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Review

Trials with 'epigenetic' drugs: An update

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ABSTRACT

Epigenetic inactivation of pivotal genes involved in correct cell growth is a hallmark of human pathologies, in particular cancer. These epigenetic mechanisms, including crosstalk between DNA methylation, histone modifications and non-coding RNAs, affect gene expression and are associated with disease progression. In contrast to genetic mutations, epigenetic changes are potentially reversible. Re-expression of genes epigenetically inactivated can result in the suppression of disease state or sensitization to specific therapies. Small molecules that reverse epigenetic inactivation, so-called epi-drugs, are now undergoing clinical trials. Accordingly, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for cancer treatment have approved some of these drugs. Here, we focus on the biological features of epigenetic molecules, analyzing the mechanism(s) of action and their current use in clinical practice.

1. Introduction

It is well established that both genetic and epigenetic changes contribute to the development of human diseases, such as cancer (Hahn and Weinberg, 2002; Jones and Baylin, 2002).

The term "epigenetics" refers to all heritable alterations in gene expression and chromatin structure due to chemical modifications that do not involve changes in the primary gene nucleotide sequence (Nightingale et al., 2006). The correct regulation of these alterations ensures appropriate cell growth (Bernstein et al., 2007; Jenuwein and Allis, 2001; Strahl and Allis, 2000). In contrast, deregulation of epigenetic patterns leads to induction and propagation of disease state (Feinberg et al., 2006; Hahn and Weinberg, 2002; Jones and Baylin, 2002).

Epigenetic inheritance can be classified into three distinct types: DNA methylation, histone modifications, and non-coding RNAs. All of these are crucial mechanisms that ensure the stable propagation of gene activity from one generation of cells to the next (Feinberg et al., 2006; Jenuwein and Allis, 2001). Disruption of any of these three distinct and mutually reinforcing epigenetic mechanisms leads to inappropriate gene expression, resulting in cancer development and other "epigenetic diseases" (Egger et al., 2004; Feinberg et al., 2006; Feinberg and Tycko, 2004; Jones and Baylin, 2002).

Although precise underlying mechanisms are not yet clear, in recent years, scientific interest in epigenetics has increased insofar as it represents an important tool to advance our understanding of pathogenesis, in particular tumorigenesis, and to help in the development (Egger et al., 2004; Strahl and Allis, 2000) of strategies for cancer treatment and prevention.

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2. Chromatin structure and regulation

In eukaryotic cells, nuclear DNA associates with histones to form a compact complex called chromatin. Histones constitute a family of basic proteins, which are generally highly conserved across eukaryotic species. The core histones, termed H2A, H2B, H3, and H4, associate to form an octamer structure comprising two molecules each of H2A, H2B, H3 and H4 histones. DNA winds around this protein core, with the basic amino acids of the histones interacting with negatively charged phosphate groups of the DNA. Approximately 146 bp of DNA wrap around a histone core to make up a nucleosome particle, the repeating structural motif of chromatin. Histone H1 assembles such nucleosomes into a higher ordered structure. The N-terminal tails of nucleosomal histones are subject to different modifications including acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation. Of these, acetylation has been the most extensively investigated (Shukla et al., 2008). Recent studies in the field of chromatin research provided experimental evidence that led to a concept known as the "histone code" (Jenuwein and Allis, 2001; Strahl and Allis, 2000). It postulates that different histone modifications are combinatorial and consistent, generating a code that is read by cellular machineries to dictate functional outcomes. Different histone modifications were shown to be essential for normal cellular processes because crucially involved in transcriptional regulation. In view of this fact, attempts have been made to regulate the transcription of disease-related genes (e.g., oncogenes and tumor suppressor genes (TSGs)) by using epigenetic regulators to treat cancers and other diseases.

3. Histone modificators in clinical trials

Epimutations play a role in the etiology of human cancers. In contrast to DNA mutations, which are passively inherited through DNA replication, epimutations must be actively maintained because they are reversible. The reversibility of epimutations by small-molecule inhibitors provides the foundation for their use in novel cancer therapy strategies. Among the compounds that inhibit epigenetic processes, the most extensively studied are DNA methyltransferase inhibitors and HDAC inhibitors (HDACi). Here, we focus on epigenetic modulators used in clinical trials (either completed or terminated) to treat human diseases (www.clinicaltrials.gov).

3.1. *HDAC inhibitors*

Histone deacetylases are known to play a key role in the transcriptional machinery for regulating gene expression, to induce histone hyperacetylation and to affect gene expression. Therefore, they represent the target of therapeutic or prophylactic agents, HDACis, for diseases caused by abnormal gene expression such as inflammatory disorders, diabetes, diabetic complications, homozygous thalassemia, fibrosis, cirrhosis, acute promyelocytic leukemia (APL), autoimmune diseases and tumors as well as organ transplant rejections and protozoal infections.

Acetylation and deacetylation of histones are carried out by "writers" (histone acetyl transferases (HATs)) and "erasers" (histone deacetylases (HDAC)) enzymes. The state of acetylation of histones is an important determinant of gene transcription. Deacetylation is generally associated with reduced transcription of genes whereas increased acetylation of histones induced by the action of HDACi results in greater transcription of genes. Thus, HDACi affect multiple processes in the cell, which are likely to depend upon its dynamic state with respect to its capabilities of replication and differentiation.

The term "HDAC inhibitors" is commonly used for compounds that target the classical class I (HDAC1, 2, 3 and 8), II (HDAC4, 5, 6, 7, 8, 9 and 10), and IV (HDAC11) HDACs and are currently being evaluated in clinical trials. A number of structurally diverse HDACi have been identified, many of which are or derive from natural products. These can be classified, according to their chemical structure, into the following categories:

(a) hydroxamic acids (such as trichostatin A (TSA) (Yoshida et al., 1990) and hydroxamic acid-based hybrid polar compounds e.g., suberoylanilide hydroxamic acid (SAHA) (Richon et al., 1998) and pyroxamide (Butler et al., 2001));
(b) cyclic tetrapeptides with the epoxyketone-containing amino acid (2S,9S)-2-amino-8-oxo-9,10-epoxy-decanoyl (Aoe) (such as trapoxin A and B) (Kijima et al., 1993);
(c) cyclic tetrapeptides without Aoe (such as apicidin (Darkin-Rattray et al., 1996) and the depsipeptide FR-901228 (Romidepsin) (Nakajima et al., 1998));
(d) short-chain and aromatic fatty acids (such as butyrate (Newmark et al., 1994), 4-phenybutyrate (Warrell et al., 1998) and valproic acid (Phiel et al., 2001));
(e) benzamides (such as MS-275 (Entinostat)) (Saito et al., 1999) and MGCD0103 (Mocetinostat) (Fournel et al., 2008);
(f) miscellaneous compounds (such as depudecin (Kwon et al., 1998)).

Increasing evidence supports distinct biological roles for each of the mammalian HDACs and it is probable that inhibition of specific members of the HDAC family will have specific functional consequences such as on gene expression, cell cycle regulation, proliferation, differentiation and apoptosis. A number of HDAC isoform-selective or -specific inhibitors are currently in development (Butler and Kozikowski, 2008; Estiu et al., 2008; Haggarty et al., 2003; Jones et al., 2008; Khan et al., 2008; Kozikowski et al., 2007; Moradei et al., 2008; Rasheed et al., 2007; Somoza et al., 2004). One question in the field of HDACi development, which remains unanswered, is whether selective inhibition of HDACs would be advantageous over broader-acting HDACi as anti-cancer agents.

HDACi have a plethora of biologic effects resulting from alterations in patterns of acetylation of histones and many non-histone proteins, which include proteins involved in regulation of gene expression, pathways of extrinsic and intrinsic apoptosis, cell cycle progression, redox pathways, mitotic division, DNA repair, cell migration, and angiogenesis (Blackwell et al., 2008; Bolden et al., 2006; Dokmanovic et al., 2007; Glozak and Seto, 2007; Jones and Baylin, 2007; Minucci and Pelicci, 2006; Shankar and Srivastava, 2008; Xu et al.,

2007). HDACi also have immunomodulatory activity that may contribute to mediating their anti-cancer effects. Furthermore, in contrast to most cancer-therapy agents, HDACi can induce the death of transformed cells in both proliferative and non-proliferative phases of cell cycle (Burgess et al., 2004). The mechanism(s) of action of HDACi are complex and not completely clear.

Mechanism(s) of resistance to HDACi are not well elucidated. Resistance to HDACi may reflect drug efflux, epigenetic alterations, stress response mechanisms, and anti-apoptotic and pro-survival mechanisms (Rosato et al., 2006).

According to results of pre-clinical studies and early clinical trials, HDACi in combination therapy with targeted anti-cancer drugs, cytotoxic agents, anti-angiogenesis drugs or radiation is potentially very useful (Bolden et al., 2006; Dokmanovic et al., 2007; Glozak and Seto, 2007; Jones and Baylin, 2007; Minucci and Pelicci, 2006; Nolan et al., 2008; Xu et al., 2007). In pre-clinical studies, HDACi have been shown to be synergistic with diverse chemical and biological therapeutic agents. HDACi will likely be most effective therapeutically when used in combination with other anti-cancer agents (Carew et al., 2008; Nolan et al., 2008).

Based on published reports, there are at least 20 structurally different HDACi currently in clinical trials as monotherapy and combination therapy for hematologic and solid cancers.

3.1.1. *Short-chain fatty acids*

Their mechanism of action of short-chain fatty acids is based on the carboxylic group, occupying the acetate escaping tunnel, that can have a zinc-binding function or compete with the acetate group released in the deacetylation reaction (Mai and Altucci, 2009).

Two short-chain fatty acids, valproic acid (VPA) and sodium phenylbutyrate, are in clinical trials (Table 1).

VPA, first used as an anticonvulsant and mood-stabilizing agent, is a pan-HDACi. Phase 1–2 clinical trials tested VPA alone or in combination treatment for lymphocytic leukemia (Bouzar et al., 2009; Lagneaux et al., 2007; Stamatopoulos et al., 2009), AML and myelodysplastic syndromes (MDS) in combination with 5-azacytidine (Kuendgen et al., 2011), melanoma (Daud et al., 2009), HIV infection (Archin et al., 2010), autoimmune lymphoproliferative syndrome (ALPS) (Dowdell et al., 2009), human T-lymphotropic virus type-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Olindo et al., 2011).

Some phase 2 clinical trials involved the combination therapy of hydralazine, a DNA demethylating agent, and *magnesium valproate* for treatment of cervical cancer (Candelaria et al., 2010), breast cancer (Arce et al., 2006) and refractory solid tumors (Candelaria et al., 2007).

There are also two clinical trials with *sodium phenylbutyrate*: one in phase 2 for treatment of Huntington's disease (Gardian et al., 2005; Hogarth et al., 2007) and one in phase 1–2 for amyotrophic lateral sclerosis (ALS) (Cudkowicz et al., 2009; Ryu et al., 2005).

Pivaloyloxymethyl butyrate (Pivanex, AN-9) is in phase 1–2 clinical trials for chronic lymphocytic leukemia and lymphoma (Rabizadeh et al., 2007), malignant melanoma (Tarasenko et al., 2008), and non-small cell lung carcinoma (NSCLC) (Reid et al., 2004).

3.1.2. *Hydroxamic acids*

Hydroxamic acids include the majority of HDACi currently in clinical trials for the treatment of several human diseases such as cancer. Peculiarities of hydroxamate-based molecules are the polar hydroximic group, a 4–6 carbon hydrophobic methylene spacer (CU, polar connection unit), a second polar site, and a terminal hydrophobic group. In the majority of HDACi, the CU can interact with amino acids in the tunnel, and a linker

Table 1 – Short-chain fatty acids in clinical trials.

Drug	Conditions	Recruitment	NCT number	Phases
Valproic acid & fludarabine	Chronic lymphocytic leukemia	Terminated	NCT00524667	Phase 2
Azacitidine, valproic acid	Advanced cancers	Completed	NCT00496444	Phase 1
Temozolomide + valproic acid	Brain metastases	Terminated	NCT00437957	Phase 1
5-Azacytidine + valproic acid + ATRA	Myelodysplastic syndromes	Completed	NCT00439673	Phase 2
5 azacytidine + valproic acid + retinoic acid	AML, MDS	Completed	NCT00339196	Phase 2
Enfuvirtide + valproic acid	HIV infections	Terminated	NCT00312546	Phase 1
Valproic acid	HTLV-I-associated myelopathy	Terminated	NCT00519181	N/A
Karenitecin + valproic acid	Malignant melanoma	Terminated	NCT00358319	Phase 1, 2
Valproic acid	ALPS|hypersplenism|lymphadenopathy	Completed	NCT00605657	Phase 1, 2
Raltegravir + valproic acid	HIV infections	Terminated	NCT00614458	Phase 2
Hydralazine + magnesium valproate	Cervical cancer	Completed	NCT00404326	Phase 2
Hydralazine + magnesium valproate	Locally advanced breast cancer	Terminated	NCT00395655	Phase 2
Hydralazine + magnesium valproate	Refractory solid tumors	Completed	NCT00404508	Phase 2
Sodium phenylbutyrate	Huntington's disease	Completed	NCT00212316	Phase 2
Sodium phenylbutyrate	Amyotrophic lateral sclerosis	Completed	NCT00107770	Phase 1, 2
Pivanex	Leukemia, lymphocytic, lymphoma, small lymphocytic	Terminated	NCT00083473	Phase 2
Pivanex	Malignant melanoma	Terminated	NCT00087477	Phase 1, 2
Pivanex/docetaxel	Carcinoma, non-small-cell lung	Completed	NCT00073385	Phase 2

of 4 or 6 units of carbon causing the following zinc-binding group to bind the zinc ion inhibiting the enzyme (Mai et al., 2005).

The HDACi *Vorinostat* is at the most advanced stage in the clinical development (Tables 2 and 3) (Siegel et al., 2009). Vorinostat, a second-generation polar-planar compound, binds to the catalytic domain of histone deacetylases (HDACs). This allows the hydroxamic moiety to chelate zinc ion located in the catalytic pockets of HDACs, thereby inhibiting deacetylation and leading to an accumulation of both hyperacetylated histones and transcription factors. Vorinostat was the first HDACi approved by the Federal Drug Administration in 2006 for clinical use in treating patients with advanced cutaneous T-cell lymphoma (Duvic and Vu, 2007; Marks and Breslow, 2007). Tables 2 and 3 show about 60 Vorinostat clinical trials, either alone or in combination, completed or terminated against multiple myeloma (MM) (Badros et al., 2009; Mazumder et al., 2010; Richardson et al., 2008), head and neck cancer (Borbone et al., 2010; Gillenwater et al., 2007), pelvic cancer (Bratland et al., 2011; Ree et al., 2010), lymphoma (Dummer et al., 2012; Kirschbaum et al., 2011; Stathis et al., 2011), leukemia and myelodysplastic syndromes (MDS) (Garcia-Manero et al., 2008b; Prebet and Vey, 2011), breast cancer (Munster et al., 2011; Shi et al., 2010), small cell lung cancer (SCLC) (Gray et al., 2012), brain and central nervous system tumors (Friday et al., 2012), prostate and urothelial cancers (Schneider et al., 2012), colorectal cancer (Fakih et al., 2012), kidney cancer (Sato et al., 2010), pancreatic cancer (Tinari et al., 2012), and ovarian cancer (Modesitt et al., 2008).

Panobinostat (*LBH589*) is currently in phase 1 and 2 clinical trials as monotherapy and combination therapy for hematologic tumors such as non-Hodgkin's lymphoma (Ellis et al., 2008; Shao et al., 2010), acute lymphoblastic or acute myeloid leukemia (Fiskus et al., 2009; George et al., 2005; Rosato et al., 2012; Scuto et al., 2008), MM (Maiso et al., 2006), for advanced solid tumors (Fukutomi et al., 2012), breast cancer (Tate et al., 2012; Zhou et al., 2007) and lung cancer (Crisanti et al., 2009) (Table 4).

CHR-3996 is a second-generation hydroxamic acid-based HDACi. Specifically, it is a selective class I HDAC inhibitor with potential antineoplastic activity (Moffat et al., 2010), currently in clinical trials for the treatment of advanced tumors (Banerji et al., 2012) (Table 4).

CHR-2845 is a novel hydroxamic acid derivative HDACi, which is a selective substrate for the intracellular carboxylesterase hCE-1, whose expression is restricted to cells of the monocyte–macrophage lineage. One phase 1 clinical trial has been completed for this drug in treatment of hematological diseases and lymphoid malignancies (Table 4).

SB939 is a new hydroxamic acid-based HDACi with improved physico-chemical, pharmaceutical and pharmacokinetic properties. *In vitro*, SB939 inhibits class I, II, and IV HDACs, with no effects on other zinc-binding enzymes, and shows significant antiproliferative activity against a wide variety of tumor cell lines (Novotny-Diermayr et al., 2010). It is in phase 1 clinical trials for treatment of solid tumors (Razak et al., 2011), hematologic malignancies and MDS (Novotny-Diermayr et al., 2012) (Table 4).

ITF2357 (Givinostat) is a novel hydroxamic acid-based HDACi that inhibits both class I and II HDACs. It has been used in five phase 1–2 clinical trials for the treatment of hematological diseases (Galli et al., 2010; Rambaldi et al., 2010) (Table 4).

PXD101 (Belinostat) is another inhibitor of class I and II HDACs that has been included in phase 2 clinical trials for

Table 2 – Vorinostat in clinical trials.

Drug	Conditions	Status	NCT number	Phases
Vorinostat	Glioblastoma, gliosarcoma, brain tumor	Completed	NCT00641706	Phase 2
Vorinostat	Ovarian cancer, primary peritoneal cavity cancer	Completed	NCT00132067	Phase 2
Vorinostat	Lymphoma	Completed	NCT00771472	Phase 1
Vorinostat	Leukemia, myelodysplastic syndromes	Completed	NCT00305773	Phase 2
Vorinostat	Advanced cancer relapsed and refractory	Completed	NCT00632931	Phase 1
Vorinostat	Carcinoma, non-small-cell lung	Terminated with result	NCT00251589	Phase 1, 2
Vorinostat	Glioblastoma, gliosarcoma, brain tumor	Completed	NCT01647100	Phase 2
Vorinostat	Mesothelioma|lung cancer	Completed	NCT00128102	Phase 3
Vorinostat	Breast cancer	Completed	NCT00132002	Phase 2
Vorinostat	Lung cancer	Completed	NCT00138203	Phase 2
Vorinostat	Brain and central nervous system tumors	Completed	NCT00238303	Phase 2
Vorinostat	Myelodysplastic syndromes	Completed	NCT00776503	Phase 1, 2
Vorinostat	Cancer	Completed	NCT00045006	Phase 1
Vorinostat	Head and neck cancer	Completed	NCT00134043	Phase 2
Vorinostat	Pelvic cancer|radiotherapy	Completed	NCT00455351	Phase 1
Vorinostat	Hematologic and solid cancer	Terminated	NCT01116154	Phase 1
Vorinostat	Tumors	Completed	NCT00127127	Phase 1
Vorinostat	Brain, CNS, intestine tumors, lymphoma	Completed	NCT00499811	Phase 1
Vorinostat	MDS, blood and bone marrow disease	Terminated	NCT00486720	Phase 2
Vorinostat	Advanced cancer	Completed	NCT00907738	Phase 2
Vorinostat	Lymphoma	Completed	NCT00127140	Phase 1
Vorinostat	Colorectal cancer	Completed	NCT00336141	Phase 1
Vorinostat	Hematologia and solid cancer	Completed	NCT00005634	Phase 1
Vorinostat	Kidney cancer	Completed	NCT00354250	Phase 2

Table 3 – Vorinostat in combineted clinical trials.

Drug	Conditions	Status	NCT number	Phases
Isotretinoi + vorinostat	Brain, neuroblastoma, CNS, hematologic tumors	Completed	NCT00217412	Phase 1
Idarubicin + vorinostat	Leukemia, myelodysplastic syndromes	Completed	NCT00331513	Phase 1
Vorinostat + gemcitabine + platinum	NSCL cancer	Completed	NCT00423449	Phase 1
Vorinostat + pemetrexed + cisplatin	Advanced cancer	Completed	NCT00106626	Phase 1
MK0683 + vorinostat	Breast cancer, colorectal cancer, NSCL	Terminated	NCT00126451	Phase 2
Vorinostat + docetaxel	NSCL, prostate, bladder, urothelial cancers	Terminated	NCT00565227	Phase 1
Vorinostat + tamoxifen	Breast cancer	Terminated	NCT01194427	Phase 2
MK0683 + vorinostat	B-cell lymphoma	Completed	NCT00097929	Phase 2
Carboplatin + paclitaxel + vorinostat	Unspecified adult solid tumor, protocol specific	Completed	NCT00287937	Phase 1
Vorinostat, vinorelbine	Malignant solid tumor	Terminated	NCT00801151	Phase 1
Azacitidine + vorinostat	Leukemia, myelodysplastic syndromes	Suspended	NCT00392353	Phase 1, 2
Gemtuzumab ozogamicin + vorinostat	Hematologic cancer	Terminated	NCT00673153	Phase 2
MK0683, vorinostat	Cutaneous T-cell lymphoma, mycosis fungoides	Completed	NCT00091559	Phase 2
Vorinostat + decitabine	Leukemia, acute myelodysplastic syndromes	Completed	NCT00479232	Phase 1
Fluorouracil, leucovorin calcium, oxaliplatin, vorinostat	Colorectal cancer	Completed	NCT00138177	Phase 1
Carboplatin + paclitaxel + vorinostat	Lung cancer	Completed	NCT00481078	Phase 2
Vorinostat + paclitaxel + carboplatin	Stage IIIB or IV non-small cell lung cancer	Terminated	NCT00473889	Phase 2, 3
Chemoradiation + vorinostat	Pancreatic cancer	Terminated	NCT00831493	Phase 1, 2
Vorinostat – bexarotene	Lymphoma	Terminated with with result	NCT00127101	Phase 1
Vorinostat + tamoxifen	Breast cancer	Completed with result	NCT00365599	Phase 2
Gemcitabine + vorinostat	Unspecified adult solid tumor, protocol specific	Completed	NCT00243100	Phase 1
Vorinostat + alvocidib	Unspecified adult solid tumor, protocol specific	Completed	NCT01645514	Phase 1
Vorinostat + alvocidib	Leukemia, myelodysplastic syndromes	Completed	NCT00278330	Phase 1
Topotecan + vorinostat	Small cell lung cancer	Terminated	NCT00697476	Phase 1, 2
Vorinostat + alvocidib	Unspecified adult solid tumor, protocol specific	Completed	NCT00324480	Phase 1
Fluorouracil + leucovorin calcium + vorinostat	Hematologic and solid cancer	Completed	NCT00942266	Phase 2
MK0683, vorinostat	Neoplasms	Terminated	NCT00424775	Phase 1
MK0683, vorinostat	Cancer, advanced	Completed	NCT00750178	Phase 1
Vorinostat + bortezomib + dexamethasone	Relapsed or refractory multiple myeloma	Completed	NCT00773838	Phase 2
Vorinostat + bortezomib	Lymphoma, sarcoma, Wilms tumor, neuroblastoma	Completed	NCT01132911	Phase 1
Vorinostat + bortezomib	Unspecified adult solid tumor, protocol specific	Completed	NCT00227513	Phase 1
Vorinostat + bortezomib	Multiple myeloma	Completed	NCT00111813	Phase 1
Bortezomib + dexamethasone + vorinostat	Multiple myeloma and plasma cell neoplasm	Completed	NCT00310024	Phase 1
Vorinostat + bortezomib	Multiple myeloma	Completed	NCT00858234	Phase 1
Vorinostat + bortezomib	Leukemia, myelodysplastic syndromes	Terminated	NCT00818649	Phase 2
Vorinostat + bortezomib	Lymphoma	Terminated	NCT00810576	Phase 2
Dasatinib + vorinostat	Leukemia	Completed	NCT00816283	Phase 1

MDS (Cashen et al., 2012), MM (Feng et al., 2007; Gimsing et al., 2008) and phase 1–2 for NSLC (Force et al., 2011; Luchenko et al., 2011) (Table 4).

JHJ-26481585 is a pyrimidyl-hydroxamic acid analog showing a pan-HDACi activity and is in phase 1 for leukemia and MDS (Arts et al., 2009; Stuhmer et al., 2010; Tong et al., 2010) (Table 4).

3.1.3. *Benzamides*

This class is composed of HDACi containing a characteristic 2′-aminoanilide moiety able to contact specific aminoacids in the tube-like active site of the HDAC core, with or without coordination/chelation of zinc ion (Pontiki and Hadjipavlou-Litina, 2012).

At least two benzamides, MS-275 (SNDX-275, Entinostat) and MGCD0103 (Mocetinostat) are in clinical development. These agents are in trials as single agents and in combination with other drugs.

Clinical trials with *MS-275*, a class I selective inhibitor, include patients with a wide variety of hematologic and solid neoplasms (Knipstein and Gore, 2011) such as leukemia (Gojo et al., 2007; Lucas et al., 2004), melanoma (Gore et al., 2008), MDS (Fandy et al., 2009) and colorectal cancer (Bracker et al., 2009) (Table 5).

MGCD0103 is an isotype-selective HDACi that potently targets human HDAC1 but also exerts inhibitory activity against HDAC2, HDAC3, and HDAC11 *in vitro* (Fournel et al., 2008). Some phase 1–2 clinical trials are for treatment of hematological diseases such as leukemia (Blum et al., 2009; El-Khoury et al., 2010; Garcia-Manero et al., 2008a), lymphoma (Buglio et al., 2010; Younes et al., 2011) and also for solid cancers (Siu et al., 2008) (Table 5).

Table 4 – Hydroxamic acids in clinical trials.

Drug	Conditions	Recruitment	NCT number	Phases
Panobinostat (LBH589)	Breast cancer	Terminated	NCT00993642	Phase 0
Panobinostat (LBH589)	Cutaneous T-cell lymphoma	Completed	NCT00490776	Phase 2, 3
Panobinostat (LBH589)	Non-Hodgkin's lymphoma	Terminated	NCT01090973	Phase 2
Panobinostat (LBH589)	Leukemia	Completed	NCT00723203	Phase 2
Panobinostat (LBH589)	Cancer, advanced solid tumor	Completed	NCT00739414	Phase 1
Panobinostat (LBH589)	Lymphoma, leukemia, multiple myeloma	Completed	NCT00621244	Phase 1, 2
LBH589 + trastuzumab + paclitaxel	HER-2 positive breast cancer, metastatic breast cancer	Completed	NCT00788931	Phase 1
Panobinostat + trastuzumab	Breast cancer	Completed	NCT00567879	Phase 1
Panobinostat (LBH589)	Cancer	Completed	NCT00570284	Phase 1
Panobinostat (LBH589)	Cutaneous T-cell lymphoma, leukemia–lymphoma	Terminated	NCT00699296	Phase 2
Panobinostat (LBH589)	Tumors, cutaneous T-cell lymphoma	Completed	NCT00412997	Phase 1
Panobinostat (LBH589)	Recurrent malignant gliomas	Terminated	NCT00848523	Phase 2
Panobinostat (LBH589)	Non-Hodgkin lymphoma, neoplasms	Completed	NCT00503451	Phase 1
Panobinostat (LBH589)	Small cell lung carcinoma	Completed	NCT01222936	Phase 2
CHR-3996	Solid tumor	Completed	NCT00697879	Phase 1
CHR-2845	Hematological disease, lymphoid malignancies	Completed	NCT00820508	Phase 1
ITF2357 (givinostat)	Hodgkin's lymphoma	Terminated	NCT00496431	Phase 1, 2
ITF2357 (givinostat)	Myeloproliferative diseases	Completed	NCT00606307	Phase 2
ITF2357 (givinostat)	Chronic lymphocytic leukemia	Terminated	NCT00792831	Phase 2
ITF2357 (givinostat)	Hodgkin's lymphoma	Completed	NCT00792467	Phase 1, 2
ITF2357 (givinostat)	Polycythemia vera	Completed	NCT00928707	Phase 2
Belinostat or PXD101	De novo myelodysplastic syndromes	Completed	NCT01647009	Phase 2
Belinostat	Myelodysplastic syndromes	Completed	NCT00357162	Phase 2
PXD101|dexamethasone	Multiple myeloma	Completed	NCT00131261	Phase 2
Belinostat, carboplatin, paclitaxel and bevacizumab	Non-small-cell lung carcinoma	Terminated	NCT01090830	Phase 1, 2
Belinostat + erlotinib	Non small cell lung cancer	Terminated	NCT01188707	Phase 1, 2
JNJ-26481585	Hematologic cancer	Terminated	NCT00676728	Phase 1
SB939	Solid tumor	Completed	NCT00504296	Phase 1
SB939 + azacitidine	Solid tumors and hematologic malignancies	Completed	NCT00741234	Phase 1

3.1.4. *Cyclic peptides*

Romidepsin (Depsipeptide or FK228) acts as an HDACi with the disulfide bond undergoing reduction within the cell to release a zinc-binding thiol. Thiol interacts with a zinc atom in the binding pocket of zinc-dependent HDAC to block its activity. Romidepsin is a natural product obtained from the bacteria *Chromobacterium violaceum*. On November 5, 2009, it was approved by the FDA for the treatment of cutaneous T-cell lymphoma (CTCL). Terminated clinical trials show the activity of this compound in treating lymphoma (Coiffier et al., 2012), MM (Khan et al., 2004; Kikuchi et al., 2010; Niesvizky et al., 2011), CTCL (Piekarz et al., 2011; Prince and Dickinson, 2012), MDS (Klimek et al., 2008), and solid tumors such as pancreatic (Sato et al., 2004), colorectal (Whitehead et al., 2009), lung (Otterson et al., 2010; Schrump et al., 2008), prostate (Lai et al., 2008), renal (Stadler et al., 2006), bladder (Karam et al., 2007), brain (Iwamoto et al., 2011), thyroid (Kitazono et al., 2001) and ovarian cancers (Son et al., 2010) (Table 6).

3.2. *Sirtuins*

Class III HDACs, also known as sirtuins, are the silent information regulator 2 (Sir2) family of proteins. Distinct from other HDACs, sirtuins are NAD^+-dependent protein deacetylases, not modulated by HDACi. The mammalian sirtuin family comprises seven proteins (SirT1-7), which differ widely in their cellular localization, activity and functions, and are subdivided into 4 classes (Carafa et al., 2012). The deacetylase activity of sirtuins is controlled by the cellular $[NAD^+]/[NADH]$ ratio; NAD^+ works as an activator, whereas nicotinamide and NADH inhibit their activity. Two reactions are catalyzed by sirtuins: deacetylation and ADP-ribosylation. In both, the cleavage of NAD^+ is the initial chemical step. SirT1, 2, 3, 5 and 7 catalyze a deacetylation reaction, in which they deacetylate lysine residues of target proteins using NAD^+ as cofactor and releasing nicotinamide with the production of 2′-O-acetyl-ADP ribose. In contrast, SirT4 and 6, catalyze ADP-ribosylation reaction, during which ADP-ribosyl moiety is transferred to the protein substrate (Yamamoto et al., 2007).

Expressed from bacteria to humans (Vaquero, 2009), sirtuins seem to preferentially target non-histone proteins, though little is known about target specificity and selectivity. Sirtuins are connected to chromatin regulation as they are responsible for controlling two post-translational histone modifications crucial for chromatin structure: H4K16ac and H3K9ac. SirT1, 2, 3 and 6 are involved in chromatin regulation (McGuinness et al., 2011). SirT1, the best-studied family

Table 5 – Benzamides in clinical trials.

Drug	Condition	Status	NCT number	Phases
Entinostat	Melanoma	Completed	NCT00185302	Phase 2
Entinostat	Cancer	Completed	NCT00020579	Phase 1
Entinostat	Leukemia, multiple myeloma and plasma cell neoplasm, MDS	Completed	NCT00015925	Phase 1
Entinostat + isotretinoin	Lymphoma, small intestine cancer	Completed	NCT00098891	Phase 1
Azacitidine + entinostat	Leukemia, myelodysplastic syndromes	Completed	NCT00101179	Phase 1
Azacitidine + entinostat	Colorectal cancer	Completed	NCT01105377	Phase 2
MGCD0103 + docetaxel	Solid cancer	Terminated	NCT00511576	Phase 1
Azacitidine + MGCD0103	Acute myeloid leukemia (AML)|myelodysplastic syndrome (MDS)	Terminated	NCT00666497	Phase 2
MGCD0103	Hodgkin's lymphoma	Terminated	NCT00358982	Phase 2
MGCD0103	Myelodysplastic syndrome, acute myelogenous leukemia	Completed	NCT00324220	Phase 1, 2
MGCD0103	Leukemia, myelodysplastic syndromes	Completed	NCT00324129	Phase 1
MGCD0103	Tumors, non-Hodgkin's lymphoma	Completed	NCT00323934	Phase 1
MGCD0103	Lymphocytic leukemia, chronic	Completed	NCT00431873	Phase 2
MGCD0103	Lymphoma	Completed	NCT00359086	Phase 2
MGCD0103	Leukemia, myelodysplastic syndromes	Completed	NCT00324194	Phase 1
MGCD0103	Myelogenous leukemia, acute, myelodysplastic syndromes	Terminated	NCT00374296	Phase 2
MGCD0103 + gemcitabine	Tumors	Completed	NCT00372437	Phase 1, 2
MGCD0103 + azacitidine	Hodgkin lymphoma	Terminated	NCT00543582	Phase 2

member, is responsible for heterochromatin formation by a deacetylating process.

In the last decade interest for sirtuins has grown, mainly due to their critical role in several biological processes, such as regulation of gene expression, control of metabolic processes, apoptosis and cell survival, DNA repair, development, neuroprotection and inflammation. Sirtuins control many vital functions and are involved in many disorders such as metabolic diseases, neurodegenerative diseases and cancer (Stunkel and Campbell, 2011). SirT1 displays contradictory roles, and has been suggested

Table 6 – Romidepsin in clinical trials.

Drug	Conditions	Recruitment	NCT number	Phases
Romidepsin + bortezomib	Multiple myeloma	Terminated	NCT00765102	Phase 2
Romidepsin + ketoconazole	Hematologic cancer	Completed	NCT01324310	Phase 1
Romidepsin (FR901228)	Mycosis fungoides, CTCL, neoplasms	Terminated	NCT01445340	Phase 1
Romidepsin	Peritoneal, epithelial, ovarian cancer	Completed	NCT01645670	Phase 2
Romidepsin + rifampin	Hematologic cancer	Completed	NCT01324323	Phase 1
Romidepsin (FR901228)	Lymphoma	Completed	NCT00383565	Phase 2
Romidepsin + gemcitabine	Pancreatic cancer	Completed	NCT00379639	Phase 1, 2
Romidepsin (depsipeptide, FK228)	Cutaneous T-cell lymphoma	Completed	NCT00106431	Phase 2
Depsipeptide, FR901228, FK228	Neoplasms	Completed	NCT00048334	Phase 1
Romidepsin + rituximab + fludarabine	Lymphoma	Completed	NCT00079443	Phase 2
Romidepsin	Brain and nervous system tumors	Completed	NCT00085540	Phase 2
Romidepsin	Lung cancer	Completed	NCT00086827	Phase 2
Romidepsin	Colorectal cancer	Completed	NCT00077337	Phase 2
Romidepsin	Bladder, renal pelvis and urethral cancer	Terminated	NCT00087295	Phase 2
Romidepsin	Leukemia, lymphoma	Completed	NCT00024180	Phase 1
Romidepsin	Lymphoma	Completed	NCT00019318	Phase 1
Romidepsin	Leukemia, lymphoma, MDS syndrome	Completed	NCT00042822	Phase 2
Romidepsin + decitabine	Leukemia, lymphoma, MDS syndrome	Completed	NCT00114257	Phase 1
Romidepsin	Breast cancer	Completed	NCT00098397	Phase 2
Romidepsin	Esophageal cancer, gastric cancer	Suspended	NCT00098527	Phase 2
Romidepsin	Ovarian, primary peritoneal cavity cancer	Completed	NCT00091195	Phase 2
Romidepsin	Lymphoma	Completed	NCT00077194	Phase 2
Romidepsin	Head and neck cancer	Completed	NCT00098813	Phase 2
Decitabine + depsipeptide	Small cell lung carcinoma	Completed	NCT00037817	Phase 1
Romidepsin	Nervous system tumors, leukemia	Completed	NCT00053963	Phase 1
Romidepsin (depsipeptide, FK228)	Prostate cancer, metastases	Completed	NCT00106418	Phase 2
Romidepsin (FR901228)	Small cell lung carcinoma	Completed	NCT00020202	Phase 2
Romidepsin (depsipeptide, FK228)	Renal cell carcinoma, metastases	Completed	NCT00106613	Phase 2

either as tumor suppressor or tumor promoter (Bosch-Presegue and Vaquero, 2011; Deng, 2009). The initial evidence of SirT1 as a tumor promoter derived from its repressive effect on the tumor suppressor p53. However, a potential tumor suppressor role has also been proposed for other human sirtuins (McGuinness et al., 2011). This hypothesis is mainly sustained by reduction of SirT2 in a large number of human brain tumor cell lines, and its involvement in cell cycle progression. SirT3 is the only mitochondrial sirtuin implicated in tumorigenesis. Its reduction in several cancers leads to an increase in reactive oxygen species (ROS) production, which results in enhanced tumor growth. SirT5 overexpression has been implicated in a study of pancreatic cancer (Kim et al., 2010). Recently, the role of SirT6 and SirT7 in tumorigenesis has been demonstrated. SirT6 controls NF-kB pathway and plays a role in DNA double-strand repair, indicating that this sirtuin has a key function in tumorigenesis. However, very little is known about the specific correlation with cancer. mRNA levels of SirT7 have been inversely correlated with the ability to undergo tumorigenesis in mouse cell lines, and levels of SirT7 have been found elevated in some forms of breast cancer (Ashraf et al., 2006).

Sirtuins are involved in aging diseases such as metabolic disease, neurodegeneration and aging itself. It is well known that overexpression of Sir2 (or its orthologs) can extend organism lifespan in a wide range of lower eukaryotes (Bosch-Presegue and Vaquero, 2011; Vaquero, 2009). Sir2 functions are often correlated to calorie restriction (CR). The link between the role of sirtuins, CR and longevity was first described in *Saccharomyces cerevisiae*. In yeast, CR leads to increased replicative lifespan. Lifespan extension was not observed in yeast lacking the Sir2 gene. Currently, the role of sirtuins in the regulation of mammalian lifespan is not clear. However, starting from the premise that sirtuins are an evolutionary conserved protein family, it is fair to assume that they play a role in the modulation of aging-related processes in higher organisms (Brooks and Gu, 2009; Westphal et al., 2007). In humans, the aging process is associated to telomere erosion. SirT1 and 6 are involved in the maintenance of telomeres and telomeric function, and are implicated in the aging process. Recent studies have demonstrated that reduction or removal of SirT6 results in telomere dysfunction and end-to-end chromosomal fusions. In terms of symptoms, the absence of SirT6 is similar to a disease characterized by premature aging, Werner's syndrome. Although very little is known about other sirtuins, no evidence suggests their involvement with telomere function, formation and stability (McGuinness et al., 2011).

Given that SirT1 has been reported to increase tumorigenesis, and despite its role as tumor suppressor or promoter, it is important to identify small chemical compounds that inhibit or activate SirT1. To date, a number of specific inhibitors of SirT1 have been proposed for cancer therapy (Alcain and Villalba, 2009). Moreover, both activators and inhibitors of sirtuins might act beneficially against different types of neurodegenerations. Thus, in addition to nicotinamide, the physiological inhibitor, some specific inhibitors have been characterized, including splitomicin and its analogs, tenovins, AGK2, sirtinol, suramin, the indole derivative EX-257, salermide and UVI5008.

SIRT activators are being studied in the hope of providing benefit to patients with neurodegenerative, inflammatory, metabolic and autoimmune diseases, and some tumor types.

Phenol derivates, including quercetin, piceatannol, and resveratrol, have been shown to posses SirT1-activating properties (Alcain and Villalba, 2009). The most potent SirT activator, and the first to be characterized, is resveratrol, a polyphenol found in grapes, and grape products. Subsequently, much more potent and efficacious SirT1 activators were reported as potential therapeutics for treatment of metabolic diseases (SRT1720, SRT2183, and SRT1460) (Saunders and Verdin, 2007). However, their activity is still debated. The question is whether they are SIRT activators or assay artifacts. Given that the activation of SirT1 by activators requires the use of fluorescently labeled substrate in a fluorescence assay, it was demonstrated that they do not activate SirT1 when using native peptide or protein substrate conjugated with a non-physiological fluorophore (Borra et al., 2005). To establish their effective modulation of SirT1, other technical approaches are necessary. Despite these issues, *resveratrol* is now in clinical trials (Table 7). Resveratrol can exhibit benefits against cardiovascular diseases (CVDs) or in its prevention, although its cardioprotective role as part of the human diet is not yet clear (Ruana et al., 2012). CR is a low calorie diet (about 30% fewer calories than the American Dietetic Association (ADA) recommends). CR has also been linked to health benefits (enhanced cardiovascular and metabolic health) and an extended lifespan. Many studies have compared the health benefits of both resveratrol and CR to determine whether resveratrol mimics some of the health benefits shown with CR. The consumption of this polyphenol could modulate cerebral blood flow and this in turn could influence cognitive performance by increasing access to blood-borne metabolic fuel. Research shows that resveratrol is able to induce vasodilation (and therefore blood flow) by interacting with nitric oxide (NO). Because tumors develop resistance to chemotherapeutic agents, the aim of cancer research is to discover effective chemosensitizers. One promising possibility is to use dietary agents, such as resveratrol, that sensitize tumors to chemotherapeutics (Aggarwal et al., 2004). Through its ability to modulate multiple cell-signaling molecules such as cell survival proteins, members of NF-kB and STAT3 pathways, resveratrol is able to prevent cancer (Gupta et al., 2011). Tumors shown to be sensitized by resveratrol include lung and breast cancer, AML, promyelocitic leukemia, MM, prostate, pancreatic and epidermoid cancers (Fulda and Debatin, 2004). Patients with colon cancer received treatment with resveratrol, and correlative laboratory studies examined its effects directly on colon cancer and normal colonic mucosa. Resveratrol may stop the growth of tumor cells by blocking some of the enzymes required for cell growth, and by inducing cell cycle arrest, apoptosis, inhibition of cell proliferation, stimulation of antiangiogenic responses and increased antioxidant and anti-inflammatory activity (Talero et al., 2012). The activity of resveratrol shows great potential in the prevention and therapy of a wide variety of human diseases.

3.3. *HAT inhibitors*

Of the several known covalent histone modifications, the reversible acetylation of key lysine residues in histones holds a pivotal position in transcriptional regulation. Histone

Table 7 – Resveratrol in clinical trials.

Drug	Conditions	Status	NCT number	Phases
Resveratrol and midazolam	Healthy	Completed	NCT01173640	
Resveratrol	Cognitive and cerebral blood flow	Completed	NCT01010009	
Resveratrol	Cardiovascular diseases	Completed	NCT01449110	Phase 2
Resveratrol	Colon cancer	Completed	NCT00256334	Phase 1
Resveratrol	Chronic subclinic inflammation	Completed	NCT01492114	Phase 3
Resveratrol	Type 2 diabetes mellitus	Completed	NCT01038089	
Resveratrol	Healthy	Completed	NCT01331382	
Resveratrol	Sports concussion	Completed	NCT01321151	Phase 1
Resveratrol	Neoplasms, colorectal cancer	Completed	NCT00920803	Phase 1
Resveratrol	Sedentary lifestyle	Completed	NCT01615445	Phase 2
Resveratrol	Melanoma (skin)	Completed	NCT00721877	Phase 1
Resveratrol	Metabolic syndrome, obesity	Completed	NCT01150955	
Resveratrol	Multiple myeloma	Terminated	NCT00920556	Phase 2
Resveratrol	Healthy	Completed	NCT01640197	Phase 1
Resveratrol	Colorectal cancer	Completed	NCT00433576	Phase 1
Resveratrol	Unspecified adult solid tumor	Completed	NCT00098969	Phase 1
Resveratrol	Obesity, metabolic syndrome, diabetes	Completed	NCT00823381	
Resveratrol	Obesity	Completed	NCT00998504	
Resveratrol	Cardiovascular disease	Completed	NCT01199549	
Resveratrol	Cellulite (orange peel skin)	Terminated	NCT01321268	
Resveratrol	Type 2 diabetes	Completed	NCT00937222	

acetylation is a distinctive feature of transcriptionally active genes, whereas deacetylation indicates the repressed state of a gene. The balance between the acetylation and deacetylation states of histones regulates transcription. Dysfunction of enzymes involved in these events is often associated with the manifestation of several diseases, including cancer, cardiac hypertrophy and asthma (Kramer et al., 2001; McKinsey and Olson, 2004; Yang, 2004). These enzymes are therefore potential new targets for therapy. Acetyl transferases (HATs) modulate gene expression by catalyzing targeted acetylation of the ε-amino group of lysine residues on histone and non-histone proteins. HATs can be classified into several families on the basis of number of highly conserved structural motifs. These include the GNAT family (Gcn5-related *N*-acetyltransferase, e.g., Gcn5, PCAF), the MYST group (MOZ, YBF2/SAS3 and TIP60) and the p300/CBP family (Kramer et al., 2001; Sterner and Berger, 2000; Yang, 2004).

Although a wide number of transcriptional co-activator proteins are now recognized to possess HAT activity, very few HAT inhibitors (HATi) have been identified to date. Availability of recombinant HATs made it possible to synthesize and test more target-specific inhibitors: Lys-CoA for p300 and H3-CoA-20 for PCAF (Lau et al., 2000). Though Lys-CoA has been extensively employed for *in vitro* transcription studies, it is unable to permeate cells (Cebrat et al., 2003). In the early 2000s, two important HATis were isolated: anacardic acid from cashew nut shell liquid and garcinol from *Garcinia indica*, which are both non-specific inhibitors of p300/CBP and PCAF but are capable of easily permeating cells in culture (Balasubramanyam et al., 2004a, 2003). Different chemical modifications of these inhibitors were attempted to identify enzyme-specific inhibitors, but it serendipitously led to the synthesis of the only known p300-specific activator, *N*-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide (CTPB) (Balasubramanyam et al., 2003) (Souto et al., 2010, 2008). None of the above is in clinical trials.

Curcumin (diferuloylmethane) is the major curcuminoid of turmeric, *Curcuma longa*, a characteristically orange-yellow colored spice often found in curry powder. In recent years, considerable interest has been focused on this substance due to its use in treating a wide variety of disorders without any side effects. It was used in ancient times to treat various illnesses such as rheumatism, body ache, skin diseases, intestinal worms, diarrhea, intermittent fevers, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammation, constipation, leukoderma, amenorrhea, and colic. Curcumin has the potential to treat a wide variety of inflammatory diseases including cancer, diabetes, cardiovascular diseases, arthritis, Alzheimer's disease and psoriasis through modulation of numerous molecular targets. Curcumin was identified as the first p300/CBP-specific cell permeable HATi (Balasubramanyam et al., 2004b). It does not affect the HAT activity of PCAF or histone deacetylase and methyltransferase activities. However, p300 HAT activity-dependent chromatin transcription is efficiently repressed by curcumin but not transcription from DNA template. Curcumin could also inhibit histone acetylation *in vivo*. It is the only HATi in clinical trials (Table 8) and exhibits great promise as a therapeutic agent. Its applications include atopic asthma (Kobayashi et al., 1997), chronic obstructive pulmonary disease (Rennolds et al., 2012), multiple myeloma (Ghoneum and Gollapudi, 2011), irritable bowel syndrome (Binion et al., 2008; Rapin and Wiernsperger, 2010), ulcerative colitis (Baliga et al., 2012), Crohn's disease (Mouzaoui et al., 2012), breast cancer (Nagaraju et al., 2012), Alzheimer's disease (Darvesh et al., 2012; Huang et al., 2012), pancreatic cancer (Dandawate et al., 2012; Veeraraghavan et al., 2011), colorectal cancer (Guo et al., 2012; Lin et al., 2011), diabetes (Abdel Aziz et al., 2012), and psoriasis (Kurd et al., 2008).

Table 8 – Curcumin in clinical trials.

Drugs	Conditions	Recruitment	NCT number	Phases
Curcumin	Atopic asthma	Completed	NCT01179256	N/A
Curcumin + bioperine	Multiple myeloma	Completed	NCT00113841	N/A
Curcumin	Irritable bowel syndrome	Completed	NCT00779493	Phase 4
Curcumin	Healthy	Completed	NCT00181662	N/A
Curcumin	Healthy	Completed	NCT00895167	Phase 1
Curcumin	Breast cancer	Completed	NCT01042938	Phase 2
Curcumin	Alzheimer's disease	Completed	NCT00164749	Phase 1 and 2
Curcumin	Inflammatory bowel disease|ulcerative colitis|Crohn's disease	Completed	NCT00889161	Phase 1
Curcumin + bioperine	Chronic obstructive pulmonary disease	Completed	NCT01514266	N/A
Curcumol	Chemotherapy induced mucositis	Completed	NCT00475683	Phase 3
Curcumin + bioperine	Mild cognitive impairment	Completed	NCT00595582	N/A
Curcumin C3 complex	Alzheimer's disease	Completed	NCT00099710	Phase 2
Curcumin + fluoxetine	Major depressive disorder	Completed	NCT01022632	N/A
Curcumin + gemcitabine	Pancreatic cancer	Completed	NCT00192842	Phase 2
Curcumin	Familial adenomatous polyposis	Terminated	NCT00248053	Phase 2
Curcumin	Colorectal cancer	Completed	NCT00027495	Phase 1
Curcumin	Aberrant crypt foci	Terminated	NCT00176618	N/A
Curcumin	Healthy, no evidence of disease	Completed	NCT00768118	N/A
Curcumin + quercitin + sulindac	Colorectal cancer	Terminated	NCT00003365	N/A
Observational study	Diabetes	Completed	NCT01029327	N/A
Curcumin C3 complex	Psoriasis	Completed	NCT00235625	Phase 2
Curcuminoids	Oral lichen planus	Completed	NCT00525421	Phase 2
Standardized turmeric root extract	Cystic fibrosis	Completed	NCT00219882	Phase 1
Carbohydrate drink	Nutrition processes	Completed	NCT00799630	N/A

3.4. *Histone methyltransferases*

Histone methylation has been shown to play a key function in the regulation of gene-expression patterns and DNA repair; this kind of post-transcriptional (epigenetically controlled) modification can affect lysine (K) or arginine (R) residues of histone tails (Greer and Shi, 2012). In contrast to histone acetylation, histone methylation does not alter the charge of the histone tail, but influences the basicity, hydrophobicity of histones and their affinity to certain proteins such as transcription factors (Rice and Allis, 2001). A methyl group is relatively small and its addition to lysine or arginine residues does not neutralize their charge, and it is therefore unlikely that methylation alone will significantly affect the chromatin structure (Bannister and Kouzarides, 2011). Lysine side chains may be mono-, di- or tri-methylated, whereas the arginine side chain may be mono-methylated or (symmetrically or asymmetrically) di-methylated (Smith and Denu, 2009).

On the basis of target residue for methylation, histone methyltransferases (HMTase) can be grouped into two different enzymatic classes: lysine methyltransferases and arginine methyltransferases.

Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by the family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4). In contrast, a more diverse set of histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the Drosophila Su[var]3–9, enhancer of zeste, and Trithorax proteins. Lysine methylation has been implicated in both transcriptional activation (H3 Lys4, 36, 79) and silencing (H3 Lys9, 27, H4 Lys20).

At present, there are many well-known methylation sites on histones. Taking into consideration all three possible methylation states of lysine and arginine, an enormous number of methylation states of histones exist. This might explain the difficulty in studying the methylation pattern on histones.

Conversely, due to the complex pattern of histone methylation, it may be possible to interfere with this enzyme in a promising manner.

Although attempts to interfere with DNA methylation (e.g., by DNMTIs) and histone deacetylation (e.g., by HDACIs) have received the bulk of attention, recent efforts have begun to focus on pharmacologic disruption of other epigenetic regulatory processes. Histone methylation represents one such target, but to date studies on specific HMTi classes are far from the clinic.

3.4.1. *Histone methyltransferase inhibitors*

The first applied inhibitor used as an anticancer drug was *S*-adenosylmethionine (SAM) and its analogs (e.g., SAH). However, these compounds target not only HMTs but also other enzymatic classes using AdoMet as methyl-donor (such as DNMTs). Their use is therefore limited by low specificity (Spannhoff et al., 2009a).

As a lysine methyltransferase, chaetocin, a fungal mycotoxin, has been reported to act against G9a at low concentration without inhibition of other KMT enzymes (such as EZH2 or SET7/9) (Copeland et al., 2009; Greiner et al., 2005; Spannhoff et al., 2009b). Inhibition mediated by chaetocin is competitive against the co-substrate SAM. Chaetocin potently induces cellular oxidative stress, selectively killing cancer cells and rapidly proliferating primary cells (Isham et al., 2007). The effects of chaetocin on oxidative stress are at least in part due to its capacity to act as a competitive and selective substrate for theoredoxin reductase-1 ($K_m = 4.6\ \mu M$) (Tibodeau et al., 2009).

Another specific inhibitor of G9a is BIX-01294 (and its derivative BIX-01338, both hydrochloride hydrates), which is effective *in vitro* at a concentration of 2.7 μM with no effects on SUV39H1 and PRMT1 (Kubicek et al., 2007; Shi et al., 2008). Cell lines treated with BIX-01294 showed a reduction in histone H3 lysine 9 (H3K9) dimethylation, while the mono- or tri-methyl levels were unaffected. Other lysine methylation sites such as H3K27 or H4K20 were not altered. Based on a kinetic inhibition model, BIX-01294 showed an uncompetitive pattern compared to the co-substrate SAM, suggesting that it only binds to G9a complexed with SAM. BIX01294 has been used in combination with the calcium channel activator BayK8644 to facilitate the generation of induced pluripotent stem cells from somatic cells *in vitro*.

UNC0224 is another potent and relatively selective G9a HMTi, exhibiting an IC_{50} value of 15 nM. Isothermal titration calorimetry revealed that UNC0224 binds to G9a with a K_d value of 29 nM. UNC0224 also inhibits G9a-like protein (GLP), a closely related H3K9 HMTase, with assay-dependent IC_{50} values of 20–58 nM, but is over 1000-fold selective against SET7/9 (a H3K4 HMTase) and SET8 (a H4K20 HMTase) (Liu et al., 2009).

The lysine methyltransferase EZH2 (KMT6), part of the polycomb repressive complex 2, catalyzes trimethylation of lysine 27 on histone H3 and is involved in proliferation and aggressive cell growth associated with neoplastic cells. Given its importance in cell proliferation and homeostasis maintenance, intense efforts have been directed toward discovering specific EZH2 inhibitors (Simon and Lange, 2008). 3-Deazaneplanocin A (DZnep) is a cyclopentenyl analog of 3-deazaadenosine, originally synthesized as an inhibitor of S-adenosyl-L-homocysteine hydrolase (Tseng et al., 1989). It has been shown to deplete EZH2 levels and to inhibit trimethylation of lysine 27 on histone H3 in cultured human acute myeloid leukemia (AML) HL-60 and OCI-AML3 cells and in primary AML cells in a dose-dependent manner (0.2–1 μM) (Fiskus et al., 2009). DZnep treatment of cultured human AML cells induces increased expression of the cell-cycle regulators p21, p27, and FBXO32, leading to cell cycle arrest and apoptosis. When used in combination with the pan-HDACi Panobinostat (10 mg/kg), the antileukemic effects of DZnep (1 mg/kg) are synergistically enhanced in mice implanted with AML cells.

The arginine methyltransferase enzyme AMI-1 (and subsequently its derivatives AMI-2 to AMI-6) is the first and to date best-known inhibitor. It is cell-permeable, symmetrical sulfonated urea compound that acts as a potent, specific and non-AdoMet (SAM)-competitive inhibitor of protein arginine N-methyltransferases (PRMTs; IC_{50} = 8.81 μM for PRMT1 and 3.03 μM for yeast-RMT1p) with minimal effect on lysine methyltransferases. It inhibits nuclear receptor reporter gene activation in MCF-7 cells, and HIV-1 RT polymerase (IC_{50} = 5 μM).

In summary, although many of these inhibitors are able to reduce or abolish HMT activity *in vitro* or in cell-based assays, they are still at pre-clinical stage due to the low specificity and toxicity observed in different cell lines.

3.5. *Histone demethylase enzymes and their inhibitors*

Two families of histone demethylating enzymes (HDs) have recently been discovered. Lysine-specific demethylase 1 (LSD1) is a flavin-dependent monoamine oxidase which can demethylate mono- and di-methylated lysines, specifically histone 3, lysines 4 and 9 (H3K4 and H3K9) (Forneris et al., 2005). Jumonji domain-containing (JmjC) histone demethylases are able to demethylate mono-, di-, or tri-methylated lysines. Two specific JmjC HDs are PHF8 and JHDM1D.

LSD1 shares similar catalytic sites with monoamine oxidases (MAO) A and B, the inhibition of which is used clinically to treat depression, anxiety, and Parkinson's disease (Lee et al., 2006).

2-PCPA (Tranylcypromine) is an inhibitor of LSD1 with an IC_{50} value of 20.7 μM and a K_i value of 242.7 μM, which effectively inhibits histone demethylation *in vivo*. Although not as selective, 2-PCPA also irreversibly inhibits MAO A and MAO B with IC_{50} values of 2.3 and 0.95 μM and K_i values of 101.9 and 16 μM, respectively (Schmidt and McCafferty, 2007). Given these collateral effects, Tranylcypromine has not yet entered clinical trials.

4. DNA methyltransferases enzymes and their inhibitors

DNA methylation refers to a covalent modification of the cytosine base (C), localized at 5′ of a guanidine base (G) in a CpG dinucleotide (Das and Singal, 2004; Robertson, 2001; Robertson and Wolffe, 2000). DNA methylation is involved in the control of gene expression, regulation of parental imprinting and stabilization of X chromosome inactivation as well as maintenance of genome integrity. It is also implicated in the development of immune system, cellular reprogramming, brain function and behavior (Di Croce et al., 2002).

The transfer of methyl groups from SAM to cytosine in CpGs is catalyzed by a family of enzymes called DNA methyltransferase (DNMTs) (Iyer et al., 2011; Jurkowska et al., 2011; Peedicayil, 2012; Xu et al., 2010). In mammals, three DNMTs have so far been identified, including two "de novo" methyltransferases (DNMT3A and DNMT3B) and "maintenance" methyltransferase (DNMT1), generally the most abundant and active of the three (Goll and Bestor, 2005). The protein DNMT2 can also be found in mammalian cells. Although the structure of DNMT2 is very similar to other DNMTs, its role is less understood (Schaefer and Lyko, 2010). It has been reported that DNMT2 does not methylate DNA but instead methylates aspartic acid transfer RNA (tRNAAsp) (Jurkowski and Jeltsch, 2011). Recent evidence suggests that DNMT2 activity is not limited to tRNAAsp and that DNMT2 represents a non-canonical enzyme of the DNMT family (Schaefer et al., 2010). Given the critical role of DNMTs, intense interest has focused on developing drugs able to interfere with aberrant DNMT activities, and using them to correct epigenetic defects such as tumor suppression gene (TSG) silencing (Rajendran et al., 2011).

DNMTs modulators represent a useful tool in epigenetic therapies. Several epi-drugs interfering with DNMT activity are currently in pre-clinical and clinical trials (Foulks et al., 2012; McGovern et al., 2012). Most of these trials have involved various types of cancer, such as solid and hematological tumors (Chaib et al., 2011; Fandy, 2009; Ren et al., 2011; Song et al., 2011). Currently, however, the main challenge in using epigenetic modulators for therapy, especially for interfering

with DNMT enzymes is their specificity (Veeck and Esteller, 2010).

It is well known that abnormal patterns of DNA methylation are often displayed in human neoplasms (Chin et al., 2011; Fonseca et al., 2012; Lokk et al., 2012; Peedicayil, 2012). A combination of regional promoter hypermethylation with the concomitant silencing of important genes involved in cell death, surveillance and proliferation is always present. However, demethylation per se is not sufficient to induce gene expression, as it is regulated by a combination of different epi-mutations (Choo, 2011; Eglen and Reisine, 2011). DNA methylation and histone modifications are tightly correlated because inactive chromatin is enriched with hypermethylated DNA and active chromatin is associated with hypomethylated DNA. In contrast, genomic hypomethylation mediates tumorigenesis (via chromosomal instability) and supports the metastatic process (Hatziapostolou and Iliopoulos, 2011; Kulis and Esteller, 2010; Plitta et al., 2011; Watanabe and Maekawa, 2010). Consequently, induction of global DNA hypomethylation using DNMTi may help speed tumor progression from cancer cells surviving DNA demethylation therapy (Guz et al., 2010; Villa et al., 2004; Wild and Flanagan, 2010).

Moreover, the interrelation between drugs targeting chromatin and those targeting DNA methylation could be utilized therapeutically by combining different epigenetic drugs against different epigenetic effectors to increase the efficacy of a monotherapy.

Several clinical trials testing different DNMTi are terminated and ongoing.

4.1. *Nucleoside analogs*

This class of DNMTi includes nucleoside analogs, which are phosphorylated by cellular kinases. Once incorporated into DNA, they form a covalent bond with the DNMT trapping the enzyme and making it unavailable for further methylation, thus resulting in demethylation of replicating nascent DNA (Szyf, 2009). Two agents have been developed clinically: 5-aza-2′-deoxycytidine (decitabine) and 5-azacytidine (azacitidine or Vidaza). These agents have been tested in numerous trials (Goffin and Eisenhauer, 2002; Sorm et al., 1964) (Tables 9–12).

Decitabine is incorporated into DNA, while *azacitidine* is incorporated preferentially into RNA (Leone et al., 2002). Demethylation by decitabine has been shown to allow re-expression of silenced genes and cellular differentiation. Additionally, incorporation of these agents into RNA causes ribosomal disassembly, defective tRNA function and inhibited protein production. However, azacitidine exhibits greater cytotoxicity during S-phase, supporting the greater importance of its DNA effects. Both these drugs are inactivated via deamination by cytidine deaminase (Galmarini et al., 2001) and have been approved by the FDA for the treatment of myelodysplastic syndromes (Kadia et al., 2011; Marks, 2012).

To date, decitabine and azacitidine have been studied as single drug treatment (Tables 9 and 11) for many malignancies, though the best results have been obtained for MDS and various types of leukemia.

These DNMTis have been in clinical trial for hematological diseases, such as MDS and leukemias (Chen et al., 2012; Garcia-Manero, 2012; Keating, 2012; Kimura et al., 2012; Oki et al., 2012; Platzbecker et al., 2012; Ritchie, 2012), thalassemia (Mabaera et al., 2008; Olivieri et al., 2011; Rose et al., 2011; Sauntharajah et al., 2003) and solid tumors, such as prostate (Gravina et al., 2011; Shabbeer et al., 2012), colon (Al-Salihi et al., 2011; Hagemann et al., 2011; Ikehata et al., 2012; Yang et al., 2012), bladder (Shang et al., 2008), breast (Mirza et al., 2010), melanoma (Alcazar et al., 2012; Liu et al., 2011), esophageal (Dong et al., 2012; Liu et al., 2005; Meng et al., 2012; Schrump et al., 2006), lung cancers (Kaminskyy et al., 2011; Wu and Hu, 2011).

The most promising results have been obtained with combination therapy, especially with HDACi (Tables 10 and 12).

4.2. *Small molecules*

Blocking the enzymatic activity of DNMTs by using small molecule inhibitors is another strategy to achieve gene demethylation (Datta et al., 2009). Hydralazine and procainamide are FDA approved for the treatment of hypertension and cardiac arrhythmia, respectively (Peng et al., 2010). Recently, their ability to inhibit DNMT activity has been discovered and is associated with direct binding to CpG-rich sequences (Amatori et al., 2010). More precisely, these molecules act as partial competitive inhibitors of DNMT1, decreasing the affinity of DNMT for its substrates (DNA and *S*-adenosyl-L-methionine), reducing the processivity of the enzyme and favoring the dissociation of DNMT1 from hemimethylated DNA. No data are currently available for their use in clinical trails as DNMT inhibitors; however, they are in pre-clinical studies.

The antihypertensive (Singh et al., 2009) hydralazine was tested as a DNMT inhibitor due to its capability to induce (as a side effect) a lupus-like syndrome known to be related to disorders associated with DNA methylation (Candelaria et al., 2011; Lu et al., 2005). Although its mechanism of action is still under investigation, some evidence indicates that hydralazine, similar to procaine and procainamide, binds to CpG-rich sequences and interferes with translocation of DNMTs along the DNA strand. A phase 1 study has shown that hydralazine is able to induce re-expression of various tumor suppressor genes, including p16 and RAR-β, in cervical cancer patients, even at lower doses than those considered safe for the treatment of cardiovascular disorders. On the basis of promising data arising from phase 1, hydralazine is currently under phase 2 and 3 investigations. Table 1 reports three clinical trials in which hydralazine is used in combination with magnesium valproate against solid tumors (discussed in “Short-chain fatty acid”).

A major drawback of these drugs is the high concentrations required for their demethylating activity, which can elicit undesired toxic effects if administered clinically.

Rational design of DNMTi that interact noncovalently with the active catalytic site of DNMTs, utilizing a three-dimensional model of the human DNMT1 catalytic pocket, is a sound alternative approach to silence the DNA methylation machinery. RG108 [2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-3-(1H-indol-3-yl)propanoic acid] is the first rationally designed DNMT1i that demonstrates demethylating activity both *in vivo* and *in vitro* (Braun et al., 2010; Brueckner et al., 2005; Chestnut et al., 2011; Schirrmacher et al., 2006; Suzuki et al., 2010).

Table 9 – Decitabine in clinical trials.

Drug	Conditions	Status	NCT number	Phases
Decitabine	MDS|CML	Completed	NCT00067808	Phase 2
Decitabine	Thalassemia	Completed	NCT00661726	Phase 2
Decitabine	Lymphoma, intestinal neoplasms	Completed	NCT00089089	Phase 1
Decitabine	AML	Completed	NCT00866073	Phase 2
Decitabine	AML, MDS	Completed	NCT00760084	Phase 2
Decitabine	MDS, CML	Terminated	NCT00113321	Phase 2
Decitabine	Leukemia	Completed	NCT01378416	Phase 1
Decitabine	CML	Completed	NCT01098084	Phase 2
Decitabine	AML	Completed	NCT00398983	Phase 2|3
Decitabine	MDS	Completed	NCT00043381	Phase 3
Decitabine	AML	Completed	NCT00538876	Phase 1
Decitabine	MDS	Terminated	NCT00282399	Phase 1|2
Decitabine	CML	Completed	NCT00042003	Phase 2
Decitabine	CML	Completed	NCT00042016	Phase 2
Decitabine	CML	Completed	NCT00041990	Phase 2
Decitabine	MDS	Completed	NCT00260065	Phase 2
Decitabine	MDS	Completed	NCT01041846	N/A
Decitabine	MDS	Completed	NCT00796003	Phase 1
Decitabine	AML	Completed	NCT00358644	Phase 2
Decitabine	Leukemia, MDS	Completed	NCT00003361	Phase 2
Decitabine	Leukemia, MDS	Completed	NCT00049582	Phase 1
Decitabine	MDS, secondary myelofibrosis	Terminated	NCT00630994	Phase 2
Decitabine	Bladder cancer, breast cancer, melanoma	Completed	NCT00030615	Phase 1
Decitabine	Esophageal, lung cancer, malignant mesothelioma	Completed	NCT00019825	Phase 1
Decitabine	Leukemia	Completed	NCT00042796	Phase 1
Decitabine	MDS, leukemia, lymphoma, MM	Completed	NCT00002980	Phase 1

This molecule was found to induce the re-expression of different hypermethylation-silenced genes, such as p16 and the putative tumor suppressor genes SRFP1 and TIMP-3, in colon cancer cells. Despite its encouraging activity, RG108 is still in pre-clinical phase.

4.3. *Natural molecules*

The use of natural products in cancer chemoprevention is currently receiving much attention.

Psammaplins, for example, are bisulfide bromotyrosines derived from a marine sponge and are able to inhibit both DNMT1 and HDAC *in vitro*. Although psammaplin A administration at low doses was found to exert a strong cytotoxic effect in human tumor cell lines and to limit tumor cell growth in a A549 lung xenograft mouse model, DNMT inhibition was not followed by DNA demethylation and re-expression of tumor suppressor genes, suggesting that an intracellular target different from DNMT1 is responsible for the cytotoxic effect of the molecule (Baud et al., 2012).

Tea polyphenols are strong antioxidants and tea preparations demonstrate inhibitory activity against carcinogenesis. (−)-Epigallocatechin-3-gallate (EGCG), the major polyphenol from green tea, is a potent inhibitor of catechol-O-methyltransferase activity (COMT) (Fang et al., 2007; Lee et al., 2005; Nandakumar et al., 2011). The structural similarity between DNMTs and COMT suggests possible inhibition of DNMTs by EGCG. EGCG inhibits DNMT activity in a dose-dependent manner and induces re-expression of hypermethylated genes such as CDKN2A, RARbeta and MGMT (Nandakumar et al., 2011). In many pre-clinical studies, EGCG exhibits a very strong DNMT inhibitory action and this natural molecule is able to induce cell death and apoptosis in many cancer types (Gu et al., 2009).

Interestingly, other dietary catechol-containing polyphenols, such as different tea catechins (catechin, epicatechin) and bioflavonoids (quercetin, genistein, fisetin), were also found to inhibit DNMT activity *in vitro* through mechanisms different to that of EGCG (Fang et al., 2007; Lee et al., 2005). The results obtained from the use of natural compounds are intriguing, especially considering their ease of use and low costs. However, further investigation is necessary to establish their real efficacy as DNMTi, as well as to evaluate the toxicity induced by their administration at pharmacological doses.

A major concern associated with the use of natural products is product standardization. Multiple sources can provide extracts with different activities and therefore create discrepancies in their reported demethylating activity.

4.4. *Antisense oligonucleotide inhibitors of DNMTs*

Antisense oligonucleotides are short, defined sequences of nucleotides that are complementary to mRNAs. They hybridize mRNAs, making them inactive and thereby blocking translation. Antisense oligonucleotides that are complementary to mRNAs for human DNMT1 are undergoing pre-clinical (Yan et al., 2003) as well as clinical trials (Davis et al., 2003).

The idea to target directly DNMT1 enzyme arises from the observation that, excluding some rare exceptions, tumor cells generally show increased expression levels of DNMTs.

Table 10 – Decitabine in combinated clinical trials.

Drug	Conditions	Status	NCT number	Phases
Decitabine + vorinostat	Myeloproliferative disorders, leukemia	Completed	NCT00357708	Phase 1
Decitabine + interferon alfa-2b	Renal cell carcinoma	Terminated	NCT00561912	Phase 2
Decitabine + filgrastim + cyclosporine	Leukemia, MDS	Completed	NCT00002832	Phase 1|2
Decitabine + VPA	Leukemia, MDS	Completed	NCT00075010	Phase 1|2
Decitabine + cytarabine or supportive care	AML	Completed	NCT00260832	Phase 3
Decitabine + carboplatin	Fallopian tube, ovarian, peritoneal cavity cancer	Terminated	NCT00748527	Phase 2
Decitabine + azacitidine	MDS	Terminated	NCT01011283	Phase 4
Decitabine + arsenic trioxide + ascorbic acid	MDS	Completed	NCT00621023	Phase 2
Decitabine + PEG-interferon alfa-2b	Unspecified adult solid tumor	Completed	NCT00701298	Phase 1
Decitabine + pegylated interferon-alfa 2b	Cancer	Terminated	NCT00886457	Phase 1
Decitabine + chemotherapy	AML	Terminated	NCT00943553	Phase 2
Decitabine + VPA	Lymphoma	Completed	NCT00109824	Phase 1
Decitabine + romidepsin	SCC|mesothelioma|NSCLC	Completed	NCT00037817	Phase 1
Decitabine + vorinostat	Leukemia|MDS	Completed	NCT00479232	Phase 1
Decitabine + filgrastim + pegfilgrastim + cyclophosphamide + doxorubicin hydrochloride	Neuroblastoma, childhood solid tumor	Completed	NCT00075634	Phase 1
Decitabine + imatinib mesylate	Leukemia	Completed	NCT00054431	Phase 2
Decitabine + romidepsin	Leukemia, MDS	Completed	NCT00114257	Phase 1
Decitabine + filgrastim + busulfan + cyclophosphamide + cyclosporine + methotrexate + methylprednisolon + tacrolimus	Leukemia	Completed	NCT00002831	Phase 1|2
Decitabine + AMG 531 (romiplostim) + azacitidine	MDS, thrombocytopenia	Completed	NCT00321711	Phase 2
Decitabine deferoxamine + deferiprone + arginine + sildenafil	Hematologic diseases, osteoporosis, hypertension	Completed	NCT00000623	N/A

The most interesting isotypic-specific DNMT1 inhibitor tested in clinical trials is MG98 (Amato et al., 2012; Patutina et al., 2009; Winquist et al., 2006), a second-generation antisense oligonucleotide that specifically targets DNMT1 mRNA. This agent eliminates the expression of DNMT1 protein resulting in the inhibition of DNA replication, triggering of damage response, and induction of TSGs. The immediate blockage of replication by DNMT1 knockdown dramatically limits demethylation induced by DNMT1 inhibition, thus avoiding the potential deleterious impact of global demethylation. The main issue with antisense oligonucleotides is delivery to solid tumors (Davis et al., 2003; Klisovic et al., 2008; Plummer et al., 2009).

Some clinical trials utilizing MG98 were stopped because of lack of objective response in the last phase 2 trials in metastatic renal cancer. Nevertheless, this strategy carries great promise. A new trial has been completed (Stewart et al., 2003) in which pharmacodynamic evaluations were performed to explore and validate the biological mechanisms of MG98 in solid tumors (Clinical trial identifier: NCT00003890).

Interesting results are provided by miR-29b, a microRNA (miRNA) that directly targets DNMT3A and DNMT3B expression. MiR-29b induces a decrease in methylation levels and induces the re-expression of hypermethylated TSGs, FHIT and WWOX in lung cancer cells, as well as of p15 and ER in AML

Table 11 – Azacitidine in clinical trials.

Drug	Conditions	Status	NCT number	Phases
Azacitidine	MDS|AML	Completed	NCT00795548	Phase 2
Azacitidine	Ovarian cancer	Terminated	NCT00842582	Phase 1
Azacitidine	MDS|leukemia	Completed	NCT00350818	Phase 1
Azacitidine	Myelofibrosis	Completed	NCT00569660	Phase 2
Azacitidine	MDSs	Completed	NCT00118287	Phase 1|2
Azacitidine	Leukemia	Completed	NCT00739388	Phase 2
Azacitidine	MDSs	Completed	NCT01186939	Phase 3
Azacitidine	AML|MDSs	Terminated	NCT00446303	Phase 2
Azacitidine	MDSs	Completed	NCT00102687	Phase 2
Azacitidine	MDSs	Completed	NCT00071799	Phase 3
Azacitidine for injectable suspension	Prostate cancer	Completed	NCT00384839	Phase 2
Azacitidine	Beta thalassemia	Completed	NCT00005934	Phase 2
Azacitidine	CMPD, secondary myelofibrosis	Terminated	NCT00381693	Phase 2

Table 12 – Azacitidine in combinated clinical trials.

Drug	Conditions	Status	NCT number	Phases
Azacitidine + VPA + ATRA	AML|MDS	Completed	NCT00339196	Phase 2
Azacitidine + VPA + ATRA	MDSs	Completed	NCT00439673	Phase 2
Azacitidine + Ara-C	AML, MDS, leukemia	Completed	NCT00569010	Phase 1|2
Azacitidine + sodium phenylbutyrate	Leukemia, MDSs	Completed	NCT00004871	Phase 1
Azacitidine + arsenic trioxide	Leukemia, MDSs	Terminated	NCT00234000	Phase 1
Azacitidine + sodium phenylbutyrate	Lymphoma, small intestine cancer	Completed	NCT00005639	Phase 1
Azacitidine + entinostat	Colorectal cancer	Completed	NCT01105377	Phase 2
Azacitidine + arsenic trioxide	Leukemia, MDSs	Completed	NCT00118196	Phase 2
Azacitidine + amifostine trihydrate	MDSs	Completed	NCT00005598	Phase 2
Azacitidine + sodium phenylbutyrate	Hematologic and solid tumors	Completed	NCT00006019	Phase 2
Azacitidine + liothyronine sodium + radiation: iodine I 131	Head and neck cancer	Completed	NCT00004062	Phase 1
Azacitidine + sodium phenylbutyrate	Thalassemia major	Terminated	NCT00007072	Phase 2
Azacitidine + AMG 531 (romiplostim) + decitabine	MDS, thrombocytopenia	Completed	NCT00321711	Phase 2
Azacitadine and hematopoietic growth factors	Leukemia, MDSs	Terminated	NCT00398047	N/A
Intravenous azacitidine	MDSs	Completed	NCT00384956	Phase 2
Azacitidine	AML, MDS	Completed	NCT00422890	Phase 3
Azacitidine + VPA + ATRA	MDS, AML	Completed	NCT01575691	Phase 1
Azacitidine + MGCD0103	AML, MDS	Terminated	NCT00666497	Phase 2
Azacitidine + cisplatin	Squamous cell carcinoma	Terminated	NCT00443261	Phase 1
Azacitidine + entinostat	Lung cancer	Suspended	NCT00387465	Phase 1|2
Azacitidine + erythropoietin	MDSs	Terminated	NCT00379912	Phase 2
Azacitidine + VPA + ATRA	MDS, AML	Completed	NCT00326170	Phase 2
Azacitidine + midostaurin	Hematologic cancers	Suspended	NCT01093573	Phase 1|2
Azacitidine + VPA	Advanced cancers	Completed	NCT00496444	Phase 1
Azacitidine + Ara-C + VPA	AML, MDS, leukemia	Completed	NCT00382590	Phase 2
Azacitidine, erlotinib	Advanced solid tumor malignancies	Completed	NCT00996515	Phase 1
Azacitidine, decitabine	MDSs	Terminated	NCT01011283	Phase 4
Azacitidine, MGCD0103	Hodgkin and non-Hodgkin lymphoma	Terminated	NCT00543582	Phase 2
Azacitidine, entinostat	Leukemia, MDSs	Completed	NCT00101179	Phase 1

cells. Although these studies are all still at pre-clinical level, the above findings support the possibility of miRNA-based approaches.

5. Non-coding RNAs

MicroRNAs (miRNAs) are short RNA molecules, 19–25 nucleotides long, that bind to complementary sequences in the 3′ UTR of multiple target mRNAs, usually resulting in their silencing. miRNAs were recently identified as key players in regulating gene expression by inhibiting translation and/or triggering degradation of their target mRNAs (Bartel, 2004). miRNAs target ~60% of all genes, are abundantly present in all human cells and are each able to repress hundreds of targets. Most miRNAs are transcribed by RNA polymerase II and subsequently processed by multiple maturation steps resulting in mature double-stranded miRNA duplexes. miRNAs are implicated in a wide range of basic biological processes, including development, differentiation, apoptosis and proliferation (Bartel, 2004; Harfe, 2005) and their misregulation is linked to the development of diseases in humans and other organisms. Researchers worldwide have validated the theory of "miRNA replacement therapy", which involves introducing synthetic miRNAs or miRNA mimetics into diseased tissues in an attempt to restore normal proliferation, apoptosis, cell cycle, and other cellular functions that have been affected by the misregulation of one or more miRNAs (Raver-Shapira et al., 2007).

Over the last 5 years, a particularly important role for miRNAs in cancer pathogenesis has emerged. Many tumor types are characterized by globally abnormal miRNA expression patterns. Up to now, 23 studies have been completed (Table 13), and describe the use of miRNAs in cancer therapy. miRNA expression profiles are highly informative for tumor classification, prognosis, and response to therapy. Moreover, recent results have documented a functional contribution of specific miRNAs to cellular transformation and tumorigenesis. The pioneering groups of specialized pharmaceutical companies have initiated studies on creating viable therapeutic candidates with miRNA inhibitors and miRNA mimetics in diverse fields such as cancer, cardiovascular diseases, neurological disorders, and viral infections.

6. Summary and concluding remarks

The present review highlights the enormous impact that small molecules such as HDACi and DNMTi have and will continue to have in the treatment of human diseases, especially cancer. Their key importance is due to the differentiation- and apoptosis-inducing activity of this new class of anticancer

Table 13 – miRNAs in clinical trials.

Drug	Conditions	Status	NCT number	Phases
Observational study	Sepsis	Completed	NCT00862290	N/A
Observational study	Cutaneous melanoma	Completed	NCT01444560	N/A
Observational study	Aspirin-exacerbated respiratory disease	Completed	NCT01631773	N/A
Observational study	Periodontal disease	Completed	NCT01399034	N/A
Observational study	Basal cell carcinoma	Completed	NCT01498250	N/A
Observational study	Cutaneous squamous cell carcinoma	Completed	NCT01500954	N/A
Observational study	Malignant melanoma	Completed	NCT00862914	N/A
Observational study	Skin cancer	Completed	NCT00849914	N/A
Observational study	Leukemia	Completed	NCT01057199	N/A
Observational study	Barrett's esophagus, esophagea, adenocarcinoma	Completed	NCT00909350	N/A
Observational study	Cutaneous malignant melanoma	Completed	NCT01482260	N/A
Observational study	Cancer of the skin	Terminated	NCT01143311	N/A
Observational study	Melanoma	Completed	NCT00536029	N/A
Observational study	Skin cancer	Completed	NCT01345760	N/A
Observational study	The study focused on reducing tobacco smoke exposure for child	Completed	NCT01317628	N/A
Observational study	Muscle anabolism	Completed	NCT00812396	Phase 1
Observational study	Hepatitis C	Completed	NCT00688012	Phase 1
Observational study	Lung cancer	Completed	NCT00897234	N/A
Observational study	Influence of genotype of drug	Completed	NCT01503658	Phase 4
Observational study	Awaiting organ transplant|infection in solid organ transplant recipients	Completed	NCT01558037	N/A
Observational study	Melanoma (skin)	Completed	NCT01216787	Phase 2
Observational study	Neuroblastoma	Completed	NCT01058798	N/A
Observational study	Recurrent calcic urolithiasis	Completed	NCT01022060	Phase 3

drugs. By targeting histone (chromatin) modulators, epi-drugs activate a complex transcriptional reprogramming, exemplified by reactivation of TSGs and repression of oncogenes.

Widespread changes in post-translational modifications of histones and DNA determine diseases and represent marks that play a crucial role in chromatin packaging and gene expression. The aberrant recruitment of HDACs and/or DNMTs or the activation of miRNAs in normal cells are involved in a number of pathologies, primarily cancer. It has been reported that HDACis/HMTis or DNMTis induce strong re-expression of TSGs and generally induce transcription-regulating activity on various genes, cell cycle inhibitory activity and apoptosis. Hence, their importance in epigenetic therapy. The vast number of clinical trials underlines the promising use of these drugs in diagnostic therapeutics of human diseases. However, their application is more effective in combination therapy. Trials related to combination therapies are based on the recognition that certain combinations of compounds combine more effectively with each other, improve tolerance, require lower dosages of each agent, and/or reduce side effects caused by at least one of the compounds in the combination. Although many anti-cancer therapies exist, there is a need to develop therapeutics that are safe and effective, circumvent resistance to hormonal and other therapies, do not cause the onset of other pathologies, and extend the disease-free survival of patients.

Cells that have developed mutations within the drug-binding pocket display a growth advantage in the presence of the drug, eventually leading to disease progression. Current clinical strategies aimed at combining these molecularly targeted drugs with standard chemotherapeutics, radiation, or other targeted agents will lead to novel strategies aimed at improving the overall response rate and increasing the number of complete remissions.

A good synergistic effect is obtained by the combination of drugs that inhibit DNMTs and HDACs.

In recent years, we have been facing the new concept of "personalized therapy", which takes into consideration individual differences between patients, and represents an attempt to ethically and medically improve cost performance of medication treatment by administering a pharmaceutical agent to patients after verification in advance of the probability of its effect, thereby enhancing efficacy as well as avoiding toxicity of the agent, and reducing inappropriate use of drugs. In cancer treatment, the development of a method for predicting the efficacy of anti-cancer agents is highly desirable as it may be an important way to bridge the gap between basic research and clinical application.

The clinical benefits of epi-therapy are currently being investigated in several human diseases but better results could undoubtedly be obtained with the development of new-generation epi-drugs. It is therefore essential to identify a characteristic profile for successful candidates as epi-drugs.

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