliferation, whereas *in vivo* results showed that animals treated with romidepsin exhibit a low IFN-γ + $CD8^+$ T cell percentage and Th1/Th2 cell ratio with a high $FOXP3^+$ Treg percentage. Interestingly, in both *in vitro* and *in vivo*, this HDAC inhibitor increased the expression of PD-L1 by regulating the transcription factor BRD4 and increasing the levels of acetylation of histones H3 and H4. More interestingly, the combination of romidepsin with an anti-PD-1 antibody enhanced the anticancer effects and reversed the influence on $CD4^+$ and $CD8^+$ T cells; consequently, presenting the romidepsin/anti-PD-1 immunotherapy combination as a promising therapeutic strategy against colon cancer.

Among the most recently developed combination therapies in the treatment of bladder cancer is the combination between inhibitors of HDAC and HMG-CoA reductase [28]. In this study, the antitumor effect of the combination between romidepsin and simvastatin was examined *in vitro* on human bladder cancer cells and *in vivo* on mice carrying this type of cells. Indeed, both molecules are able to induce histone acetylation with the activation of AMP-activated protein kinase (AMPK) for simvastatin. The authors of this study showed that this combination kills bladder cancer cells and induces apoptosis synergistically. In addition, oral administration for 15 days of romidepsin (0.5 mg/kg) plus simvastatin (15 mg/kg) inhibited tumor growth *in vivo*. In a cooperative manner, this association induced histone acetylation *via* the decrease in HDAC expression and the activation of the AMPK pathway. In addition, the combination increased expression of peroxisome proliferator-activated receptor (PPAR)γ, responsible for ROS production, and caused endoplasmic reticulum (ER) stress, associated with histone acetylation and increased expression of AMPK. Taken together, a treatment combining romidepsin and simvastatin killed bladder cancer cells *via* histone acetylation, activation of the AMPK pathway, increased expression of PPARγ, and induction of ER stress.

In 2021, the radiosensitizing effect of romidepsin was assessed for the first time in a study conducted by Rossetti et al. [30] against alveolar (*in vitro* and *in vivo*) and embryonal (*in vitro*) rhabdomyosarcoma (ARMS and ERMS, respectively). *In vitro*, romidepsin alone induced DNA damage, oxidative stress, down-regulation of HDAC-1 expression, and growth arrest related to non-apoptotic cell death. An up-regulation of the expression of cyclin A, B, D1, p27, Myc, and an activation of MAPK and PI3K/Akt/mTOR signaling pathways were observed in surviving cells, testifying to a cancer chemo-resistant effect. Interestingly, it radiosensitized ARMS cells by impairing antioxidant repair pathways. *In vivo*, romidepsin (1.2 mg/kg) combined with radiotherapy (2 Gy) reduced tumor mass in murine xenografts (Fig. 3). From these facts, this strategy can be adopted in the treatment of the most aggressive alveolar phenotype subtype.

Cellular immunotherapy is strongly implicated in cancer therapy, and CAR-T cells (adoptive cellular immunotherapy) identify tumor target antigens to directly destroy tumor cells *via* cytotoxic effects. However, in ovarian cancer, the benefits of this therapy remain very limited. In addition, the effect of CAR-T cells on a single target remains ineffective given the difficulty of identifying a single antigenic marker on the surface of cancer cells [36]. Therefore, to improve the treatment, it is necessary to develop the therapeutic application of CAR-T cells by enhancing the expression of tumor antigen. Additionally, a target family, the NKG2D ligand, is expressed specifically in many solid tumors, including ovarian cancer. Indeed, the NKG2D/NKG2DL binding is responsible for the activation of NK cells.

A very recent study was performed with the aim of improving the expression of NKG2DL as a target antigen on the surface of SKOV-3 ovarian cancer cells treated *in vitro* with romidepsin, and consequently obtaining an improved cytotoxic effect of CAR-T cells [32]. Indeed, Wang et al. [32] showed that this treatment promotes the expression of NKG2DL on the surface of SKOV-3 cells, improves the cytotoxic activity of NKG2D-CAR-T cells, and increases the secretion of IFN-γ. This suggests that the combination of epigenetic therapy and immune cell therapy improves the pattern of limited application of CAR-T cells against ovarian cancer, by enhancing the expression of the tumor target antigen.

## 3. Clinical studies of romidepsin

Even before its official FDA approval in November 2009 for the treatment of cutaneous T-cell lymphoma (CTCL), the anticancer potential of romidepsin was clinically investigated previously (Table 2) against lung [37] and pancreatic [38] cancers, as well as acute myeloid leukemia (AML) [39].

Effectively, given the mechanisms of action highlighted above in preclinical studies using romidepsin against different types of lung tumor cells, Schrump and collaborators conducted a phase II study to assess its clinical molecular responses in patients suffering from lung cancer [37]. In 19 patients receiving this HDAC inhibitor at a dose of 17.8 mg/$m^2$ (I.V.) on days 1 and 7 of a 21-day cycle, only 9 patients showed transient stabilization of disease. Additionally, romidepsin increased p21 expression and enhanced histone H4 acetylation in lung tumor cells, without any significant cardiac toxicity. This indicates that romidepsin, at this schedule and dose, is responsible for several biological effects despite its minimal clinical efficacy in lung cancer patients, warranting further investigations of the association of this HDAC inhibitor with other new compounds for cancer therapy.

Indeed, a combination of romidepsin and gemcitabine, a chemotherapeutic, was examined in a phase I clinical trial for the treatment of patients suffering from pancreatic cancers and other advanced solid cancers (breast, ovaries, NSCLC, others) [38]. The evaluation of the tolerability and safety of this combination was demonstrated by administering, at different dose levels, romidepsin for 4 h in infusion followed by gemcitabine for 30 min; on days 1, 8, and 15 of a 7-month cycle. The results showed that most dose levels examined exhibit dose-limiting toxicity (DLT) of neutropenia and/or thrombocytopenia. However, 12/800 mg/$m^2$ every 14 days for the romidepsin/gemcitabine combination, respectively, were the recommended phase II doses having reduced DLT in the majority of patients with a disease-stabilizing effect.

The efficacy and safety of romidepsin alone was also evaluated in the other study against advanced acute myeloid leukemia (AML) [39]. In fact, in some patients with core binding factor (CBF)-AML receiving intravenously this HDAC inhibitor (13 mg/$m^2$/day) on days 1, 8, and 15 of a 4 month cycle, molecular and antileukemic effects were revealed in addition to a significant increase in the expression of *MDR1* with, however, common adverse events such as fatigue, anorexia, and nausea. Based on these pharmacodynamic results, development of romidepsin against CBF-AML should focus on combination with DNMT inhibitors for more improved efficacy and reduced chemoresistance.

On the other hand, the therapeutic options for patients with metastatic cancers evolving under conventional chemotherapy are very limited, hence the need to develop new harmless and effective agents. For this reason, Whitehead et al. [40] tested the antitumor potential of romidepsin in a phase II trial in patients with advanced colorectal cancer who had previously failed standard chemotherapy regimens. To determine the response probability in these patients with metastatic colorectal cancer (MCC), they were administered romidepsin 13 mg/$m^2$ (I.V.) over 4 h on days 1, 8, and 15 of a 4-month cycle. As a result, no grade 4 toxicities were observed in 25 treated patients, while 14 of them had grade 3 toxicities, in particular anorexia and fatigue. However,

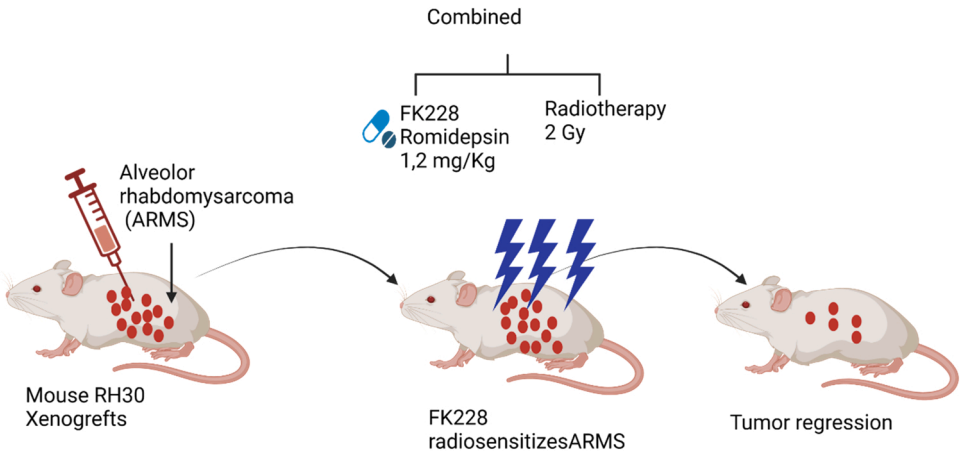


**Fig. 3.** Representation indicating that FK228 (romidepsin) successfully radiosensitizes the alveolar rhabdomyosarcoma (ARMS) subtype *in vivo*. In mouse RH30 xenografts with ARMS, romidepsin (1.2 mg/kg) combined with radiotherapy (2 Gy) was found to reduce tumor mass leading to its regression. Therefore, romidepsin effectively radiosensitizes the ARMS subtype.

according to the experimental protocol followed, romidepsin was ineffective in the therapy of patients with MCC after a prior standard chemotherapy regimen.

In a different context, the pharmacokinetics of this HDAC inhibitor was investigated in patients with CTCL and relapsed peripheral TCL (PTCL) by determining pharmacogenetic, clinical, and demographic covariates [41]. This was performed by administering a 14 or 18 mg/m$^2$ dose of romidepsin intravenously over 4 h on the first day of the first cycle. The authors noted mediocre interindividual variability in the pharmacokinetics of this molecule without a statistically significant link with the selective covariates in the patients. Furthermore, this pharmacokinetics was reassessed the same year in patients with CTCL who had received a median of 4 prior cytotoxic treatments and 87% of them presented with a metastatic state [64]. Accordingly, romidepsin monotherapy showed durable and significant clinical activity and biological responses with a median response duration of 13.7 months. In addition, some tolerable toxicities were recorded such as fatigue, vomiting, nausea, as well as transient granulocytopenia and thrombocytopenia. One month after these findings, the FDA approved the use of romidepsin against CTCL in clinical investigations for patients who had received prior conventional therapy.

In contrast, among the new therapeutic approaches adopted in the treatment of metastatic prostate cancer, figure castration consisting of inhibiting the synthesis of sex hormones likely to stimulate tumor growth. However, treatments targeting hormone suppression in patients with metastatic castration-resistant prostate cancer are limited and only an alkaloid with anticancer properties, docetaxel, has shown improved overall patient survival [65]. Importantly, in addition to the suppression of hormonal secretion as a therapeutic option against castration-resistant prostate cancer, the irreversible association with the androgen receptor (AR) has shown a marked anticancer effect in some patients suffering from this cancer (60%) [66,67]. It is known that HDAC inhibition can mediate the acetylation of heat shock protein 90 (HSP90), nullifying AR signaling. To verify the therapeutic potential of HDAC inhibitors against this type of cancer, Molife et al. [42] conducted a two-stage, phase II study in patients with metastatic castration-resistant prostate cancer by administering a single intravenous dose of romidepsin (13 mg/m$^2$) for 4 h on days 1, 8, and 15 every 4 weeks. Defining disease control rate as the primary endpoint based on determining signs of radiological progression at 6 months, the authors detected partial radiological response ($\geq$ 6 months) and reduction in prostate-specific antigen ($\geq$ 50%) in 2 out of 37 patients, while toxicity was observed in 11 patients prompting early discontinuation of treatment. No cardiac toxicity was observed except for manageable adverse events (nausea, vomiting, fatigue, and anorexia). This suggests that romidepsin at this schedule and formulation exhibits minimal anticancer effect in patients with castration-resistant prostate cancer. However, these results are not consistent with those obtained in the phase I/II study conducted by Iwamoto et al. [43] using romidepsin to treat subjects with recurrent glioblastomas. Indeed, for phase II, patients received a dose of this HDAC inhibitor (13.3 mg/m$^2$/day I.V.) following the same schedule of the previous study, but without recording any objective radiographic response or therapeutic efficacy.

In view of the marked and durable responses demonstrated in the previous study by Piekarz et al. [64] in patients with CTCL as well as official FDA approval for romidepsin use, the same research team performed another phase II study in 2011 to assess the antitumor potency of this compound in patients with different PTCL subtypes [44]. Therefore, with an objective response rate (ORR) of 38% with a median duration of 8.9 months, some patients experienced complete responses while others experienced partial responses. In addition to manageable toxicity, all responses were durable in patients suffering from relapsed PTCL.

This was in perfect agreement with the findings of a study carried out the following year evaluating the same potential against refractory/relapsed PTCL [45]. Indeed, to confirm this therapeutic efficacy after at least one previous failed systemic treatment, the patients were subjected to a four-hour treatment (I.V.) of romidepsin (14 mg/m$^2$), on days one, eight, and 15 every four weeks. Romidepsin monotherapy showed an ORR of 25% with a median duration of 17 months in patients with relapsed/refractory PTCL as well as durable and complete responses with manageable toxicity (infections, neutropenia, and thrombocytopenia) regardless of the type or number of previous treatments. With limited effective treatments, this significant activity presents another very important option in this type of indications.

From the preclinical data discussed above, it was shown that romidepsin as a single agent or combined with immune cell therapy [32] could induce effective anticancer effects *via* direct immune responses [24] or by potentiating the effect of NK [23], by suppressing immune cell functions by killing dendritic cells (DCs) and helper T cells, which may reduce the effectiveness of the immune response [68]. This was previously clinically investigated by Kelly-Sell et al. [46] by testing, in 8 patients with CTCL, the cellular immune function following treatment with 3 cycles of romidepsin. Consequently, the measurement of NK cell cytolytic activity of patients showed a decrease after the 3rd cycle of treatment. However, this activity was increased by a toll-like receptor (TLR) agonist. In addition, the first cycle of romidepsin suppressed IL-12 production and DC activation mediated by a TLR agonist. This confirms the suppressive effect of romidepsin on cellular immune functions in patients with CTCL.

To expand this potential to other solid cancers with limited treatment

**Table 2**
Clinical effect of Romidepsin against human cancers.

| Methods | Key results | References |
|---|---|---|
| Phase II trial<br>19 lung cancer patients<br>I.v. administration of romidepsin (17.8 mg/m$^2$) over 4 h on days 1 and 7 of a 21-day cycle | Minimal clinical efficacy | [37] |
| Phase I trial<br>Patients with pancreatic and other advanced solid tumors (AST)<br>Administration as a 4-hour infusion of romidepsin (10 mg/m$^2$) followed by gemcitabine (800 mg/m$^2$) over 30 min on days 1, 8, and 15 of a 4-week cycle | Thrombocytopenia and neutropenia | [38] |
| 20 patients with advanced acute myeloid leukemia (AML)<br>I.v. administration of romidepsin (13 mg/m$^2$/day) on days 1, 8, and 15 of a 28-day cycle | Differential anti-leukemic and molecular activity | [39] |
| Phase II trial<br>Patients with pathologically verified, measurable, metastatic or locally advanced colorectal cancer<br>I.V. administration of 13 mg/m2 over 4 h on days 1, 8, and 15 of a 28-day cycle | Ineffective treatment in patients with metastatic colorectal cancer | [40] |
| A multi-institutional phase II trial<br>Patients with cutaneous T-cell lymphoma (CTCL)<br>14 or 18 mg/m$^2$ romidepsin as a 4-h infusion on day 1 | Moderate inter-individual variability in the pharmacokinetics of romidepsin | [41] |
| Phase I/II trial<br>71 patients with CTCL | Clinical activity with significant and durable responses<br>The median duration of response = 13.7 months | [41] |
| Phase II, two-stage, single-arm trial<br>Patients with progressing, metastatic, castration-resistant prostate cancer (CRPC)<br>I.v. administration of romidepsin (13 mg/m$^2$) over 4 h on days 1, 8, and 15 every 28 days | Minimal antitumor activity | [42] |
| Phase I/II trial<br>Patients with recurrent glioma on enzyme-inducing antiepileptic drugs (EIAEDs)<br>In a phase I trial (8 patients): 13.3 and 17.7 mg/m$^2$/day<br>In a phase II trial (35 patients): 13.3 mg/m$^2$/day on days 1, 8, and 15 of each 28-day cycle | 34 patients (97%) died<br>Romidepsin was ineffective for patients with recurrent glioblastoma | [43] |
| Phase II trial<br>Patients with peripheral T-cell lymphoma (PTCL) | Clinical activity associated with durable responses<br>Objective response rate (ORR) = 38% | [44] |
| A pivotal, single-arm, phase II trial<br>Patients with relapsed/refractory PTCL<br>An infusion (I.V.) with romidepsin (14 mg/m$^2$) over 4 h on days 1, 8, and 15 every 4 weeks | Induced complete and durable responses<br>Manageable toxicity<br>ORR = 25% | [45] |
| 8 CTCL patients<br>Treatment with three cycles of romidepsin | Decreased cytolytic activity of patients' natural killer (NK) cells<br>Suppressed interleukin-12 production | [46] |
| | Suppressed dendritic cell (DC) activation<br>Increased specificity for CD41 tumor cell apoptosis | |
| Phase II trial<br>Patients with squamous cell carcinoma of the head and neck (SCCHN)<br>Administration by intravenous infusion of romidepsin (13 mg/m$^2$) over 4 h on days 1, 8, and 15 of 4-week cycles | Frequent severe fatigue<br>Induced limited activity for the treatment of SCCHN<br>Inhibited tumor-associated HDAC | [47] |
| Phase I trial<br>Patients with AST<br>Dosing started at 10 mg/m$^2$ of romidepsin and 800 mg/m$^2$ of gemcitabine given on days 1, 8, and 15 of a 28-day cycle | Days 1 and 15 every 3 weeks:<br>Romidepsin phase II dose = 12 mg/m$^2$<br>Gemcitabine phase II dose = 800 mg/m$^2$ | [48] |
| Phase I trial<br>28 patients with solid tumors, including 11 patients with thyroid cancer<br>Administration (I.V.) as a 4-hour romidepsin (7 mg/m$^2$) on days 1, 3, and 5 of a 3-week cycle | Induced acetylation of histones in peripheral blood mononuclear cells<br>Well tolerated treatment | [49] |
| Phase II trial<br>20 patients with radioactive iodine (RAI)–refractory thyroid cancer<br>I.v. administration of romidepsin (13 mg/m$^2$) on days 1, 8, and 15 of 28-day cycle | Grade 4–5 adverse events<br>No major responses were observed<br>Median overall survival (OS) = 33.2 (1–71 +) months | [50] |
| Phase I trial<br>An unselected advanced NSCLC population<br>Romidepsin administration (I.V.) (8 or 10 mg/m$^2$) on days 1, 8, and 15 every 4 weeks + oral administration of erlotinib (150 mg/day) | Combined treatment (Romidepsin 8 mg/m$^2$ + Erlotinib) well tolerated<br>PFS = 3.3 months | [51] |
| Phase I trial<br>An unselected advanced NSCLC population<br>Romidepsin administration (I.V.) (8 or 10 mg/m$^2$) on days 1, 8, and 15 every 4 weeks + oral administration of erlotinib (150 mg/day) | Combined treatment (Romidepsin 8 mg/m$^2$ + Erlotinib) well tolerated<br>PFS = 3.3 months | [52] |
| Phase II trial<br>Romidepsin + Gemcitabine relapsed/refractory PTCL patients<br>Six 28-day cycles<br>The primary endpoint: ORR<br>The secondary endpoints: regimen safety, response duration, and survival | ORR = 30%<br>Two-year OS = 50%<br>Progression-free survival (PFS) = 11.2%<br>Adverse events were anemia, neutropenia, and thrombocytopenia<br>Disappointing clinical results | [53] |
| Phase I trial<br>relapsed/refractory PTCL patients enrolled in dose escalation arms<br>Romidepsin + Duvelisib<br>Maximum tolerated dose (MTD) established once the optimal combination was found | High effectiveness level<br>ORR = 17%<br>Median PFS = 8.8 months<br>Relatively low rates of adverse events | [54] |
| Phase I trial<br>Patients with relapsed/refractory PTCL or DLBCL lymphoma<br>Gemcitabine (1000 mg/m$^2$ I.V. d1, d8) + Cisplatin (75 mg/m$^2$ I.V. d1) + Dexamethasone (40 mg po d1–4) + Romidepsin on days 1 and 8 every 3 weeks to a maximum of six cycles | On the 3-week schedule at 6 mg/m$^2$ of romidepsin: 3 DLTs in 4 patients<br>On the 4-week schedule at 6, 8 or 10 mg/m$^2$ romidepsin: no DLT<br>At 12 mg/m$^2$ romidepsin: 4 grade 3 DLTs were noted | [55] |

(*continued on next page*)

**Table 2** (*continued*)

| Methods | Key results | References |
|---|---|---|
| Phase II trial<br>Patients with relapsed/ refractory PTCL<br>Using romidepsin after Cisplatin, Dexamethasone, and Gemcitabine (CDG) therapy<br>CDG therapy administered every 3 weeks<br>PFS rate was the primary endpoint | Similar or better results than conventional therapies | [56] |
| Phase I trial<br>Patients with virally mediated cancers | Well-tolerated combination treatment<br>No significant anticancer activity was observed | [57] |
| Phase I dose-escalation trial<br>Patients with relapsed/ refractory CTCL (11) and PTCL (12)<br>20 mg/m$^2$ I.V., once every 4 weeks | Romidepsin + liposomal doxorubicin demonstrated a promising clinical efficacy and acceptable safety profile with deep skin responses in relapsed/refractory CTCL<br>MTD = 12 mg/m$^2$<br>ORR = 70% in CTCL<br>ORR = 27% in PTCL | [58] |
| Phase I trial<br>Open label single study<br>Romidepsin + Alisertib relapsed/refractory lymphoma (PTCL, DLBCL, Burkitt's lymphoma, and Hodgkin lymphoma) patients | A safe regimen | [59] |
| Phase II trial<br>Patients with untreated PTCL<br>Romidepsin (10 mg/m$^2$ I.V.) on days 1, 8, and 15 + Lenalidomide (25 mg p.o.) on days 1–21 of a 28-day cycle | Effective combination therapy<br>ORR = 75%<br>One-year PFS = 54.3%<br>One-year OS = 76% | [60] |
| 66 Patients with relapsed/ refractory lymphoma<br>Romidepsin (10 mg/m$^2$) + Duvelisib (75 mg BID) on days 1, 8, 15 of a 28-day cycle | Safe and highly active combined treatment<br>In PTCL:<br>ORR = 58%<br>Complete response (CR) = 42%<br>Median PFS = 6.9 month | [61] |
| 2 phase I trials with expansion cohorts<br>Patients with relapsed/ refractory lymphoma<br>The MTD of regimen A (49 patients):<br>Romidepsin (14 mg/m$^2$ I.V.) on days 1, 8, and 15 + Lenalidomide (25 mg p.o.) on days 1–21 of a 28-day cycle<br>The MTD of regimen B (27 patients):<br>Romidepsin (8 mg/m$^2$) on days 1 and 8 + Lenalidomide (10 mg p.o.) on days 1–14 + Carfilzomib (36 mg/m$^2$ I.V.) on days 1 and 8 of a 21-day cycle | Both regimens were effective<br>ORR = 49% (study A)<br>ORR = 48% (study B)<br>Median PFS = 5.7 months (study A)<br>Median PFS = 3.4 months (study B)<br>Most common adverse events: neutropenia, thrombocytopenia, and electrolyte abnormalities | [62] |
| Phase I/II trial<br>Patients with relapsed AML<br>Romidepsin (12 mg/m$^2$) + 5-azacytidine (75 mg/m$^2$) administered on days 8 and 15, for a cycle of 7/28 days | Safe and clinically active combination therapy | [63] |

options, Haigentz et al. [47] conducted a phase II investigation of 14 patients with advanced squamous cell carcinoma of the head and neck (SCCHN) by administering intravenously a 4-hour dose of romidepsin (13 mg/m$^2$) to days 1, 8, and 15 of 4-week cycles. Despite a brief stabilization of the disease in 2 patients, no ORR was observed with frequent severe fatigue. In SCCHN tumors, romidepsin treatment did not alter DNA methylation of candidate genes. However, this treatment effectively inhibited tumor-related HDAC, but despite this, it can be seen that romidepsin monotherapy has a limited effect against SCCHN.

On the other hand, we have previously discussed the molecular mechanism of action of this substance in a triple combination (romidepsin/gemcitabine/cisplatin) against UC, which was attributed to a synergistic potency inducing UC cell death in a way dependent on ERK-Nox pathway, ROS prodyction, and drug resistance [26]. In fact, the romidepsin/gemcitabine combination was examined in 2012 by Jones and co-workers in clinic (phase I) for two schedules in patients with AST, where the recommended phase II doses were 12 and 800 mg/m$^2$ for romidepsin and gemcitabine, respectively, on days one and 15 every four weeks, with an anticancer potential warranting further studies to assess the efficacy and safety of this combination [48].

The following year, another phase I trial evaluated the impact of this HDAC inhibitor on radioactive iodine (RAI) absorption in thyroid tumors [69]. The exploration of 6 dose levels of this molecule administered (I.V.) over 4 h on days 1, 3, and 5 of a 3-week cycle, identified 7 mg/m$^2$ as the tolerable dose able to induce histone acetylation in peripheral blood mononuclear cells without any significant increase in RAI. In the same framework, Sherman et al. [50] reassessed RAI reuptake in patients with RAI-refractory thyroid tumors by administering (I.V.) romidepsin (13 mg/m$^2$) on days 1, 8, and 15, in 4-week cycles. Despite RAI avidity restoration and disease stabilization in some patients, the absence of major responses and the recording of a case of pulmonary embolism and sudden death made it very difficult to estimate the real efficiency of this HDAC inhibitor. Based on these data, we cannot recommend further in-depth studies in this direction.

In contrast, it has already been established from preclinical results (*in vivo/in vitro*) that romidepsin potentiates the anticancer activity of erlotinib against NSCLC by various mechanisms [13]. The clinical verification of these obtained was later carried out by Gerber and colleagues through two phase I trials based on the combined administration of both molecules with the aim also of evaluating the pharmacodynamics and safety of this therapy in advanced NSCLC population [51, 52]. Consequently, despite the occurrence of certain adverse events, this romidepsin (8 mg/m$^2$ I.V.)/erlotinib (150 mg/day p.o.) association seems to be well tolerated through stabilization of the disease in some cases, inhibition of epidermal growth factor receptor (EGFR) phosphorylation, and increased histone acetylation.

Furthermore, in the mechanism section, romidepsin/gemcitabine combination demonstrated an effective and synergistic control power against the development of urothelial tumors [26]. The same combination was already investigated in a phase II clinical trial by Pellegrini et al. [53] to treat patients with relapsed/refractory PTCL *via* a regimen of six 4-week cycles. The study showed that the combination therapy resulted in additional hematological toxicities compared to romidepsin monotherapy, but no deaths were reported. However, the authors found the clinical results of this bitherapy disappointing against relapsed/refractory PTCL since they were inferior to those of romidepsin monotherapy. This led researchers to explore other combinations with romidepsin for the treatment of relapsed/refractory lymphomas. In fact, the combination of romidepsin with duvelisib was found to be highly effective with manageable side effects in patients with relapsed/refractory PTCL [54]. It is worth noting that duvelisib is a drug that belongs to phosphoinositide 3-kinase (PI3K) inhibitors, which inhibit certain enzymes promoting cell growth and survival, and is used in the treatment of several types of cancer and autoimmune diseases [70]. Furthermore, this HDAC inhibitor/PI3Ki combination demonstrated better outcomes than the combination of duvelisib and bortezomib (PI3Ki/proteasome inhibitors) at a higher dose. Similarly, in patients with relapsed/refractory PTCL or DLBCL lymphomas, a treatment combining cisplatin, dexamethasone, and gemcitabine at different doses with romidepsin (1000 mg/m$^2$ I.V.) on days 1 and 8 every 3 weeks has been tested in a phase I trial [55]. DLTs of different grades were observed in some patients depending on the schedule adopted and the administered dose of romidepsin. While full combination doses of all

selected molecules provided a recommended phase II dose of 10 mg/m$^2$ for romidepsin on days one and 15 every four weeks. This study only determined the recommended phase II dose for romidepsin by highlighting certain possible DLTs in cases of relapsed/refractory PTCL or DLBCL lymphomas without evaluating treatment tolerability. Therefore, it is clear that further studies are needed to better investigate this type of combinations. This is why Yamasaki et al. [71] examined the anticancer effect of this HDAC inhibitor after a treatment combining some of these molecules, namely cisplatin, dexamethasone, and gemcitabine against relapsed/refractory PTCL. In fact, chemotherapy combining these three molecules was carried out every three weeks and when the disease stabilizes after two to four cycles of this combined chemotherapy, patients receive romidepsin every four weeks. According to the two-year PFS rate, it has been revealed that patients following this therapeutic protocol can have superior results compared to conventional therapies.

In an effort to enhance the modest anticancer activity of romidepsin as well as that of an anthracycline, liposomal doxorubicin, as single agents in the treatment of patients with relapsed/refractory CTCL and PTCL, a phase I translational study combined the two drugs [58]. With an acceptable safety profile characterized by some manageable hematological and non-hematological toxicities related to the treatment, this bitherapy has demonstrated important clinical efficacy, expanding the field of investigation of this combination against lymphomas. The same safety was noted by combining this HDAC inhibitor with alisertib, an aurora kinase inhibitor, in the therapy of other relapsed/refractory lymphomas, namely PTCL, DLBCL, Burkitt's lymphoma, and Hodgkin lymphoma [59]. Interestingly, the combination of romidepsin with another promising drug for PTCL treatment, lenalidomide, was highly effective in the one-year treatment of patients with advanced-stage, previously untreated PTCL. However, as expected, there were adverse events associated with the treatment [60]. The efficacy of this combination against relapsed/refractory lymphoma was supported in the same year by two phase I studies focused on using these two drugs in addition to carfilzomib, a proteasome inhibitor [62]. Despite the high incidence of adverse events, the results showed that these regimens are effective and generally well-tolerated, with non-accumulating toxicity over time. This suggests that the combination of romidepsin, lenalidomide, and carfilzomib constitutes a cutting-edge therapeutic strategy against relapsed/refractory lymphoma. In this context, Horwitz et al. [61] conducted a more recent study using the same romidepsin/duvelisib combination in the treatment of relapsed/refractory lymphoma patients with low levels of transaminitis, including 55 with PTCL and 11 with CTCL. Their results were consistent with their own previous study. In this context, Horwitz et al. re-performed a more recent study using the same previous romidepsin/duvelisib combination in the treatment of relapsed/refractory lymphoma patients with low levels of transaminitis, including 55 with PTCL and 11 with CTCL. Their results were consistent with those of their own previous study [54]. The combination of HDAC inhibitor and PI3Ki was highly effective in treating relapsed/refractory PTCL, although there were some drug-related adverse events. Additionally, the addition of romidepsin to duvelisib was safe and reduced transaminitis levels compared to using the drugs alone. Patients who received duvelisib monotherapy before starting the combination treatment had higher levels of ALT/AST and diarrhea compared to those who began the combined treatment from the first cycle. Overall, romidepsin shows promise as a therapeutic option for patients with relapsed/refractory PTCL, with high rates of complete response and frequent transition to allograft.

On the other hand, the anticancer potential of the romidepsin/ 5-azacytidine (HDAC inhibitor/DNMTi) combination was evaluated in preclinical studies in the treatment of ovarian caner that has shown good efficacy as indicated above [18]. The efficacy of this combination has also been reported clinically in the treatment of advanced AML [39], which has encouraged additional and more recent investigations to re-evaluate it against advanced solid tumors [57] and AML [63]. The combination of romidepsin and 5-azacytidine was not only well tolerated, but also clinically active in high-risk AML patients who are ineligible for intensive chemotherapy. This offers hope for these patients and paves the way for further research and development. However, the romidepsin/5-azacytidine combination did not show a significant antitumor effect against advanced solid tumors, despite a tolerable profile [57].

From the clinical outcomes of all the trials discussed above, it can be inferred that despite the therapeutic potential of romidepsin as a single agent or combined with other therapeutic compounds or standard treatments, further investigations are still necessary in order to reach more tumors with poor therapeutic prognosis and improve the clinical efficacy of standard treatments with a largely tolerable toxicity profile.

## 4. Concluding remarks and future perspectives

Based on the data from this investigative review, it is deduced that the HDAC inhibitor romidepsin possesses significant potency to modulate signaling pathways that indirectly mediate cell cycle regulation, leading to cell death. Accordingly, it is expected through these findings that romidepsin could be used as a chemopreventive drug in the management of various types of cancers. Nevertheless, two salient counterarguments are romidepsin should be used in combination with other drugs in targeted therapy while studying the possible synergistic effects. Secondly, the mechanistic understanding of the anticancer action of these molecules that it is romidepsin or others would allow downstream to better understand the cellular system, namely the mechanisms that regulate tumor transformation. Indeed, cancer drugs have allowed scientists to understand the behaviour of cells because inhibition of a molecular pathway is the result of sequential events that transition from a normal cell to a cell tumor. The exploration of these types of molecules already used clinically will improve our understanding of the system and the biological order of normal and tumor cells.

There are multiple potential future perspectives for the clinical use of romidepsin as a chemopreventive drug for managing various types of cancer. Firstly, the review suggests that romidepsin may be more effective when used in combination with other drugs in targeted therapy. Therefore, future clinical studies could investigate the possible synergistic effects of romidepsin in combination with other drugs to optimize its use in cancer treatment. Secondly, a better understanding of the anticancer action of romidepsin and other HDAC inhibitors could lead to a greater understanding of the cellular system and the mechanisms that regulate tumor transformation. By understanding the sequential events that lead from a normal cell to a tumor cell, researchers may be able to develop new therapies and better target the underlying molecular pathways of cancer. Overall, exploring molecules such as romidepsin, which are already used clinically, may improve our understanding of the system and the biological order of normal and tumor cells, ultimately leading to the development of more effective cancer treatments.

## CRediT authorship contribution statement

**Asaad Khalid, Long Chiau Ming, Abdelhakim Bouyahya**: Conceptualization. **Nasreddine El Omari, Learn-Han Lee, Saad Bakrim, Hassan A. Alhazmi, Syam Mohan, Asaad Khalid, Long Chiau Ming**: Methodology. **Abdelhakim Bouyahya**: Software. **Nasreddine El Omari, Learn-Han Lee, Saad Bakrim, Hafiz A. Makeen, Hassan A. Alhazmi, Syam Mohan**: Validation. **Saad Bakrim, Asaad Khalid, Hassan A. Alhazmi**: Resources. **Nasreddine El Omari, Learn-Han Lee, Saad Bakrim, Hafiz A. Makeen, Hassan A. Alhazmi**: Writing – original draft preparation. **Syam Mohan, Asaad Khalid, Long Chiau Ming, Abdelhakim Bouyahya**: Writing – review & editing. **Abdelhakim Bouyahya, Nasreddine El Omari**: Visualization. **Learn-Han Lee, Abdelhakim Bouyahya, Long Chiau Ming**: Supervision. **Learn-Han Lee, Hafiz A. Makeen, Hassan A. Alhazmi**: Project administration. All authors have read and agreed to the published version of the

manuscript.

## Declaration of Competing Interest

None.

## Data availability

No data was used for the research described in the article.

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