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Review Article

Strategic Therapies with Epigenetic Drugs: A Review

Daniela Maria Capuano[1,3], Rosanna Cipolla[3] and Roberto Verna[1-3]*

[1]*World Association of Societies of Pathology and Laboratory Medicine, IL, USA*

[2]*UniCamillus University, Rome, Italy*

[3]*Center for Sports Medicine and Management, Sapienza University of Rome, Italy*

***Corresponding Author:** Roberto Verna, World Association of Societies of Pathology and Laboratory Medicine, IL, USA and UniCamillus University, Center for Sports Medicine and Management, Sapienza University of Rome, Italy.

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Abstract

With recent advances in DNA sequencing (high-throughput analysis), researchers have been able to examine epigenetic changes across the whole genome, and recent studies have shown that epigenetics play a central role in many types of diseases. Epigenetic variations have been found in various pathologies and their involvement in the cancerous transformation has been demonstrated. Given that epigenetic dysregulation is potentially reversible and given that many diseases have an epigenetic etiology, the researchers hypothesized that inhibition epigenetic changes may have therapeutic potential. This is what has encouraged the development of new pharmacological opportunities that can be defined with the term "epigenetic therapy".

A number of epigenetic drugs have already been approved or are currently undergoing clinical trials. This paper is a review of the literature to illustrate the therapeutic potential of these drugs and evaluate their clinical application.

Keywords: DNA Sequencing; Epigenetic Therapy; Epigenetic Dysregulation

Introduction

Cellular functions are regulated by epigenetic mechanisms, in particular by DNA methylation and histone modifications. Alterations of these processes can lead to abnormalities that result in pathologies (Table 1) [1-18]. In particular, the loss of the methylation pattern has been observed as a cause of disease in studies concerning the inactivation of the X chromosome, genomic imprinting and cancer. In these diseases, chromosomal anomalies are present, which suggest the importance of chromosomal architecture and the central role of epigenetic mechanisms for its maintenance.

Specific mutations affecting components of epigenetic pathways are responsible for many syndromes: hereditary mutations of the ATRX gene are associated with an X-linked mental retardation (XLMR) syndrome most often accompanied by alpha-thalassemia (ATR-X) syndrome, in FMR15 'in the fragile X syndrome, in DNMT3b in ICF centromeric instability immunodeficiency, in the MECP2 gene in Rett syndrome. Chromatin-associated enzyme alterations have been correlated with the etiology of different hemopathologies, e.g. the presence of chromosome translocations characteristic of some Leukemias (Table 2) [19-21].

Normal Functions	
Correct organization of chromatin	Controls active and inactive states of embryonic and somatic cells
Specific DNA methylation and histone modifications	Controls gene- and tissue- specific epigenetic patterns
Silencing repetitive elements	Ensures that chromatine order and proper gene expression patterns are maintained
Genomic imprinting	Is essential for development
X chromosome inactivation	Balances gene expression between males and females
Abnormalities	
DNA hypermethylation	Results in chromatin condensation and silencing of tumor suppressor and other genes
DNA hypomethylation	Activates oncogenes, results in chromosomal instability, activates transposons
Mutations at methylated cytosines	Results in inappropriate gene expression
Imprinting defects	Results in loss of parental identity

Table 1: Normal cellular functions regulated in part by epigenetic mechanisms and abnormalities caused by epigenetic errors.

Disease	Symptom	Aethiology
ATR-X syndrome	Intellectual disabilities, alpha thalassemia	Mutations in ATRX gene, hypomethylation of certain repeat and satellite sequences
Fragile X syndrome	Chromosome instability, intellectual disabilities	Expansion and methylation of CGG repeat in FMR1 5' UTR, promoter methylation
ICF syndrome	Chromosome instability, immunodeficiency	DNMT3b mutations, DNA hypomethylation
Angelman's syndrome	Intellectual disabilities	Deregulation of one or more imprinted genes at 15q11-13 (maternal)
Prader-Willi syndrome	Obesity, Intelluctual disability	Deregulation of one or more imprinted genes at 15q11-13 (paternal)
BWS	Organ overgrowth	Deregulation of one or more imprinted genes at 11p15.5 (e.g.IGF2)
Rett syndrome	Intellectual disabilities	MeCP2 mutations
Alpha-thalassemia (one case)	Anemia	Methylation of alpha2-globin CpG island, deletion of HBA1 and HBQ1
Various cancers	Microsatellite instability	De novo methylation of MLH1
	Disruption of Rb, p53 pathway, uncontrolled proliferation	De novo methylation of various gene promoters
	Disruption of SWI-SNF chromatin remodeling complex	Mutation in SNF5, BRG1, BRM
	Overexpression of IGF-2, silencing of CDKN1C	Loss of imprinting
Leukemia	Disturbed hemopoiesis	Chromosomal translocations involving HATs and HMTs
Rubinstein-Taybi syndrome	Intellectual disabilities	Mutation in CREB-binding protein (histone acetylation)
Coffin-Lowry syndrome	Intellectual disabilities	Mutation in Rsk-2 (histone phosphorylation)

Table 2

Aberrant epigenetic patterns alter gene expression and genome stability and may contribute to the onset and progression of malignancies [10]. The methylation pattern of genes has been shown to be altered in cancer cells and the methylation of the CpG islands of certain genes is associated with their specific silencing, resulting in aberrant regulation of genes involved in cell cycle control, cell differentiation and/or apoptosis. Various studies show that methylated CpG islands are unable to initiate transcription [22-24]. Aberrant hypermethylation of the promoter genes can lead to the suppression of suppressor genes and this can lead to the beginning and progression of the cancer process [25]. In fact, it has been seen that cancer cells show different and apparently contradictory epigenetic modifications, as has been observed by the coexistence of global hypomethylation of the genome and hypermethylation of the promoter of a specific gene [26]. Hypermethylation in the CpG islands of the promoter regions has been described as a feature of human malignancies. The frequency of aberrant DNA hypermethylation can be used to differentiate tumors into two classes: the highly methylated ones defined as CIMP + (CpG island methylator phenotype) and those that are not called CIMP. These two types of tumors are characterized by different clinical and molecular patterns. The incidence of hypermethylation, particularly in sporadic malignancies, varies with respect to the gene involved and the tumor type. Hypermethylation of the promoter gene P16 occurs in 9-49% in many types of neoplasm, while BRCA1 hypermethylation is associated with 10-20% of sporadic breast cancer and ovarian cancer [25]. A high percentage of patients with sporadic colon cancer with microsatellite instability show methylation and silencing of the hMLH1 coding gene [27]. Many of these alterations are used today in the diagnostic field [28].

Alterations of epigenetic regulatory patterns are also observed in chronic, neuropsychiatric, immunological and aging diseases.

Epigenetic therapies

Recent advances in genome and bio-informatics analysis are providing information on the deregulated cellular network and epigenetic modifications involved in these processes [29-37]. Given the reversible nature of the epigenetic events involved in the development of various pathologies, it has been hypothesized that epigenetic factors may be potential targets of new therapeutic strategies to combat diseases with aberrant gene expression such as cancer. In this context, interest has been focused on the study of molecules that have the ability to inhibit or activate histone-modifying enzymes, DNA methyltransferases or can interfere with reader protein complexes. In particular, DNMT and histone deacetylase HDAC inhibitors have shown anti-tumor activity [8]. To date, epigenetic therapies are few and only some drugs have been approved for specific types of cancer, many other drugs are under study in clinical trials.

Drugs

The enzymes that catalyze the methylation and acetylation of histones have been identified as the first types of epigenetic modifications considered targets for a possible therapy. To date, the most studied drugs are the inhibitors of the DNA methyltransferase enzymes (DNMTi) and the inhibitors of deacetylases (HDACi).

HDACi are drugs that block the function of HDAC enzymes (histone deacetylase). These drugs are capable of making the chromatin structure more relaxed, allowing transcriptional activation. They have many biological effects on histone and non-histone proteins. The latter include proteins involved in the regulation of gene expression, apoptosis, the cell cycle, and angiogenesis. They have the role of inhibiting cell growth and giving rise to differentiation and apoptosis. They consist of a zinc chelating portion, a linker and a "cap" that interacts with the enzyme through hydrophobic interactions. Based on the chemical structure, different classes of HDACi are distinguished: short chains of fatty acids; hydroxamic acids; epoxyketones; benzamides.

The ability of structurally different HDACs to inhibit the activity of different classes of HDAC depends on the domains of the protein to which they bind. A large number of these drugs have been synthesized in recent years and many are the subject of clinical trials. Some have been second-line approved for the treatment of refractory, persistent or recurrent cutaneous T-cell lymphoma. Preclinical studies investigate the use of these drugs in clinical practice in combination with chemotherapy or other epigenetic drugs [38].

Short chain fatty acids: The most accepted hypothesis is that the carboxylic group acts as a binding group of Zn and competes with the acetate group released in the deacetylation reaction.

Valproic acid, a drug used as an anticonvulsant, is an HDAC inhibitor. It is a synthetic derivative of Propylpentanoic acid with antiepileptic properties. It has antineoplastic potential and has antiangiogenic activity. There are various trials alone or in association with other drugs in diseases such as chronic lymphocytic leukemia, breast cancer, myeloproliferative syndromes, Alzheimer's (www.clinical.gov). It belongs to the class of drugs called latency-reversing agent and has also been studied as part of a strategy in the treatment of HIV virus infection. In the latent status the virus remains inside the T cells (CD4) and is not recognized by the immune system. At this stage, anti-viral therapy (ATR) is not effective. These agents reactivate the virus that can replicate and after reactivation, the virus is more likely to be recognized by the immune system and killed. ART therapy is more effective, reducing the possibility of infection of other cells and increasing the probability of elimination of the virus [39]. It has insignificant side effects and is well tolerated (TOXNET®: Toxicology Data Network). Recent research has had excellent results, many clinical trials have been completed and other are still underway (ClinicalTrials.gov). (Https://druginfo.nlm.nih.gov/drugportal/name/Valproic+acid).

PBA sodium phenylbutyrate is a salt of an aromatic fatty acid used to treat urea disorders. It has been approved for the treatment of ammonia in patients with urea cycle disorders (rare genetic diseases due to enzyme deficiencies). The urea cycle is the major route of elimination of nitrogen groups including ammonium. Its dysfunction leads to an increase in ammonium which causes neurological damage and cognitive deficiency. Phenylbutyrate and sodium benzoate promote an alternative nitrogen elimination pathway. It is a prodrug metabolized to phenylacetate which binds with glutamine to form phenylacetylglutamine excreted by the kidney and which does not require urea cycle metabolism. Approval as an orphan drug took place in 1996. Sodium phenylbutyrate is also an inhibitor of histone deacetylase and this has led to research into its use as an antineoplastic agent and in protein misfolding diseases such as cystic fibrosis. It is available in pills and as powder (trade name Buphenyl). Side effects are bitter taste, loss of appetite, nau-

sea, vomiting, diarrhea, fever, rash. In 2013, glycerol-tri-phenylbutyrate (trade name Ravicti) with improved taste was approved. Phase 1 clinical studies are underway in association with AZA and FU for neurodegenerative diseases, hematological and solid tumors (https://www.clinicaltrials.gov/ct2/results?term=PBA&Search=Search).

Hydroxamic acids: They are the majority; Vorinostat, Panobinostat, TSA, Belinostat belong to this class.

Vorinostat: Suberanylhydroxamic acid (SAHA) (http://hedds.org/drug.) Is a synthetic hydroxamic acid with antineoplastic activity. It inhibits the enzymatic activity of HDAC 1, HDAC 2, HDAC 3 (class I) and HDAC 6 (class II) in quantities of nanomoles. It binds the catalytic domain of histone deacetylase HDACs by inhibiting deacetylation and leading to the accumulation of hyperacetylated histones and transcription factors. Hyperacetylation of Histone proteins leads to up-regulation of kinase-cyclic dependent p21, followed by arrest in G1. Hyperacetylation of non-historical proteins such as tumor suppressor factor p53, alpha tubulin, heat-shock protein 90, produces other additional antiproliferative effects. It also induces apoptosis. In 2006 it was approved by the FDA (Zolinza) for the treatment of patients with cutaneous T-cell lymphoma with progressive, persistent or recurrent disease or following 2 systemic therapies (https://www.fda.gov). Many trials are underway alone and in association with other drugs in diseases such as multiple myeloma, breast cancer, lymphoma, small cell lung cancer, myelodysplastic syndromes, prostate, pancreatic cancer, ovary (https://www.clinicaltrials.gov/ct2/results? term = Vorinostat + & search = Search). Administration is oral. The observed side effects are general (asthenia), hematological (thrombocytopenia and anemia), and on the gastrointestinal stem (nausea, vomiting). The most serious side effect is the increased thromboembolic risk. Excretion occurs in 35 - 52% via the urinary tract.

Panobinostat: hydroxamic acid with antineoplastic activity. Selectively inhibits class I, class II and class IV HDACs. Induces hypermethylation of histone proteins which leads to modulation of cell cycle expression, arrest in G2/M phase and apoptosis. It also appears to modulate the expression of genes related to angiogenesis such as HIF-1a (hypoxia inducible 1al factor) and VEGF (vascular endothelial growth factor), compromising endothelial chemotaxis and tissue invasion. It was approved by the FDA in 2015 (Farydak) for the treatment of adult patients with relapsed and/or refractory multiple myeloma (MM) who received at least two previous treatment regimens including Bortezomib and an immunomodulating agent. Efficacy was demonstrated by the Panorama-1 study (Panobinostat or Placebo with Bortezomib and Dexamethasone in patients with relapsed Multiple Myeloma https://clinicaltrials.gov/ct2/show/NCT01023308?term=Panorama1&rank=1) in which the participants who had received the combination with Panobinostat had increased survival (10.6 months compared to 5.8 months in the other group) and response rate (59% against 41% of those treated only with Bortezomib and dexamethasone). It has been approved with a boxed warning which warns that severe diarrhea, serious and/or fatal cardiac events, arrhythmias and changes in the electrocardiogram have occurred in patients who received Panobinostat. The most common side effects are diarrhea, tiredness, nausea, swelling in the arms or legs, decreased appetite, fever, vomiting and weakness. Numerous trials are underway on hematological malignancies and solid tumors (https://www.clinicaltrials.gov/ct2/results?term=Panobinostat+&Search=Search).

Trichostatin a (TSA) is a natural derivative of the dienohydroxamic acid isolated from the bacterial species *Streptomyces*. TSA specifically and reversibly inhibits histone deacetylase resulting in hyperacetylation of the histones of the core and modulation of the chromatin structure. Increased histone acetylation promotes transcription and inhibits cell growth. It is a powerful inducer of cell growth arrest, differentiation and apoptosis in culture and animal models. Preclinical studies are underway.

Belinostat (Beleodaq) has been approved by the FDA for patients with peripheral T-cell lymphoma who had received other treatments without improvement or who had relapsed. The safety and efficacy of Belinostat were evaluated in a clinical study involving 129 patients with relapsing or refractory PTCL who had presented disease progression after at least one previous treatment. Belinostat treatment resulted in a general response rate of 25.8% of the participants, with a complete or partial response [40].

Benzamides: Entinostat and Mocetinostat belong to this class.

Entinostat: Synthetic benzamide derivative with antineoplastic activity. Class I deacetylase inhibitor. This agent appears to exert dose-dependent effects in human leukemia cells including cyclin kinase dependent inhibitor 1a (p21/CIP1/WAF) at low concentration produces marked induction of reactive oxygen species (ROS),

mitochondrial damage, activation of caspases and at high concentrations induce apoptosis. In normal cells the expression of the dependent cyclic inhibitor-kinase (p21) has been associated with cell cycle exit and cell differentiation (https://pubchem.ncbi.nlm.nih.gov/compound/4261#section=Top). Various Trials are underway in monotherapy and in association with other drugs for non Hodking lymphoma, advanced breast cancer (in combination with aromatase) and metastatic lung cancer (in combination with Erlotinib). It has been studied in association with Azacitidine for lung, metastatic colon (phase 2), Non Hodgkin lymphoma (https://clinicaltrials.gov/ct2/results?term=Entinostat+&Search=Search).

Mocetinostat (MGCDO103): Is a selective benzamide for HDAC class 1 with potential antineoplastic activity. The mechanism is still to be defined. Leads the cancer cell to death through apoptosis, DNA repair inhibition, up-regulation of suppressor genes and down-regulation of growth factors (https://pubchem.ncbi.nlm.nih.gov/compound/9865515#section= Information-Sources). Various clinical studies are underway on hematological and solid tumors (https://clinicaltrials.gov/ct2/results?term=Mocetinostat+&Search=Search).

Cyclic peptides

Romidepsin: Bicyclic depsipeptide antibiotic isolated from the bacterium *Chromobacterium violaceum*, with antineoplastic activity. After intracellular activation it binds and inhibits HDAC causing blockage of cell growth and angiogenesis (http://hedds.org/drug.jsp?id=13). In 2009 it was approved by the FDA for cutaneous T-cell lymphoma in patients who had received at least one other systemic treatment. The administration is parenteral (1 ampoule in 4 hours on days 1, 8 and 15) in cycles of 28 days repeatable until the appearance of side effects (severe). It is metabolized by the liver. Common side effects are nausea, vomiting, anorexia. It is studied alone and in combination in various trials on hematological and solid tumors (https://clinicaltrials.gov/ct2/results?term=Romidepsin).

Sirtuins: They are the III class of deacetylase enzymes. All Sirtuins contain a catalytic core of 275aa and have stoichiometric requirements for the NAD + cofactor. They include 7 proteins [1-7] which differ in their location and functions and are divided into 4 classes (Table 3).

Sirtuine	Location
SIRT1	Core
Sirt 2	Cytoplasm/nucleus
Sirt 3,4,5	Mitochondria
Sirt 6.7	Nucleoli

Table 3: Classes of Sirtuins.

They have many biological functions including the control of metabolism, senescence and cell proliferation. They participate in a wide spectrum of activities and are implicated in various pathologies of metabolism, inflammation and cancer and in recent years they have emerged as potential targets for the treatment of these pathologies. Sirt1 is the best known. It acts in various cellular processes and its role in cancer and aging has been demonstrated [41].

Resveratrol (Sirt1 activator) class III HDACs (sirtuins) is a phytoalexin derived from grapes, with antioxidant and pro-apoptotic activities in cancer cells *in vitro*. It is a polyphenol found in grapes. It mediates anti-inflammatory effects, induces differentiation and shows activity in various stages of carcinogenesis (http://hedds.org/drug.jsp?id=60). It is used in clinical trials for cardiovascular disease, diabetes and cancer (https://clinicaltrials.gov/ct2/results?term=Resveratrol+&Search=Search).

HDACi have also shown great potential for the treatment of neurodegenerative and psychiatric diseases. In many studies they have been shown to reduce memory loss and have neuroprotective effects. One group has shown that the administration of valproic acid in post cerebral ischemia has significantly reduced the size of the heart attack and the neurological deficit score, suggesting that HDCAi are drugs that can be useful in preventing permanent post stroke damage [42].

Nucleosidal analogue compounds are the oldest inhibitors of the DNA methyltransferase (DNMTi) enzymes and many of these compounds have been approved by the FDA for the treatment of some cancers. Nucleoside analogues such as AZA are embedded in DNA inhibit methylation and reactivate the silenced gene [43]. This class of drugs includes: 5-Azacitidine, Decitabine, Zebularine.

5-Azacitidine is a DNMT analogue pyrimidine inhibitor. Its cytotoxic effects in cancer had been known since 1968, but the mecha-

nism of action has only recently been discovered [44]. The drug is an analogue of cytidine, with a nitrogen atom instead of carbon 5. Inside the cell is phosphorylated and incorporated into DNA during replication. The analog is recognized by DNMT1 and a normal methylation reaction takes place. The nitrogen group forms an irreversible DNAMT1-AZA bond that degrades the enzyme with reduced methylation [45]. Hypomethylation can activate tumor suppressor genes with anticancer effects. Clinical trials were started in 1967 but were rejected due to high toxicity. The drug returned to trials for the treatment of low-dose myelodysplastic syndromes in the 1980s and was approved by the FDA in 2004 (trade name Vidaza), for the treatment of patients with myelodysplastic syndromes in subtypes: refractory anemia and myelomonocytic leukemia. Although it has been approved by the FDA, it is still to be improved, because it is relatively unstable and the oral formulation is not available (http://hedds.org/drug.jsp?id=2). It is used in various trials in combination with other drugs for the treatment of various cancers (https://clinicaltrials.gov/ct2/results?term=5-Azacitidine).

5-aza-2'deoxycytidine or Decitabine (DACOGEN) is an analogue of cytidine with antineoplastic activity. It binds to DNA by blocking the activity of DNA methyltransferase causing DNA hypomethylation and arrest in S phase of replication. It was approved in 2006 for the treatment of myelodysplastic syndromes. Administration e.v. Preclinical studies on solid tumors are ongoing (http://hedds.org/drug.jsp?id=1).

Zebularine or 5-fluoro-2'-deoxycytidine is another stable analogue of cytidine with antineoplastic properties. It has the mechanism of action similar to AZA, integrating itself into DNA and inhibiting DNMT1 (it forms a covalent bond with DNMT1). The drug has not yet been approved by the FDA but there have been good results in mouse models. Zebularine is more stable than AZA and can be administered orally (http://hedds.org/drug.jsp?id=4). Preclinical studies on hepatocellular carcinoma, cholangiocarcinoma, renal cell carcinoma and prostate have shown its therapeutic advantage in certain types of tumors [46,47].

Non-nucleoside DNMT inhibitors

The interest of recent years has been to synthesize molecules that directly inhibit DNMT activity to avoid the side effects caused by nucleoside analogues RG 108 was the first synthesized DNMT1 that directly inhibits the active site of DNMT1 and interacts in a non-covalent manner with the active site of DNMT 1. *In vitro* studies on human cancer cell lines on the use of RG108 have shown reduced methylation and re-expression of p-16 tumor suppressor [48]. Furthermore, many groups have demonstrated the DNMT inhibitory activity of some drugs already in use for other pathologies such as the anesthetic Procaine, the Procainamide drug used for arrhythmias and the Hydralazine drug used as an antihypertensive. Even natural products derived from tea, sponges, have shown inhibition of methylation *in vitro*. Their mechanism is not yet well known, but they have been shown to bind GpG-rich sequences and interfere with the translocation of DNMT along the DNA strand. Hydralazine has shown re-expression of P16 in cervical cancer [49]. Hydralazine is in use in conjunction with magnesium valproate in a Phase 3 study for ovarian cancer and in a Phase 2 study for breast cancer [50]. Among the natural molecules, the EGCG (Epigallocatechin-3-Gallate) polyphenol compound found in green tea showed anti DNMT activity [51]. Studies are ongoing on short-sense antioligonucleotides complementary sequences to mRNAs that cause transcription blockade.

MG98 is a second generation antisense oligonucleotide consisting of 20 base pairs, which specifically binds DNMT1mRNA, preventing gene transcription. Studies in mouse models of gallbladder and colon cancer have shown that administration of MG98 leads to re-expression of the p16 suppressor gene. Clinical trials have shown mixed results but appear to have good results on advanced kidney cancer. The most promising results occurred in combination with Roferon A. Reduced DNMT1 levels and slowed tumor growth with minimal MG98 toxicity were observed [52-54].

Recent studies have shown that treatment with DNMT inhibitor drugs can improve the response to traditional chemotherapy drugs. The ability of these agents to reactivate the expression of suppressor genes is important for sensitizing cancer cells to the response to chemotherapy and other therapeutic procedures such as radiation therapy. Furthermore, converting the gene re-expression allows to improve the immune response against the tumor. Combination therapy between these agents and other drugs may be advantageous in clinical application [55].

HAT inhibitors

Acetyltransferase (HAT) enzymes modulate gene expression by catalyzing the acetylation of lysine residues in various histone and non-histone proteins. Therefore, they are considered potential

targets for new therapies. They are classified in different families in relation to the chemical structure. They include GNAT (Gcn5-related N-acetyltransferase), the MYST group (MOZ, YBF2/SAS3 and TIP60) and the p300/CBP family [56]. A large number of transcription activating proteins have shown acetyltransferase activity, however few HAT inhibitors are known to date. In the early 2000s, two HAT inhibitors were isolated from natural substances: cashew acid, a compound found in cashew shell oil, and garcinol extracted from *Garcinia indica*. Both show HAT activity not specific for p300/CPB [57,58]. Various chemical modifications of these inhibitors have been attempted to identify new enzyme-specific inhibitors but there are no drugs in clinical studies [59].

Curcumin, a pigment (phytopolyphenol) extracted from Curcuma longa used mainly as a vegetable dye, was identified as the first specific HAT inhibitor p300/CBP. It has anti-inflammatory activities. In animal models of cancer the proliferation and growth of the tumor. Its use in the treatment of a wide variety of disorders has shown potential against inflammatory diseases, cancer, diabetes, arthritis, cardiovascular diseases, Alzheimer's and psoriasis without causing side effects [60]. It is the only HATi in clinical trials (https://clinicaltrials.gov/ct2/results?term=Curcumin).

Histone methyltransferases (HMTS)

Histone methylation influences histone basicity and hydrophobicity and their affinity for certain proteins such as transcription factors. Lysine chains can be mono, di or trimethylated while arginine chains can be mono- or di-methylated. Histone methyltransferases (HMTs) can be divided into two different classes: lysine methyltransferases and arginine methyltransferases. The methylation of arginine in histones H3 and H4 promotes transcriptional activation and is mediated by a family of arginine methyltransferase proteins (PRMTs), which include the activators PRMT1 and PRMT4. In contrast several histone lysine methyltransferases have been identified which contain a catalytic site SET domain Enhancer of Zeste (EZHZ2). Methylation is implicated in both transcription and gene silencing. These large protein complexes are considered new epigenetic targets but to date the studies on HMTi are far from clinical application [61]. Various gene mutations that activate regulatory proteins have been found in various types of cancer and can be a pharmacological target.

Methyl transferase protein inhibitors

Lysine methyltransferase EZH2 (KMT6) is the catalytic component of the protein complex PRC2 (polycomb repressive complex 2) which consists of 3 or 5 protein members and which has catalytic activity on histones, mono-nucleosomes and oligo-nucleosomes. This complex catalyzes the methylation of lysine 27 on histone H3 (H3K27) to mono-di- and trimethylation. The constant presence of mutations in this catalytic site is seen in germ cultures of B-cell lymphoma and follicular lymphoma [62]. These genomic data suggest that the activity of EZH2 MT may be a target for a valid alternative therapy in patients with lymphoma.

An EZH2 inhibitor inhibits the catalytic activity of the enzyme and decreases methylation in histone H3K27.

3-Deazaneoplacin A (DZnep) is a drug that acts both as an adenosylmonocysteine inhibitor and as a histone methyltransferase EZH2 inhibitor. *In vitro* studies have shown its activity against a variety of different cancer cell lines. In studies in mice it has been shown to be effective for the treatment of the Ebola virus, interfering with the ability of the virus to block the synthesis of interferon which allows it to evade the immune response [63]. Treatment of human AML cell cultures with DZnep induces increased expression of regulatory genes such as p21 and leads to cell cycle arrest and apoptosis. The antileukemic effects are enhanced when used in combination with Panobinostat (HDACi) [64]. Preclinical studies are underway alone or in combination with chromatin remodeling agents to investigate the role of EZH2 in regulating conditions such as breast cancer and SCLC (small cell lung cancer) [65].

GSK126 is an HMTi drug and reduces H3K27 trimethylation in both types of EZH2 mutant and wild type lymphoma cell types by 50%. Suppresses proliferation in cell cultures of B cell lymphoma [66].

Other histone methyltransferase is DOT1L which appears to be responsible for the catalytic methylation activity for histone H3K79. Aberrant activity (increase of mono- and methylation) of DOT1 was found in the MILL fusion Protein protein complex, present in leukemia cells that present chromosome 11q23 rearrangement. The MLL protein is a Histone-lysine N-methyltransferase that normally catalyzes the methylation of H3K4, a function that is lost when chromosomal translocations occur that produce oncogenic proteins. MLL translocations occur in 3% -10% of patients with lymphoblastic leukemia (ALL). Many evidences show that in many forms of leukemia associated with these mutations, DOT1L is activated and leads to the development and progression of the disease. Inhibition of DOT1L activity through small inhibitory mol-

ecules has shown to inhibit the proliferation of many types of MLL-rearranged leukemia *in vitro* [61]. This makes DOT1L a target for the discovery of new drugs and many small inhibitory molecules have been synthesized. One of these, EPZ-5676 (celgene-epizyme) has entered a phase 1 clinical study in patients with relapsed/refractory disease and carriers of MLL gene translocation on 11q23 or for advanced hematological diseases (https://clinicaltrials.gov/ct2/results? term = EPZ-5676 & search = Search).

Bromodomini and BET inhibitors

They are structural sequences associated with chromatin-modifying proteins such as HATs. The BET Bromodomain family (BET- extra-terminal protein) which includes BRD2, BRD3, BRD4 and BRDT (Bromodomain testis-protein) is made up of epigenetic proteins that bind the acetylated residues of lysine on histones. BRD4 binds to the transcription elongation factor P-TEFb and stimulates the dependent RNA polymerase II transcription (RNA-PII). Many studies have investigated the clinical importance of BRD4, a specific BET protein known for an important role in mitosis [67,68]. NUT midline carcinoma (NMC) associated in 75% with the chromosomal translocation between BET BRD3 and BRD4 proteins, and NUT (nuclear protein in testis). The NUT sequences of chromosome 15q14 merge with BRD4 and BRD3 to form a fusion protein (BRD3-NUT and/or BRD4-NUT) which blocks cell differentiation and promotes undifferentiated tumor growth of the carcinoma [69]. Given the function specific of the BET protein, it has been hypothesized that agents that block the biological activity of these oncogenic proteins, may be effective in the therapy for NMC.

BET inhibitors: JQ1 and BT 726 (IBT726)

Thienotriazole-diazepine (JQ1) is a BET inhibitor that promotes tumor differentiation, regression and prolongs survival in mouse models of NMC, which correlates with the role of BDR-NUT in this type of cancer. It is used in preclinical studies on various types of cancer alone and in association with HDACi (Mocetinostat-Panobinostat). JQ1 has many laboratory applications but is not used in clinical trials for its short life span (http://hedds.org/drug.jsp? id = 42).

Treatment with JQ1 in association with HDACi has shown synergistic action with increased suppression of genes essential for cell cycle progression. There was an increase in the expression of multiple members of the ubiquitin specific protease family 17 (USP17) and the attenuation of the RAS/MAPK pathway with a decrease in cell viability [70].

BET inhibitors structurally similar to JQ1 have been tested in various cancers. Similar activity was discovered in GSK IBET762. The BT 726 inhibitor (IBT726) binds BRD2, BRD3, BRD4 very closely and competes with the histone tetraacetylate H4 for binding with these proteins. It is in use in clinical trials for NMC and other malignancies (https://www.clinicaltrials.gov/ct2/show/NCT01587703).

The discovery that BET proteins have a direct effect in transcription is a goal for oncological therapy which aims to identify agents that directly and specifically silence oncogenes and increase the expression of suppressor genes. Much progress has been made in the discovery of DOT1L, BET and EZH2 inhibitors that have entered clinical trials [61].

Studies have started to develop therapeutic candidates with mimetic miRNAi and miRNA in different fields of diseases such as cancer, cardiovascular, neurological and viral infections. Extracellular miRNAs have been shown to be involved in intracellular communication and can be used as diagnostic indices, as non-invasive tumor markers [71].

MicroRNAs are small non-coding RNA molecules, 19-25 nucleotides long, single-stranded Stem-loops, which are capable of regulating gene expression levels at the post-transcriptional level. Some are encoded by particular genetic loci, others are contained in histone regions of protein-coding sequences. They are expressed in the nucleus as pre-miRNAs which are processed by an Endonuclease (DROSHA) in fragments of 70-100 nucleotides called Pre-miRNA. The latter pass into the cytoplasm and are further processed by another RNAsi called DICER. A single strand of these miRNAs is loaded into the induced silencing complex (RISC) together with one or more proteins of the ARGONAUTA (AGO) family and is directed towards its target with which it binds for complementarity of the bases, thus ensuring a extreme specificity of action. (Ribonucleocomplex). They play an important role in gene regulation through various mechanisms: Degradation of target mRNA known as RNA interference (iRNA), Block translation of target mRNA, Inhibition of transcription.

They therefore play a role in negatively regulating post-transcriptional gene expression.

The choice between translation inhibition and mRNA degradation depends on the type of complementarity between mRNA and

miRNA. If the filaments are perfectly paired, the mRNA is cut, if they are not paired, the reversible inhibition of the translation occurs [72]. In general, a miRNA inhibits the translation of multiple genes and each gene is regulated by multiple miRNA to form a complex pattern of regulation of gene expression [73]. In addition to the regulatory role of gene expression, miRNAs seem to play a role in DNA methylation and histone modifications. In turn, the expression of miRNAs can be regulated by epigenetic factors since it is possible that the miRNAs of the introns may be transcribed by promoters present in the CpG regions regulated by DNA methylation [74]. MiRNAs are involved in a wide range of biological processes such as development, differentiation, apoptosis and cell proliferation. Their dysregulation has been seen to be involved with the development of many diseases. A particularly important role of miRNAs in the pathogenesis of cancer has emerged in recent years. They can act as tumor suppressors or as oncogenes. In fact, in case of inactivation of the miRNA there will be overexpression of the target messenger RNA. In case of overexpression of miRNA, inactivation of the target mRNA will occur. Many types of tumor are characterized by an abnormal expression of the expression pattern of miRNA. Overexpression of mRNA-135b has been found associated with both sporadic and IBD-associated colon cancer [75]. The high presence of miRNA among neurons indicates an important function in brain health and this has encouraged the application of miRNA for the maintenance and therapeutic intervention of neuronal functions in neurodegenerative diseases [76]. One study suggests that miR-138 is a regulator of gene stability and that it is a potential therapeutic agent to increase the effectiveness of radiation therapy and chemotherapy (DNA-damaging agents). Histone H2AX forms nuclear foci at points where DNA is damaged and facilitates DNA repair. MiRNA-138 inhibits H2AX foci induced by ionizing radiation in osteosarcoma. The overexpression of miRNA-138 silences H2AX, reduces the repair of DNA foci and cis-platinum is more effective [77].

Trials in progress and approved drugs (Table 4)

In recent years, numerous clinical trials using epigenetic drugs have been conducted or are in progress [78]. The greatest use has been in the oncology field and the most promising results have been obtained in various types of hematological malignancies for which some drugs have been approved already approved by the FDA.

FDA approved drugs are: DNMTi and HDACi

DNMTi in trials: In the therapeutic context, DNA methylation was seen as a target especially in the oncology field, on various types of hematological cancer and on solid tumors. DNMTi such as Decitabine and Azacitidine have been approved by the FDA in monotherapy for myelodysplastic syndromes and are also being evaluated in clinical studies for other pathologies. These drugs have shown to be good epigenetic modulators but their clinical use is limited in the oncological field due to their toxicity. The new generation Zebularine is more stable and more promising for clinical use.

Drug	Pathology	Status	Number	Phase
5-azacitidine	Myelodysplastic syndromes			
5-azacitidine	Leukemia MDS	Completed	NCT00350818	Phase 1
5-azacitidine	Ovarian Cancer	Terminated	NCT00842582	Phase 1
5-azacitidine	Leukemia	Completed	NCT00739388	Phase 2
5-azacitidine	Myelofibrosis	Completed	NCT00569660	Phase 2
5-azacitidine	Prostate cancer	Completed	NCT00384839	Phase 2
5-azacitidine + Entinostat	Colon Cancer	Completed	NCT01105377	Phase 2
5-azacitidine + Valproic acid	Myelodysplastic syndromes	Completed	NCT00439673	Phase 2
5-azacitidine + Entinostat	Lung Cancer	Suspended	NCT00387465	Phase 1/2
Azacitidine	Myelodysplastic syndromes	Completed	NCT00071799	Phase 3
Azacitidine + Tricostatin A	Prostate Cancer			Preclinical study
Azacitidine + Tricostatin A + DNzp	Breast cancer			Preclinical study

Azacitidine	Breast cancer Ovary Cancer Colorectal cancer			Preclinical study
Decitabine	Myelodysplastic syndromes			Approved N21790
Decitabine	Bladder Cancer Breast cancer Melanoma	Completed	NCT00030615	Phase 1
Decitabine	Esophagus Lung cancer Mesothelioma	Completed	NCT00019825	Phase 1
Decitabine	Leukemia	Completed	NCT00042796	Phase 1
Decitabine + Peg-interferon	Advanced solid tumors	Terminated	NCT00701298	Phase 1
Decitabine + Romidepsin	Leukemia Myelodysplastic Syndromes	Completed	NCT00114257	Phase1
Decitabine	Breast Adenocarcinoma			Preclinical study
Zebularine	Cholangiocarcinoma			Preclinical study
Zebularine	Renal carcinoma			Preclinical study
Zebularine	Prostate cancer			Preclinical study
Zebularine	Hepatocarcinoma			Preclinical study

Table 4

HDCAi in clinical trials (Table 5)

There are at least 20 different HDACi in monotherapy trials than in association with other drugs. HDAC inhibitors have been evaluated in studies on hematological pathologies and are now also studied in solid tumors. The first approved was Vorinostat for the treatment of T cell lymphoma, and is in a study in a number of trials in the treatment of leukemia and lymphomas and solid tumors such as ovary, lung, colorectal, kidney. Panobinost has been approved for Multiple Myeloma (MM) and is evaluated in phase 1 and 2 studies in monotherapy and in combination with other drugs, for non-Hodgkin lymphoma and acute myeloid leukemia. Belinostat has been approved for peripheral T-cell lymphoma. Benzamides such as Entinostat, Mocetinostat, have been used in studies of hematological and solid tumors. Valproic acid has been evaluated in monotherapy for hypersplenism and lymphadenopathy in phase 1 and 2 studies. The cyclic peptide Romidepsin has been approved for the treatment of T cell lymphoma. Sirtuins are in use in metabolic and neurodegenerative diseases. Resveratrol has shown benefit against cardiovascular disease, and regression of tumor cell growth has been seen in patients with colon carcinoma.

Drug	Pathology	Status	Number	Phase
Vorinostat	Cutaneous T cell Lymphoma T			Approvato NO21991
Vorinostat	Lymphoma	Completed	NCT00771472	Phase 1
Vorinostat	Colorectal carcinoma	Completed	NCT00336141	Phase 1
Vorinostat	Advanced relapsing and refractory neoplasms	Completed	NCT00632931	Phase 1

Valproic Acid	Chronic Lymphatic Leukemia	Terminated	NCT00810680	Phase 2
Valproic Acid+ decitabine	Acute Myeloid Leukemia	Completed	NCT00867672	Phase 2
Valproic Acid+ 5-azacitidine	Advanced Neoplasms	Completed	NCT00496444	Phase 1
Valproic Acid+ 5-azacitidine	Myelodysplastic Syndromes	Completed	NCT00439673	Phase 2
Valproic Acid + Raltegravir	HIV	Terminated	NCT00614458	Phase 2
Valproic Acid	Cholangiocarcinoma			Preclinical study
Valproic Acid+ Vorinostat	Prostate cancer			Preclinical study
Valproic Acid	Hypersplenism/lymphadenopathy/ALPS (autoimmune lymphoproliferative syndrome)	Completed	NCT00605657	Phase 1 Phase 2
Sodium phenylbutyrate (PBA) + 5-azacytidine	Leukemia Myelodysplastic Sindromes	Completed	NCT00004871	Phase 1
Sodium phenylbutyrate (PBA) + 5-azacytidine	Hematological and solid tumors	Completed	NCT00006019	Phase 2
Sodium phenylbutyrate (PBA) + 5-azacytidine	Lymphoma Bowel neoplasms	Completed	NCT00005639	Phase 1
Vorinostat + Gentamicyn + Platinum	NSCL	Completed	NCT00423449	Phase1
Vorinostat + Idarubicine	Leukemia myelodysplastic syndromes	Completed	NCT00331513	Phase 1
Vorinostat + Radiotherapy	Pancreas	Terminated	NCT00831493	Phase 1 - 2
Panobinostat	Multiple myeloma			Approved NDA205353
Panobinostat	Breast carcinoma	Terminated	NCT00993642	Early Phase 1
Panobinostat	Advanced solid tumors	Completed	NCT00739414	Phase 1
Panobinostat	non Hodgkin Lymphoma	Completed	NCT00503451	Phase 1
Panobinostat + Trastuzumab	Breast cancer	Completed	NCT00567879	Phase 1
Panobinostat	Leukemia	Completed	NCT00723203	Phase 2
Panobinostat	SCLC	Completed	NCT01222936	Phase 2

Panobinostat + Vorinostat + Tricostatin A	Prostate cancer			Preclinical study
Panobinostat	Endometrial cancer			Preclinical study
Tricostatin A	Carcinoma of the cervix			Preclinical study
Tricostatin A	Colon cancer			Preclinical study
Tricostatin A	Lung Adenocarcinoma			Preclinical study
Tricostatin A	Burkitt Lymphoma			Preclinical study
Tricostatin A+ 5-azacitidine + DZnep	Breast cancer			Preclinical study
Tricostatin A+ 5-azacitidine	Colon cancer			Preclinical study
Tricostatin A+ 5-azacitidine	Hodgkin Lymphoma			Preclinical study
Belinostat	Peripheral T-cell lymphoma PTCL			Approvato NAD206256
Belinostat + Desametasone	Multiple Myeloma	Completed	NCT00131261	Phase 2
Belinostat + Erlotinib	NCLC	Completed	NCT01188707	Phase 1/2
Entinostat	Leukemia Multiple myeloma and plasma cell neoplasia MDS	Completed	NCT00015925	Phase 1
Entinostat	Leukemia Myelodysplastic Sindromes	Completed	NCT00324129	Phase 1
Entinostat + 5-azacitidine	Leukemia Myelodysplastic Sindromes	Completed	NCT00101179	Phase 1
Entinostat	Melanoma	Completed	NCT00185302	Phase 2
Entinostat + 5-azacitidina	Colon carcinoma	Completed	NCT01105377	Phase 2
Entinostat	Chronic lymphocytic leukemia	Completed	NCT00431873	Phase 2
Entinostat + 5-azacitidina	Lung neoplasm		NCT00387465	Phase 1/2

Entinostat + 5-azacitidina	Oesophagus neoplasm			Preclinical study
Entinostat				
Mocetinostat	Healthy			Preclinical study
Mocetinostat + JQ1	Healthy			Preclinical study
Romidepsin	T-cell cutaneous lymphoma			Approvato NDA022393
Romidepsin + Ketoconazole	Hematologic neoplasms	Completed	NCT01324310	Phase 1
Romidepsin + Gemcitabin	Pancreatic carcinoma	Completed	NCT00379639	Phase 1
Romidepsin + Bortezomib	Multiple Myeloma	Terminated	NCT00765102	Phase 2
Romidepsin	Lung carcinoma	Completed	NCT00086827	Phase 2
Romidepsin	Breast carcinoma	Completed	NCT00098397	Phase 2
Romidepsin	Prostate cancer	Completed	NCT00106418	Phase 2
Resveratrol	Colon cancer	Completed	NCT00256334	Phase 1
Resveratrol	Multiple Myeloma	Completed	NCT00920556	Phase 2
Resveratrol	Cardiovascular diseases	Completed	NCT01449110	Phase 2

Table 5

HDACi have a clinical limitation for their toxicity and for their low efficacy at low doses on solid tumors [79]. The challenge of the future will be to limit toxic effects in normal cells and ensure that the effects reach the target gene in cancer cells (increase specificity and reduce side effects). Various therapeutic strategies are in place, such as the search for new molecules or combination therapy. Clinical trials with combination therapy have shown promising results and this has led to a new approach in the development of anticancer drugs, which involves the combination of epigenetic drugs with each other or with other agents. HDACi have been used successfully in combination with chemotherapeutics such as Idarubicin and Cytarabine, in patients with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS). Studies show that HDACi sensitize breast and ovarian cancer cell lines to a variety of cytotoxic drugs [80]. A new approach to the development of anti-cancer drugs involves the link between histone modifications and DNA methylation, which prompted researchers to experiment with double therapy by combining DNA methylation inhibitors and HDAC inhibitors. The synergistic activity between DNA methylation inhibitors and HDACi has been demonstrated in various studies [81] (Table 6). DNMTi and HDACi have been shown to act synergistically in reactivating gene silencing [82]. The gene containing CpG islands in the promoter (eg P16 tumor suppressor) can be opened or closed through the alteration of the histone acetylation levels and in the presence or absence of transcription factors in the promoter. Gene silencing (closed structure) is achieved through multiple changes of Histone modifications including trimethylation of H3-K9, which binds binding proteins such as MeCP2. The silenced gene can be activated synergistically by HDACi together with DNMTi which removes the methylation of cytosine and quickly changes the structure of the chromatin by opening it for transcription [83].

Drug	Pathology	Status	Number	Phase
Hydralazine + Magnesium valproate	Carcinoma of the cervix	Completed	NCT00404326	Phase 2
Hydralazine + Magnesium valproate	Late breast carcinoma	Terminated	NCT00395655	Phase 2
Hydralazine + Magnesium valproate	Carcinoma of the cervix			Preclinical study
Hydralazine	Breast cancer		NCT00575978	Phase 2
Hydralazine	Rectal cancer		NCT00575640	Phase 2
Hydralazine	Ovarian cancer		NCT00533299	Phase 3
Epigallocatechina-3-gallate+ Resveratrol	Obesity			Preclinical study
Curcumin	IBD (RCU/CROHN)	Completed	NCT00889161	Phase 1
Curcumin	Colorectal cancer	Completed	NCT00027495	Phase 1
Curcumin	Alzheimer	Completed	NCT00099710	Phase 2
Curcumin	Irritable bowel syndrome	Completed	NCT00779493	Phase 4
Curcumin + Gemcitabine	Pancreatic cancer	Completed	NCT00192842	Phase 2
DZnep + 5-aza-2 deoxycytidine	Breast carcinoma			Preclinical study
DZnep	SCLC			Preclinical study
DZnep + tricostatin A+ 5-Azacitidine	Breast carcinoma			Preclinical study
GSK 126	Ovarian carcinoma			Preclinical study
GSK 126	Lymphoblastic leukemia			Preclinical study
GSK 126+ Tricostatin A	Colon cancer			Preclinical study
JQ1	Breast carcinoma			Preclinical study
JQ1	Multiple Myeloma			Preclinical study
JQ1+ IBT726	Prostate cancer			Preclinical study
JQ1	Ovarian carcinoma			Preclinical study
JQ1	NSCL			Preclinical study
JQ1+ Panobinostat	Neuroblastoma			Preclinical study
JQ1+ RVX-208	Hepatocarcinoma			Preclinical study

Table 6: Trials with non-nucleoside DNMT inhibitors and HATi.

Today there are ongoing clinical trials that combining epigenetic and non-epigenetic drugs together, or two epigenetic drugs with different mechanisms of action. In many cases there is *in vivo* evidence from animal models that the combination has synergistic effects. This can widen the spectrum of pathologies in addition to the hematological cancers for which they are currently in use [84,85]. Among the new therapeutic strategies the interest in recent years is in the development of small molecules that have the ability to modulate at the same time more target [86]. For example, CUDC-101 is a drug with inhibitory activity for HDAC/EGFR/HEr which has shown neoplastic activity [87,88]. The drug is in use in phase 1 clinical trials (https://clinicaltrials.gov/ct2/results?term=CUDC-101&Search=Search).

Conclusion

The increase in knowledge of the role that epigenetic factors play in pathologies has led to innovations applicable in the clinical field.

The three main changes that have been studied in this field are: DNA methylation, Histone modification, miRNAs

In the diagnostic field, miRNAs have proven to be useful as non-invasive biomarkers for diagnosis, prognosis and predictability of response to therapy, especially in cancer [89]. In the therapeutic field, the reversibility of the mechanisms has suggested an alternative approach for the discovery of new drugs. The evidence on epigenetic proteins involved in the regulation of biological processes and their dysfunctions as the cause of diseases is continuously increasing. This has led to significant results in the discovery of a number of drugs already approved by the FDA and other compounds that are being evaluated in clinical trials. The main study of these drugs is in the oncological field even if at present the interest is also focused on other pathologies such as Parkinson's and Alzheimer's and in the field of immunity [90]. Epigenetic drugs have many biological effects (pleiotropic effect), and act on apoptosis, gene expression, DNA repair, cell cycle and as immunomodulators. They are gene expression reprogrammers, reactivate suppressor genes and stimulate cell differentiation and apoptosis. They are very powerful and are able to reverse the abnormal expression of a gene found in various pathologies. The composite biological effect of epigenetic drugs occurs with the activation of various pathways and can offer advantages in the development and progression of the tumor [91].

The interest aroused by these new therapeutic possibilities has prompted researchers from various fields of scientific interest to create databases available online.

The HMED (Human Epigenetic Enzyme and Modulator Database) is dedicated to epigenetic therapy. Information on enzymes and chemical modulators is available and is useful for the classification of products as epigenetic regulators, inhibitors, activators, modulators. The database provides information on epigenetic molecules and is useful for classifying new products.

The HEDD (Human Epigenetic Drug Database) is another database specialized in the collection of information on drugs obtained from basic reference experimental laboratories [94,95].

Various research associations such as, e.g. Epigenome-wide association studies (EWSs) have started large-scale research projects on diseases associated with epigenetic variations, in particular on DNA methylation alterations. The human epigenome project aims to investigate a large number of CpGs on a large number of patients and controls to identify aberrant methylations at the population level, with the aim of generating the methylation map of the whole genome [96] (Jones PA, Martienssen R. A blueprint for a Human Epigenome Project: the AACR Human Epigenome Workshop. Cancer Res 2005; 65 (24): 11241-6. (EWASs wide association studies =). The database includes IHEC (human epigenome consortium), EU-junked blueprint project, the International cancer genome consortium (ICGC) [96] (http://www.epigenome.org/; http://www.blueprint-epigenome.eu/;http://icgc.org/).

In this project by examining both diseased and healthy tissues, genomic regions that are involved in development, specific tissue expression, environmental susceptibility and pathogenesis will be identified. The use of epigenetic maps will lead to the recognition of epigenetic alterations for complex disorders in the clinical spectrum and this makes epigenetic therapies a valid treatment option [97].

From the data reported in the literature, there is a great potential of these drugs in vast clinical areas and the opening of new scenarios for new therapeutic options that affect a wide range of pathologies seems evident. The great development in the bioinformatics and biotechnology sector has provided a huge wealth of data at the level of basic research and promising results for these drugs come

from preclinical studies. However, the evaluation of these data by means of the conducting clinical trials, which are confirmed as a useful tool for the development and use of new drugs in practice.

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