1.1 INTRODUCTION

Cancer the term commonly used where cells are abnormally divided without control as also able to invade most of the tissues and organism. These cells may spread to various parts of body by various blood circulation and fluidlymph as well. Cancer term includes so many diseases not only one.\(^1\) Medical term for cancer is malignant neoplasm includes various classes, like cell division beyond limits called uncontrolled growth, these infected cells causes intrusion on and destruction of nearby cells called invasion, and cells spread to various body parts through blood or lymph called metastasis. Cancer commonly cause tumor in the body parts except leukemia. Oncology is the term used for the branch dealing with literature review, analysis of symptoms, medical diagnosis and prevention of tumors\(^2\). Cancer can affect all the animals including human beings at any stage of life either foetus or old age. According to American Cancer Society more than 1 trillion people have died from cancer in the world during 2014\(^4\).

1.1.1 History

Carcinoma, the Greek medical term used for malignant or benign tumor obtained from epithelial human cells. Originally word calsus converted to Carcinos in Latin language. And word Oncos used for all types of tumors. And branch related to study of all cancer research called oncology in modern medical term.\(^6\)

Years ago some Egyptian surgeon discovered the description and treatment of cancer in approximate 1600 B.C. Likewise in the Canon of Medicine also describes the treatment of cancer by Ibn sina. German scientist Wilhelm Fabry has proved that milk clot in the mammary duct cause breast cancer. In the year 1775 British surgeon named Percivall Pott has firstly reported chimney sweeps causes cancer of scrotum.\(^7\)

1.1.2 Types of Cancer

Cancer may categories in more than 100 different types. According to types of cells that resemble the tumor it can be grouped in very wide category. So the tissue also causes the invention of tumors.
The main classifications of cancer include:

- Carcinoma: Tumor which are obtained from epithelial human cells called Carcinoma. Most common class of cancer includes breast, prostates, lung & colon cancer.
- Sarcoma: Tumor which are obtained from connective tissues or mesenchyma cell called Sarcoma.
- Germ cell tumor: Malignant tumors which are derived from totipotent tissue. It founds as tumors in testicle and ovary of adults, on body midline of fetuses babies and young children, while it found at the base of head of horses.
- Blastic tumor or blastoma: An immature or embryonic tissue cause this kind of cancer found mainly in children.
- Leukemia – Deformation of blood generating tissue like bone marrow results leukemia wherein generates high numbers of damaged blood cells.
- Lymphoma and myeloma – Deformation of immune system causes this kind of cancer.

1.1.3. Origin of Cancer

Human body is basically made up of cells. The origin of any type of cancer is cell the basic unit of human body. Normally cells are growing and dividing in a forbidden way to generate other cells to maintain body healthy. As cells became aged they expire and replaced by fresh cells. This is called normal cell cycle.

Sometimes this whole cell cycle disturbed and goes wrong. Due to these deformation main genes of body i.e. DNA got damaged or sequence of DNA got changes. As a result of this process cell cycle and cell division become affected and cells do not get died when they have to. So massive mass produces at that place of body called tumor. The uncontrolled cell division results into production of new cells which are actually not needed by the healthy body and due to this uncontrolled process whole cell cycle got disturbed. Sometimes mutation in the gene structures also responsible for the uncontrolled cell division.
1.1.4. Sign and Symptom

Cancer symptoms can be described into 3 groups:

- Common signs observed are unusual body massive mass i.e. tumors, sudden loss of blood i.e. hemorrhage, defragmentation of skin i.e. ulcers and compression of nearby tissues of body i.e. yellowing of eyes and skin may cause jaundice
- In benign tumors cancer cells got spreads identified by enlargement of lymph nodes, unusual cough and haemoptysis, enlargement of liver i.e. hepatomegaly, pain in the bones, may cause fracture of affected bones and neurological signs.
• Some other symptoms includes loss of weight, loss of appetite, stubbornness of bones, wasting(cachexia) abnormal sweating, deformation of red blood cells i.e. anemia, and specific paraneoplastic condition only because of cancer i.e. thrombosis and hormonal imbalance.

Common sites and symptoms of Cancer metastasis

- **Brain**
  - Headaches
  - Seizures
  - Vertigo

- **Respiratory**
  - Cough
  - Hemoptysis
  - Dyspnea

- **Lymph nodes**
  - Lymphadenopathy

- **Liver**
  - Hepatomegaly
  - Jaundice

- **Skeletal**
  - Pain
  - Fractures
  - Spinal cord compression

Figure 1.2 Common sites and symptoms of cancer metastasis

1.1.5. Diagnosis

Cancer is mainly identified by technique called tissue biopsy in which pathologist takes specimen tissue sample for histologic investigation. Investigation mainly includes initial indication of malignancy via radiographic investigation and records all the abnormalities through screening. Mostly all types of cancers initially identified by abnormal and unusual signs and symptoms appear in the body. Definitive diagnosis requires the opinion of physician or specialist of cancer via various blood reports and biopsy reports as well.
1.1.5.1 Investigations

Patients with cancer are subjected with X-Ray, CT scan and sonography medical test.

![X-ray showing left lung cancer](image)

Figure 1.3: X-ray showing left lung cancer.\textsuperscript{12}

1.1.6 Introduction to HDAC inhibitor

In the year 1996 Histone deacetylase\textsubscript{1} was discovered using the trapoxin as an subtract form nuclear extract. It was reported hdac\textsubscript{1} was highly compatible in sequence homology with yeast Rpd\textsubscript{3}, which was mainly incorporates in gene regulation and transcription co-repressor to Histone deacetylase activity\textsuperscript{13}. Subsequently, another eighteen Histone deacetylase member was discovered in the human genome. With the help of various recombinant techniques and purification procedures now it is available to identified the inhibitory symptoms of human HDAC inhibitors and it was vastly applied to in cellline culture and animal form models. That was reported all the commonly used HDAC\textsubscript{i} act unselectively and inhibit all or selective group of HDFC family\textsuperscript{14}. Now a days these unselective HDAC inhibitors have been identified and shows very effective in blood cancer i.e. leukemia and some massive body tumors also. In the year 2006 USFDA approved very first HDAC inhibitor named as Vorinostat (SAHA) for cutaneous T-cell lymphoma\textsuperscript{15}. However, just like other medicines SAHA also shows so many side effects like taste disturbances, bone marrow depression, electrolyte changes, diarrhea, discovered clotting, weight loss, fatigue and some times cardiac arrhythmias\textsuperscript{16}. These kind of observations shows potent role of HDAC in the regulation of chromatin structure and also reported as posttranslational modifier of various important proteins in cell and tissue as well. These results motivates scientists to develop selective individual HDAC inhibitors for targeting critical oncogenic function in damaged cells which is not observed in normal cell. With respect
to this aim we have described individual HDACs as target in cancer treatment. And also it was reviewed the individual function of each HDAC in cancer cells which was not observed in normal cells. This will help anyone to understand normal physiology and development of tumors and dangerous side effects associated with their regulation\textsuperscript{17}.

1.2 Classification of HDAC family member

Based on sequence similarities with yeasts orthologues HDACs are grouped into class I, class II, class III and class IV with the yeasts SIR2, HDAI and RPD3 respectively. Class 1, 2, and 4 are named as “classical (ancient)” HDAC members and including 11 family members. And class 3 members have referred as sirtuins. Both Classical HDAC members and sirtuins are mainly differs in their mechanisms. Classical HDAC members are mainly deals with Zink dependent enzymes containing a catalytic pocket with a Zink at its basement which can be inhibited by Zink chelating compounds such as hydroxamic acids. While, sirtuins have a different mechanism of action requiring NAD\textsuperscript{+} as regulatory cofactor to activate class 3 HDAC. So in general the term “HDAC inhibitors is mainly used as drugs to target the “classical” class 1, 2, and 4 HDAC and that are currently under clinical trials\textsuperscript{18}.

1.2.1. Class I HDACs

Multiprotein nuclear complexes which are mainly incorporate with transcription repressions and epigenetic landscaping are mainly grouped in the HDAC family subunit 1, 2 and 3. Just like HDACs 1 and 2 are main parts of the adjacent complex to inactivate the signals of neuronal gene at non-neuronal tissue. While other examples of complex HDACs 1 and 2 are the NURD and SIN3 suppressor units. HDAC 3 is mostly founds within the NCOR and S-MRT repressor complexes. As Class I member HDAC 8 does not observe as a component of any repressor complexes for a particular function. Classifications of Class I HDAC are as under

1.2.1.1. HDAC1

HDAC1 has lifecycle of 9.5 days by embryonic lethal, that results in uncontrolled cell division degradation of embryonic cells. The signal of the CDK (Cyclin dependant Kinase) inhibitor and global histone deacetylase activity is vice versa. One is up regulated i.e. CDKi and other is down
regulated i.e. HDACi. Lack of HDACs 1 functions in mice could not be compensate by concomitant up regulation of HDAC 2 and 3.

**Table 1.1: HDAC1**

<table>
<thead>
<tr>
<th>Molecular functions:</th>
<th>• transcriptional factors activity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>• Histone deacetylase activity</td>
</tr>
<tr>
<td></td>
<td>• transcriptional factor binding</td>
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<tr>
<td></td>
<td>• hydrolyses activity</td>
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<td></td>
<td>• enzymatic binding</td>
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<td>• simillar protein binding</td>
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<tr>
<td>Cellular components:</td>
<td>• histone deacetylase complex</td>
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<td></td>
<td>• nucleus of cell</td>
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<tr>
<td></td>
<td>• cytoplasmic cell</td>
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<tr>
<td>Biological processes:</td>
<td>• transcriptional process</td>
</tr>
<tr>
<td></td>
<td>• regulates transcription,</td>
</tr>
<tr>
<td></td>
<td>• anti apoptosis</td>
</tr>
<tr>
<td></td>
<td>• modification of chromatin</td>
</tr>
<tr>
<td></td>
<td>• Histone deacetylase</td>
</tr>
</tbody>
</table>

**1.2.1.2. HDAC2**

HDAC2 shows its role in mainly cardiac functions. As mice not having HDAC2 can live until its fetus period, but die because of cardiac defects. It shows cardio specific removals of either HDACs 1 or 2 by use of model which is not generate a similar condition. However, cardio selective removal of these genomes concurrently results in early born child dysfunction, followed by arrhythmia and increased cardiomyopathy\textsuperscript{15}. 
Table 1.2: HDAC 2

| Molecular functions: | • transcriptional activity  
|                      | • Histone deacetylation activity  
|                      | • transcriptional binding  
|                      | • hydrolysis activity  
|                      | • enzyme binding  
| Cellular components: | • Histone deacetylase complex  
|                      | • heterochromatinoid  
|                      | • cell nucleus  
|                      | • replication forks  
|                      | • cytoplasm  
| Biological processes:| • transcriptional process  
|                      | • regulates transcription  
|                      | • modification of chromatin  
|                      | • Histone deacetylase  

1.2.1.3. HDAC 3

HDAC 3 shows its role in Liver functionality. Germline removal of HDAC 3 results in early embryonic death before day 8.5. Modification of HDAC 3 may lead to a holdup in cell cycles-dependent DNA deformation, cell division progression, inappropriate repair, embryonic fibroblasts death in mouse. Removal of HDAC 3 results in hepatocytes, hypertrophy, enlargement of organ, & metabolism of fat specifically at Liver.
1.2.2. Class II HDACs

Class II HDAC family is classified into II A and II B. Class II A member grouped into HDAC 4, HDAC 5, HDAC 7, HDAC 9 which are explained by a huge and important N terminal domains regulation of nuclear cytoplasm shuttling & DNAs binding. Main functions of these HDACs are mainly associated with intrinsic nuclear transport in and out signal as well as protein binding site i.e. 14.3.3. These HDAC 4, HDAC5, HDAC7, HDAC9 are containing three conserved 14.3.3 binding site which excites the cytoplasm retentions and export of nucleolus to the class II A HDAC. These HDAC members are involving to phosphorylations to regulates the activity of transcriptional factor i.e. myocytes enhancing factor-2 (MEF2). Some signalling pathways involving protein kinase-D Ca\(^{2+}\)-calmodulin-dependent kinases (CaMKs), salt-inducible kinases, microtubule affinity regulating Kinase, and check point Kinases regulates phosphorylations of the binding site. Same wise HDAC 6 contains two movable deacetylase domains and one Carbon terminal Zn\(^{2+}\) finger. HDAC 6 is involving in a key cytoplasmic deacetylase process as tubulins.
and HSP 90 deacetyl to regulate cell mortality, chaperone function and adhesion. Moreover HDAC6 show cellular function independently from deacetylase functioning. Zinc finger domain incorporates Binding to ubiquitin which regulates autophagy, aggresome function, HSF-1 and PDGF functions. HDAC 10 is physically resembles to HDAC 6, but containing 1 supplementary catalytic inactive domain. Role of HDAC6 was not clear. Class II HDAC is grouped into two class: Class II A HDAC and Class II B HDAC.

1.2.2.1 Class IIA HDACs

(A) HDAC4

Main activity of HDAC 4 is associated to chondrocytic hypertrophy and endochondral bone tissue formation which is important as function of MEF 2c. Deletion of HDAC4 in mice shows early onsite chondrocytic hypertrophy. Excess amount of HDAC 4 results in chondrocytes proliferation leads to in vivo inhibition chondrocytic hypertrophy and differentiation. Thus, HDAC 4 is mainly incorporates with bone marrow cancer.

Table 1.4 HDAC4

| Molecular functions: | binding of DNA  
|                      | Histone deacetyllytic activity  
|                      | transcriptional binding  
|                      | hydrolytic activity  
| Cellular components: | Histone deacetylase complex  
|                      | cellnucleus  
|                      | cytoplasm  
| Biological processes: | down regulation of transcription process by RNA polymerase  
|                      | development of skeletal  
|                      | transcriptional process  

• regulates transcription
• inflammation response
• cell cycles
• multicellular organism development
• development of nervous system
• down regulation of cellular proliferation
• modification of chromatin
• β cell differentiation

(B) HDAC5

HDAC5 shows its role in cardiac function. Germline deletion of HDAC5 in mice develops enlargement of heart with pressure response and results in aortic restriction or constitutive commencement of cardio strain signals.

Table 1.5 HDAC 5

| Molecular functions:            | • transcriptional corepressor activity          |
|                                | • histone deacetylase function                 |
|                                | • transcriptional factor binding               |
|                                | • specific transcription repressor function   |
|                                | • hydrolytic activity                         |
| Cellular components:           | • histone deacetylase complex                  |
|                                | • cellnucleus                                 |
|                                | • cytoplast                                   |
|                                | • nucleous body                               |
| Biological processes:          | • regulates of progression to cell cycle       |
|                                | • down regulation of transcription to RNA polymerase |
|                                | • chromatinoid remodeling                     |
|                                | • chromatin silencing                         |
(C) HDAC7

HDAC7 is mainly consent with nerve line integrity and vascular dysfunction. Deformation of the HDAC7 genome in mice shows embryonic lethargic activity because of failure in endothelial cell–cell adhesion followed by frequent dilatation and break of blood vessel. HDAC 7 was mainly associated in the endothelial tissue in embryogenetic cycle, results in maintenance of vascular integrity by repression of the metalloproteinase ten expression and associatede with myocyte enhancer factor-2 (MEF2).

Table 1.6 HDAC7

| Molecular functions:     | • transcriptional repressor activity | • Histone deacetylase activity |
|                        | • transcriptional factor binding      | • transcriptional repressor function |
|                        | • hydrolytic activity                | |
| Cellular components:    | • histone deacetylase complex        | • cellnucleus                     |
|                        | • cytoplasm                          | • cytoklyst                       |
| Biological processes:   | • regulates progression of cell cycles| • down regulation of transcriptional |
|                        | • transcriptional processes           | • transcriptional processes        |
|                        | • inflammation response               | • inflammation response           |
|                        | • development of nerve system         | • development of nerve system      |
|                        | • chromatinoid modification           | • chromatinoid modification        |
(D) HDAC9

HDAC9 does not show any effect in early stage of mice. But at the age of 0.8 year mice develops sensitization to hypertrophic signals resulting in spontaneous cardiac hypertrophy.

**Table 1.7 HDAC9**

| Molecular functions: | • transcriptional repressor activity  
|                      | • Histone deacetylase activity  
|                      | • transcriptional factor binding  
|                      | • transcriptional repressor activity  
|                      | • hydrolytic activity  
| Cellular components: | • Histone deacetylase complex  
|                      | • cell nucleus  
|                      | • cytoplasm  
| Biological processes: | • regulates progression of cell cycles  
|                      | • down regulation of transcription from transcriptional genes  
|                      | • inflammation response  
|                      | • cardiac development  
|                      | • chromatinoid modification  
|                      | • Histone deacetylation  
|                      | • β cell differentiation  
|                      | • down regulation of striated muscle  

1.2.2.2. Class IIB HDACs

(A) HDAC 6

HDAC 6 is also categorized by tubulin deacetylase. Germline removal of HDAC 6 in mice results in changing of its viability that will lead to increase in the level of tubulin acetylase in the various body organs. Lacking of HDAC 6 also shows changes in immune response and density of bone minerals. It was observed increase in Hsp90 acetylation due to lacking of HDAC 6. Cells undergo apoptosis after treatment with arsenic due to unavailability of intact HDAC 6. Symptoms by deletion of HDAC 8, HDAC10 and HDAC11 from mice have not yet been published. This whole research proved absence of HDAC1, HDAC2 HDAC3 and HDAC7 make a lethargic response because of inappropriate early embryonic cycle of cells. Likewise absence of HDAC7 causes damaged blood vessels. In contrast, absence of HDAC4, HDAC5, HDAC6, and HDAC9 lead to defective muscle formation, cellular hypertrophy, defective bone development and differentiation in stress response.

From all these data study the whole research concludes (i) every HDAC is independently shows its role in each steps of cell cycle (ii) each HDAC member is responsible for normal development of embryo and also play a vital role in cell cycle regulation, deformation of DNA response, growth and development of cell and metabolism process. (iii) Each and every HDAC play a vital role in normal cell and tissue generation and also in various cellular functions. (iv) It was also noticed that wide spectrum HDAC inhibitor were likely to cause various side effect.

Table 1.8 HDAC6

| Molecular functions:          | • actin receptor binding |
|                             | • histone deacetylase activity |
|                             | • Zn²⁺ ion binding |
|                             | • transcriptional repressor function |
|                             | • hydrolytic activity |
|                             | • histone deacetyl binding |
|                             | • tubulin deacetyl activity |
• metal ions binding

| Cellular components: | • histone deacetylase complex  
|                      | • cellnucleus  
|                      | • cytoplasm  
|                      | • microtubule |

| Biological processes: | • protein poly ubiquitination  
|                       | • transcription process  
|                       | • regulates transcription,  
|                       | • down regulation of microtubule depolymerization  
|                       | • cell cycle  
|                       | • cellular organism development  
|                       | • chromatin modification  
|                       | • Histone deacetylation |

1.2.3 Class IV HDAC

This class HDAC consist only one member i.e. HDAC11. Class IV HDAC resembles to class I and II HDACs structurally. Very less research has done on this class. HDACs cooperate a vital position in formation and growth of various proteins like E2Fs, Bcl-6, p53, HMG, STAT, GATA1, b catenin, tubulin etc. For example, HDAC1 shows its role in regulation of transcription activity of p53 genome. Now HDACi led to deacetylation of p53 genome results in damaged cell cycle followed by interaction with DNA and transcriptional changes in the cell. Whole process finally results in the cell growth arrest and leads to apoptosis. Thus, HDAC has proved its role in various cell functioning like transcription, translation and differentiation. From this study one can easily clarified that HDACs take part in the cell functions and regulates chromatographic structures.
1.3 CLASSICAL HDACs FAMILY MEMBERS IN CANCER

Although so much important role of HDACs in cell division and clinical trials surprisingly very less knowledge of expression and mode of action of HDACs targets in cancer cells. Moreover no systematic inquiry and research of all HDACs in given animal models are available. Study of these HDAC is very much important as scientific significance, as study shows conflict to HDAC inhibitor in cells not having HDAC 2 expression. Now we will focus our study on the genetic symptoms, expression of DNA and fundamentals of the HDACs 1 to 11 in cancer.

1.3.1. HDACs polymorphism in cancer risk

Various studies reveal lung cancer and breast cancer patients show presence of Germline variants. But no any evidences found to prove role of HDAC 3, 4 and 5 in lungs cancer and HDAc2, 5 in breast cancer risk. Research shows that 18 percent of total 182 cancer samples found CAG triplet at its 50-UTR DNA while 10 percent found in total 191 normal DNA control samples. This shows how important HDACs in regulation of DNA structures. Some patients having hepatic carcinoma subjected to insertion of polymorph of HDAC10 resulted in increasing in promoter activity that was combined with hepatic carcinoma and chronic HBV.

1.3.2. Somatic HDAC mutations in cancer

Research shows mutation in the HDAC 2 genome lead to increase microsatellite instability in human epithelial cell lines. This mutation was observed in 22 percent of breast cancer cases that was leads to increase in the symptoms associated with HDAC 2 protein expression. Surprisingly this kind of mutation affects the anti proliferative and apoptotic function of the resistant cancer cell. Likewise this kind of microsatellite mutation was observed in case of HDAC 4 genome also.19
1.4 Expressions and Functions Of Classical HDACs

1.4.1 Class I HDAC

(A) HDAC 1 expression

Expression of HDAC 1 was found upregulated in clinical survey of gastric cancer patients. Its percentage reported was about 60 percent compared to normal cell. When this clinical survey applied to larger scale then along through HDAC 1 expression other HDAC 2 & HDAC 3 also found in upregulated activity. This proves the key role of HDAC 1, 2 and 3 in the gastric cancer. Same wise in case of pancreatic cancer the expression of HDAC 1 was found upregulated along with H1f1 genome in small clinical survey. While same study applied to larger clinical survey of pancreatic cancer the expression of HDAC 1 found along with high expressions of HDAC 2 and HDAC 3 also. In case of colorectal cancer clinical survey shows the elevated expressions of HDAC 1 along with HDAC 5 and HDAC 7 which was not observed in case of renal, breast and bladder cancer. While this study subjected to larger survey it shows high expressions of HDAC 2 and 3. This results in significantly decreased HDAC 2 prognostic factors. In case of prostate cancer expression of HDAC 1 was at higher level. Expressions were also observed very much clearly in benign tumor of prostate hyperplasia. While in case of liver cancer higher HDAC 1 expression was observed at cell cycle functions like differentiation and transcription. It was more prone to show expressions at TNM stage. In case of Lung cancer there is no any specific symptoms observed in expression of HDFC 1. Tissue function observed same like normal tissue. So there is no expression in lung cancer. In case of breast cancer, expressions of HDAC 1 and HDAC 3 were observed to associate with activity of progesterone and oestrogens receptors in small scale clinical survey. While this survey goes larger the role of HDAC 1 has become very clear. It was said as a positive marker for identification of breast cancer. Symptoms also noticed at various lymph nodes. Overall conclusion come out is HDAC 1 over expressions were noticed in case of gastric cancer, colorectal cancer, progesterone cancer, pancreatic cancer. These all cancer includes deformation of cell functions like invasion, differentiation, proliferations. While in case of breast cancer, renal cancer and lung cancer role of HDAC 1 was observed very less.
**HDAC1 Function**
Main function of HDAC 1 is established in cell division process and proliferation process to inhibit P21 and P27 signals. Cyclin dependant kinase inhibitions leads to deformation of stem cells of embryo. Germline removal of HDAC 1 and HDAC 3 results in blockage of cell differentiation of HELA cell line where removal of HDAC 4 and HDAC 7 do not make any difference in cell numbers. Removal of HDACs 1 result in blockage either at G1 phase of the cell cycle or G2 / M transitions, causes damage of mitotic cell, cell development inhibitor, and increase in the percent of apoptosis in bone cancer and breast cancer. While deletion of HDAC 2 not show any remarkable effect on the cancer cell line. Study shows remarkable results on removal of HDAC 1 in colon carcinoma by RNA base inhibitors. Excess levels of HDAC1 lead to increasing in proliferation process and dedifferentiation of embryonic cell in cultured prostate carcinoma. Moreover HDAC 1 also involve in multidrug resistant. Neuroblastoma cells which are resistant to chemotherapy show high level of HDAC 1. Removal of HDAC 1 also helps in the etoposide treatment by siRNA. By the use of unselective HDAC inhibitor affects RNA dependant urokinase and plasminogen expression. Recent research shows that this inhibitor plays a role in DNA transcription process and RNA recombinant process also. Main function of HDAC 1 is reported in cell Autophagy by HELA cell line.

**HDAC2 expression**
Compared to HDAC1 this HDAC2 is more robust and more frequent in case of colorectal cancer. It was observed at very early stage of cell cycle i.e. polyp stage. HDAC 2 showed a clear symptoms of high range stains at transitional region of neuron blastoma cells as compared to HDAC 1. In case of gastric, prostate and colon cancer HDAC 2 shows poor prognosis associated with HDAC 1 and HDAC 3.
HDAC 2 function
Study shows apoptosis of cell due to increase in the activity of p21/Cpi1/WAF2/p53 expressions is mainly dependant on HDAC 2 genome. Removal of HDAC 2 may lead to increase in the DNA functions like binding affinity towards p53, proliferation and induction of cell sensations in breast carcinoma. Selective activity of progesterone and estrogen receptors in breast cancer cell through down regulation was controlled to increase tamoxifen induced apoptosis, observed by selective blockage of HDAC 2. RNA induced removal of HDAC 2 & HDAC1 but not HDAC 3 decrease spreading of colonal carcinoma cell in vitro. Deletion to HDAC 2 & HDAC 1 instead of HDAC 3, HDAC 6, and HDAC 8 sensitize CLL cells to TRAIL-induce apoptosis. HDAC 2 removal induce apoptosis in neuron blastoma cells against HDAC 8. Crossing of HDAC 2 - mutation with tumor APC mouse have shown limiting role of HDAC 2 for intestine cancer in vivo.

(B) HDAC 3 expression
HDAC 3 shows its expressions in association with HDAC 1 & HDAC 2. It is poorly noticed in increasing number of colorectal, prostates and gastric cancer sample.

HDAC 3 functions
In pro myelocytic blood cancer cell, HDAC3 plays a vital role in cellular transcriptional regulation of various cells. Removal of HDAC 3 in this cell restores expressions of retinoic acid depending genes. The Amlokind fusion transcription with AML-1-ETO joined with class I HDAC. Together HDAC 1, HDAC 2, and HDAC 3 with ETO mainly incorporate to decrease transcription and disruption of cell cycle of leukemia.

(C) HDAC8 expression
It is reported at very early age neuronal cancer. Due to high HDAC 8 expressions remarkably associated with early stage of disease, poor diagnostic marker. While, all other ten HDACs family member investigation do not compare to this disease stage.
**HDAC8 function**

After deletion of HDAC 8 proliferation process was decrease in cervical, colon and lung cancer cell line. Telomerase activity is also been associated with HDAC 8 genome. Specific inhibitor of HDAC 8 selectively increases apoptosis in Tcell derived lymphosarcoma and leukemia, but not involve in solid tumor cancer cultures. When HDAC 8 was deleted from neuroblastoma cells of children, it will reduced proliferation, inhibit clonogenic growth, blockage of cell cycle and stop the differentiation process without affecting normal cell HDAC activity. Research reveals deletion of HDAC 2 increase cell death but no signals of transcription, suggest that each HDAC increase various cancer.

1.4.2 Class II HDAC

1.4.2.1 Class II A HDAC

(A) HDAC 4 expression

Expressions of HDAC 4 up regulated in breast cancer cell lines in contrast to colorectal, bladder and renal cancer cell lines.

HDAC 4 function

HDAC 4 was mainly involved in leukemic fusion protein to regulate differentiation process adjoined with genomes. Transcriptional activity in HIF 1a genome in renal cancer cell was regulated by HDAC 4 and HDAC 6. So inhibition of these HDACs results anti angiogenesis in tumors.

1.4.2.2. Class II B HDAC

(A) HDAC 6 expression

Expression of HDAC 6 was found upregulated in clinical survey of colonal cancer patients. Its percentage reported was about 55 percent compared to normal cell. When this clinical survey applied to larger scale then along with HDAC 6 expression found in upregulated activity. This proves the key role of HDAC 6 in the colonal cancer. Same wise in case of breast cancer the expression of HDAC 6 was found upregulated along with H1f1 genome in small clinical survey. While same study applied to larger clinical survey of breast cancer the expression of HDAC 6 found along with high expressions of HDAC 1 and HDAC 2 also. In case of gastric cancer clinical survey shows the elevated expressions of HDAC 6 along with HDAC 5 and HDAC 7
which was not observed in case of renal, breast and bladder cancer. While this study subjected to larger survey it shows high expressions of HDAC 1 and 2. This results in significantly decreased HDAC 2 prognostic factors. In case of renal cancer expression of HDAC 6 was at higher level. Expressions were also observed very much clearly in benign tumor of renal hyperplasia. While in case of prostate cancer higher HDAC 6 expression was observed at cell cycle functions like differentiation and transcription. It was more prone to show expressions at TNM stage. In case of bladder cancer there is no any specific symptoms observed in expression of HDFC 6. Tissue function observed same like normal tissue. So there is no expression in lung cancer. In case of pancreatic cancer, expression of HDAC 6 was observed to correlate with the activity of progesterone and oestrogens receptors in small scale clinical survey. While this survey goes larger the role of HDAC61 has become very clear. It was said as a positive marker for identification of pancreatic cancer. Symptoms also noticed at various lymph nodes. Overall conclusion come out is HDAC 6 over expressions were noticed in case of colonal cancer, breast cancer, prostate cancer, liver cancer. These all cancer includes deformation of cell functions like invasion, differentiation, proliferations. While in case of bladder cancer, renal cancer and lung cancer role of HDAC 6 was observed very less.

**HDAC6 function**

Main function of HDAC 6 is established in cell division process and proliferation process to inhibit P21 and P27 signals. Cyclin dependant kinase inhibitions leads to deformation of stem cells of embryo. Germline removal of HDAC 6 results in blockage of cell proliferation of HEPA cell line whereas removal of HDAC 5 and HDAC 7 do not make any difference in cell numbers. Removal of HDAC 6 result in blockage either G1 phase of the cell cycle or at the M transition, increases damage of mitochondrial cell, cell development inhibitor, and increases in the percent of apoptotic cells in breast cancer and colonal cancer. While deletion of HDAC 7 not show any remarkable effect on the cancer cell line. Study shows remarkable results on removal of HDAC 6 in colon carcinoma by RNA base inhibitors. Excess levels of HDAC 6 lead to increasing in proliferation process and dedifferentiation of embryonic cell in cultured prostate carcinoma. Moreover HDAC 6 also involve in multidrug resistant. Neuroblastoma cells which are resistant to chemotherapy show high level of HDAC 6. Removal of HDAC 6 also helps in the etoposide treatment by siRNA. By the use of unselective HDAC inhibitor affects RNA dependant urokinase and plasminogen expression. However, research reports that the HDAC inhibitor plays
a vital role in DNA transcription process and RNA recombinant process also. Main function of HDAC 6 is reported in cell Autophagy by HEPA cell line.

(B) HDAC 10
Removal of HDAC 10 with HDAC 6 results in decreased VEGF receptor expression in damaged cells. So it was reported these HDFC members play a vital role in proteasomal degradation of heatshock protein. It also regulates endothelial growth factors.\textsuperscript{20-26}

1.5 CLASSIFICATION OF HDAC\textsubscript{i} BASED ON STRUCTURE
The HDAC inhibitor acts properly on class I and class II HDAC family members by adhering to the Zn\textsuperscript{+2} containing positive ion domain of the HDAC. Some class of HDAC inhibitors have been classified to (a) organic hydroxamic acid e.g Trichostatin A and sub eroyl anilid hydroxamic acids (SAHA), (b) short fatty acid chain e.g. butyrate and valproic acids (VPA), (c) benzamide (d) cyclic tetra peptide e.g., trapoxin (e) sulfonamide anilide\textsuperscript{27}.

(A) Hydroxamates class
e.g. TSA (Trichostatin), SAHA (Vorinostat), CBHA, LAQ-824 (Dacinostat), PDX-101 (Belinostat), LBH-589 (Panobinostat), ITF-2357 (Givinostat)

![Trichostatin A](image1)

![SAHA](image2)
LBH-589 (Panobinostat)

ITF-2357 (Givinostat)
(B) Cyclic peptide

e.g Desipeptide (Romidepsin), Apicidin, Trapoxins, Chlamydocin

![Chemical structure of Depsipeptide (Romidepsin)]

![Chemical structure of Apisidin]
(C) **Aliphatic acids**

e.g. Valproic acid, Phenylbutyrate, Butyrate, AN-9

![Valproic acid](image)

![Phenylbutyrate](image)

![Butyrate](image)

![AN-9](image)

(D) **Benzamides**

e.g. MS-275 (Entinostat), MGCD0103, Pimelic diphenylamide, N-acetyldinaline

![MS-275](image)
(E) **Sulphonamide anilides**

e.g. N-2-aminophenyl-3-[4-(4-methylbenzenesulfonylamino)-phenyl]-2-propanamide

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1.6 Mode of action: HDAC Inhibitor

With the help of two nearby histidine molecule, 2 aspartate residues, 1 tyrosine molecule and zinc as chelating ion which binds very deeply to the receptor the acetyl group of Histone was removed by HDAC enzyme. Study shows inhibitors of HDACs mainly inhibit cell cycle and speed up the differentiation process depends on cell type and other ecological factors. Specific HDAC inhibitor like SAHA, Trichosatin, and PXD act by replacing $\text{Zn}^{2+}$ atom. Every cells follows two main pathways for apoptosis. One is death receptor pathway also named as extrinsic pathway. And other is mitochondrial pathway also named as intrinsic pathway. All the HDAC blockers follow these cell death pathways in most cancer models. Procedure of apoptosis due to inhibitors are as follow.

I. Death receptor (extrinsic) pathways of apoptosis

II. Mitochondrial (intrinsic) pathway of apoptosis

III. Inhibition of angiogenesis

IV. Generation of reactive oxygen species (ROS)

V. Autophagy etc.
Cell death stimulated by HDAC blockers is mainly affiliated with induced expressions of pro-apoptotic gene and reduced expressions of anti-apoptotic gene, this imbalance leads to cell death.\textsuperscript{32} HDAC inhibitors increase the functioning of proapoptotic anticancer agent. Other remarkable effects linked to HDAC inhibitors induced apoptosis are the production of reactive oxygen species, i.e. ROS. It plays an important role in activating the mitochondrial pathway of cell death\textsuperscript{33}. In addition to increase cell death, HDAC blockers have been reported to block tumors angiogenesis and increase autophagy, which also contributes to their mode of action. However, recent reports suggest that the induction of autophagy due to HDAC inhibitors could promote survivals and thus decrease therapeutic effect since its blockage greatly stimulates HDAC inhibitor-induced apoptosis\textsuperscript{34}.

![Fig 1.4 Mechanism of Action of HDAC Inhibitors\textsuperscript{35}]

HDACs eliminate the acetyl group from histone in nucleosides which leads to reduced chromatin, on the other hand HATs adds acetyl group. HDAC inhibitors obstruct HDAC activity,
foremost to increased amount of acetylation and available chromatin. HDACs and HATs are multicomponent protein complexes that act together with transcription factors\textsuperscript{36}.

**Fig 1.5** Mechanism of action of HDACi in cancer\textsuperscript{37}

**Fig 1.6** Mechanisms of HDAC inhibitor-induced cell death.\textsuperscript{37}
1.7 CHEMISTRY OF NOVEL HDAC INHIBITORS

The excellent pharmacophores of HDAC blockers contains 3 different structural groups (a) Zn$^{2+}$ joining group (b) lipophilic Linkers (c) identification of cap groups. HDAC inhibitors may be divided into various structural class like benzamide, aliphatic acid, cyclic peptide and hydroxamates. Trichosatin A was first natural hydroxamate invented to inhibit HDAC. Vorinostat moiety was structurally resembles to Trichosatin A. The series of amino suberoyl hydroxamic acid have been reported to block HDAC and translate cell differentiation process to nano molar concentration. Vorinostat was first HDAC inhibitor that get approval for clinical treatment by the U.S.F.D.A. Carboxy cinnamic acid bis hydroxamate was active HDAC inhibitor and established ass structural basis for various derivatives like PXD-101 (Belinostat), a sulfonamide derivative, LBH-589 and LAQ-824. These HDAD inhibitors inhibit class 1 and class 2 HDAC. Panobenostats (LBH-589) is a cinnamic hydroxamic acid varient of carboxy cinnamic acid bishydroxamate. Italfarmac was an HDAC inhibitor that contain hydroxmic acid molecule adhere to aromatic ring. Cyclic peptides group are structurally matrix groups of HDAC inhibitor, involved the usual product desipeptide (Romidesin), apicedine and the cycli hydroxamic acids peptides, all are stimulated at nano molar concentration. Aliphatic acids are like valproic, phenyl butyrate and butyrate acid are relatively less inhibitor of HDAC, Ic$_{50}$ of milli molar concentrations. Valporoic acid & phenyl butyrate molecules that are available in society for other applications like in mental disorder; psychic problems and are reported their roles HDAC inhibitors. MGD0103; dihydro bromide substituted 2-amino phenyl benzamides. 2 chemical compounds, SK741 & SK768 specifically targets HDAC 1 and HDAC 2. Tubacin like small molecules selectively inhibit HDAC 6 function and cause accumulations of acetylation tubulins, as do not affects acetylations of Histone molecules and not affect cell cycle progressions. This proves inhibition of HDAC might better over inhibition of HDAC at carcinoma. The capping groups of HDAC inhibitor was exposed to solvent and reacts with peptides nearby gateway of the main sites. The zinc adhesion groups reside in the inner side of protein, make complex with zinc ion i.e. involve in catalysis. The linker play a vital role between zinc binding group and capping site for high affinity interaction with peptides. Substitution near zinc binding site and linker may increase in the inhibitory activity of HDACs.
Other Disease that may be treated with HDAC inhibitors

Other than cancer there would be some novel therapeutic uses where HDAC inhibitor may provide medical benefits. HDAC inhibitors were identified in the activation of some HIV viruses.\textsuperscript{52-53} This study helps in the development of some target specific anti viral treatment by reactivation of some virus that are susceptible to other treatments.\textsuperscript{54} Valproic acid is the best example of this technique where HDAC inhibitors used as anti retroviral therapy by blocking of cell infection remarkably in 75 percentage of patients with such combination.\textsuperscript{55} HDAC inhibitors are also able to decrease graft vs hosts disease in bone marrow transplantation by decreasing inflammatory TNF $\alpha$ cytokin.\textsuperscript{56} HDAC inhibitors are also used in some central nervous system disease like anti epitic agents and mood changer. Again valproic acid sets best example of CNS active drugs. Recent study shows HDAC inhibitors may also play a key role in some neuron degenerative disease like Alzheimer or Huntington disease.\textsuperscript{57} HDAC inhibitors like SAHA and sodium butyrate have proved their role in increase of the memory cell generation in mice. While other HDAC inhibitors like Trichostatin A play a vital role as anti inflammatory agent, Givinostat is used in polycytemia and thrombocytemia.\textsuperscript{58-59} AR-402 has started clinical trials in 2014 for cancers like myeloma, chronic leukemia and lymphoma\textsuperscript{60}. 