Targets in anticancer research—A review

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Cancer is a complex disease characterized by a loss in the normal cell regulatory mechanisms that govern cell survival, proliferation, and differentiation. Current chemotherapeutics, as anticancer agents, are developing resistance to single drug and also to treatment therapies involving multiple drugs. Cross resistance associated with the specificity and selectivity of existing drugs has restricted the application of chemotherapy. Alternatively, these limitations have given better insight in understanding the underlying molecular mechanisms responsible for the development of various stages in cancer. In the light of this, continuous efforts are being made in order to identify and validate newer anticancer targets. This review presents some of the important targets that have been already reported, such as aromatase, farnesyl transferase, histone deacetylase, tyrosine kinase and cyclin-dependent kinase. A few molecules designed against these targets have successfully reached clinical trials. However, only limited marketed drugs are available from these classes. Besides, the review also highlights some of the other important targets and strategies that have also drawn considerable attention in the area of anticancer drug development such as, cancer stem cells and monoclonal antibodies. Further, the integration of the tools in molecular biology with the results from preclinical and clinical trials would strengthen the effectiveness of treatment regimens in cancer patients. There lies a much scope for designing promising lead compounds and treatment therapies against these established targets.

Keywords: Acute lymphoblastic leukemia, Antitumor activity, Aromatase inhibitor, Cancer, Chemotherapeutics, Chronic myelogenous leukemia, Cyclin-dependent kinase inhibitor, Farnesyl tranferase inhibitor, Histone deacetylase inhibitor, Leukaemia, Stem cells, T-cell lymphoma, Tumors, Tyrosine kinase inhibitor

Introduction

Cancer is a multifactorial disease. Numerous genes are involved in the complexities of this disease. The abnormalities in the genetic constitution at cellular and molecular levels are a consequence of accumulation of both mutational and non-mutational (epigenetic) factors. Further, an altered gene functioning affecting the cancer cells is also attributed to activation of oncogenes (phenotypically dominant genes) and the inactivation of tumor suppressor genes (phenotypically recessive genes). The characteristic changes that occur in cancerous cells have been termed as its hallmarks. Initially, processes such as growth factor independence, evasion of growth suppressors, absence of apoptosis, maintenance of replicative state, angiogenesis and invasion/metastasis, were identified as the key changes affecting the malignant transformations of normal cells. However, re-programming energy metabolism and evading immune destruction along with characteristics like genomic instability and inflammation are also well documented. Understanding the various factors associated with these hallmarks has enabled researchers in identification of potential anticancer targets. Table 1 highlights various approaches followed in the development of treatment therapies in cancer. Fig. 1 summarizes important cell signal transduction pathways involved in the development of breast cancer.
**Table 1—Various anticancer approaches and their examples**

<table>
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<tr>
<th>Approach</th>
<th>Target mechanisms</th>
<th>Examples of drug candidate/anticancer approach developed against respective targets</th>
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<tr>
<td><strong>Cell signal transduction</strong></td>
<td>Development of specific inhibitors of growth factors such as:</td>
<td>a. Herceptin and Imitinib</td>
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<td>a. EGFR and HER2 (c-erb2)</td>
<td>b. Gefitinib</td>
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<td>b. Tyrosine kinase</td>
<td>c. Tipifarnib and Lonafarnib</td>
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<td>c. Farnesyl transferase</td>
<td>d. Flavopiridol and 7-hydroxystaurosporine-UCN-01</td>
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<td>d. Cyclin-dependent kinase</td>
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<td>e. Raf and mitogen-activated protein (MAP) kinase</td>
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<td><strong>Endothelial cell proliferation</strong></td>
<td>Inhibition and destruction in tumor associated microvessels through:</td>
<td>a. Angiostatin, Endostatin, Combretastatin A-4</td>
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<td>a. Inhibition of tumor angiogenesis /neovascularisation</td>
<td>and Vitaxin</td>
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<td>b. VEGF</td>
<td>c. Bevacizumab</td>
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<td></td>
<td>c. Matrix metalloproteinase inhibitors (MMPIS)</td>
<td>c. Marimistat, CGS 27023a and BAY 12-9566</td>
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<td><strong>Direct-gene therapy</strong></td>
<td>Altering the DNA sequence responsible for malignant transformation by:</td>
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<td>a. Removal of an overexpressed oncogene</td>
<td>b. Re-expression of tumor suppressor genes p53 specifically in tumor cells</td>
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<td>b. Addition of an absent tumor suppressor gene</td>
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<td><strong>Indirect gene therapy</strong></td>
<td>Targeting intermediate steps involving non-malignant host cells by:</td>
<td>a. Inserting a foreign MHC genes and some cytokines</td>
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<td>a. Gene-directed immunotherapy -stimulation of the host anti-tumor immune response by introduction of DNA sequences,</td>
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<td>Gene-directed enzyme pro-drug therapy (G-DEPT)-insertion of pro-drug targets into the tumor environment.</td>
<td>b. Aciclovir, Ganciclovir, 5-Fluorocytosine and 6-Thioxanthine</td>
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<td><strong>Epigenetic New</strong></td>
<td>a. Histone deacetylase inhibitors (HDACi)</td>
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<td><strong>epigenetic therapy</strong></td>
<td>b. DNA methyl transferase inhibitors (DNMTi)</td>
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<td>c. Histone methyl transferase inhibitors (HMTi)</td>
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<td>d. Histone demethylase inhibitors</td>
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<td>a. Vorinostat, Romidepsin</td>
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<td>b. Azacytidine, Decitabine, RG108</td>
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<td>c. 3-deazaneplanocin A</td>
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<td>d. Tranylcypromine</td>
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Some of the previous works attempted by the authors in this research area includes the synthesis of different heterocyclics such as, 2-chloro-N10-substituted phenoxazines as potential modulators in reversing multidrug resistance in cancer cells. Benzopyrone based flavone derivatives and substituted 2-quinolones, synthesised in our laboratory have also turned out as useful anti diabetic, antioxidant and anticancer agents in *in vitro* studies. The authors continue to work in the same area of research against four anticancer drug targets, namely aromatase, farnesyl tranferase, histone deacetylase and cyclin-dependent kinase. Recently, we have detailed out the mechanism behind anticancer potential of 2-quinolone derivatives and novel cinnamyl sulphonamide hydroxamate derivative. Therefore, in the light of this, it was thought to present a review highlighting the role of these targets in the development and progression of different types of cancers. Further, the targets discussed in this review will enable researchers to understand cancer biology in terms of both genetic and epigenetic pathways, and the rationale for the development of inhibitors against them.

**Aromatase**

Most of the breast cancers are hormone dependent in nature and nearly 60-70% of them are estrogen receptor (ER) and/or progesterone (PgR) receptor positive. Estrogenic pathways have been implicated for their role in the development and growth of such tumors owing to the presence of higher concentration of estrogens in them. Estrogen levels are approximately 20-fold higher in breast cancer tissues when compared to that of the plasma circulation. Thus, tapping of estrogenic signaling mechanisms using hormonal therapy has become the main approach for treatment of breast cancers.

Among the endogenous estrogens such as estrone and estradiol, estradiol is regarded as the most potent compound. The biosynthesis of estradiol from androgens (androstenedione and testosterone) in the
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Fig. 1—Schematic presentation of important signal transduction pathways in breast cancer cells. Red colours with arrow and cross mark represent site of action of inhibitors in breast cancer where drugs may target this pathway. (Ac- Acetylated, CDK- cycline dependent kinases, DNMTi- DNA methyl transferase inhibitors, FTi- Farnesyl transferase inhibitors, HDACi- histone deacetylase inhibitors, HDM- Histone demethylases, HMTi- histone methyl transferase inhibitors, mAb- monoclonal antibodies, Me- methylated, PI3K- Phosphoinositol-3-kinase, TF- transcription factors, TKi- tyrosine kinase inhibitors. mAb bind with EGFR/HER2 or HER2/HER3 receptors and blocks the downstream pathways. Activation of EGFR/HER2 by agonist results into activation of tyrosin kinase linked downstream pathway i.e, Ras/raf/MEK/ERK pathways. ERK is a member of MAPK enzyme. The inhibitor for tyrosine kinase includes imatinib. Farnesylation is an important step in Ras activation, performed by the farnesyl transferase enzyme. Specific inhibitor of this enzyme, tipifarnib inhibits the downstream pathways. Aberrant epigenetic modifications in chromatin structure by enzymes such as HDAC, HMT, HDM and DNMT, etc. plays an important role in cancer development through repression of tumor suppressor genes such as P53, Rb and RUNX3, etc. Various preclinical and clinical agents for cancer therapy include HDACi -Vorinostat, Remidepsin; DNMTi- Azacytidine, Decitabine, RT108; HMTi- deazaneplanocin and HDMI- Tranylcypromine.)

The body is mediated by cytochrome P450 enzyme complex known as “aromatase”. This enzyme is expressed in ovaries including granulosa and luteal cells and also in various other extra-glandular sites such as placenta, brain, bone, testis and adipose tissue. In the post-menopausal phase, estrogens are synthesized in extra-gonadal tissues. Under such conditions, aromatisation of adrenal androgens in muscle and fat tissues becomes the prime source of circulating estrogens in the body. Further, increased level of estrogen in tumors is a mere indication for the overexpression of aromatase near or within the vicinity of the cancerous tissue. Inhibition of aromatase and the aromatisation process overall reduces estrogen production in the body, and thereby, prevent the growth of hormone dependent cancers. Investigations on the development of aromatase inhibitors began four decades ago and have greatly expanded over the period.

Aromatase, an enzyme complex found in the cell endoplasmic reticulum belongs to the family 19, subfamily A and polypeptide 1 of the cytochrome P450 enzymes. It comprises two major proteins namely, the cytochrome P450arom, a hemoprotein that converts C19 steroids (androgens) into C18 steroids (estrogens) and the NADPH-cytochrome P450reductase, that transfers reducing equivalents to cytochrome P450arom. Aromatization of androstenedione, the preferred substrate, is achieved after three successive oxidations.

Inhibitors of aromatase (Ai), as shown in Fig. 2, are broadly classified into two categories namely, steroidal and non-steroidal inhibitors. Steroidal inhibitors, regarded as enzyme inactivators or Type I inhibitors, result in the enzyme inactivation by covalently binding to it. It includes competitive and irreversible inhibitors. Further, they interact with the substrate (androstenedione) binding sites of the
enzyme. Chemical modifications at various positions of androstenedione nucleus have led to the development of different steroidal inhibitors. Structurally, most of them are androgens and therefore, they are also likely to possess hormonal activity. Formestane and 1-methyl-androsta-1,4-diene-3,17-dione (1-methyl-ADD), are two such inhibitors commonly used to treat breast cancers.

On the contrary, non-steroidal inhibitors or the Type II inhibitors act by reversibly binding to the enzyme. Here, the inhibition may be due to the interaction between the heteroatom on the inhibitor and the heme iron of the aromatase. These inhibitors exhibit estrogen blockade based on the continuous presence of the inhibitor. The first generation inhibitor from this class, aminogluthethimide was clinically available in late 1970’s. This prototype drug was originally developed as an antiepileptic agent. However, it was later withdrawn from the market due to its serious side effects. Furthermore, this drug also failed to completely and selectively inhibit aromatase resulting in decreased efficacy. The second generation AI are represented by fadrozole, a selective and 700 times more potent drug as compared to that of aminogluthethimide. The third generation of AI are found to be more specific and selective for aromatase inhibition and are clinically available for cancer treatment. This class of inhibitors is represented by anastrozole (Arimidex), letrozole (Femara), and exemestane (Aromasin). Naturally occurring compounds are also found to exhibit effect on hormones by interacting with the ER-α and -β receptors. Phytoconstituents such as isoflavones, coumestans, lignans and their metabolites, flavonoids and stilbenoids are considered as phyto-estrogens. They bind with high affinity to the ER-β receptor that regulates differentiation and apoptosis of steroid-sensitive cells and tissues found in bones and reproductive organs, as compared to the ER-α receptor in the vasculature that regulates cellular proliferation. Several flavonoids with estrogenic activity such as rutin, catechin, apigenin, kaempferol, luteolin, chrysin and puerarin have also been extensively studied. In particular, flavones and flavanones have shown better inhibition against aromatase as compared to isoflavones. Chrysin, a flavone, is reported to be 20 fold more potent than biochanin A, an isoflavone. Further, synthetic flavonoids such as, chromones and xanthone analogues have also been explored for selective and effective inhibition of the aromatase enzyme. The rationale for designing some of the investigational synthetic molecules as AI is based on selective modifications on the existing parent drugs such as letrozole, anastrozole, and benzopyrones analogues such as isoflavones, flavanones, and coumarins.

Scope—In the treatment of estrogen dependent cancers, continuous efforts are being made to design potent aromatase inhibitors. With the advancement in medicinal chemistry and molecular endocrinology, different classes of AI are now available and are further investigated. As adjuvants in chemotherapy, AI have shown better responsive rates as compared to the mono-drug therapy with tamoxifen. With higher therapeutic potential in terms of activity and specificity, the third generation inhibitors could be useful alternatives to tamoxifen. Regulation of metabolism and maintenance of organal functions require optimum estrogen levels. Reduction in circulating estrogen level as a result of therapy with these agents may lead to skeletal problems such as bone demineralization, decreased bone density, osteoporosis and anti-implantation effects. Therefore, there is a need for the emergence of fourth generation AI with even more target specific inhibition and lower toxicity profiles.

Farnesyl transferase

The nucleus of a cell is the store house for genes responsible for proper mammalian cell functioning. Unwanted triggering of genes/proto-oncogenes is one of the key mechanisms responsible for stimulating inappropriate signal transductions, proliferations and
malignant transformations. Among several genes studied in the cancer research, an activated Ras (rat sarcoma) is the commonest oncogene present in human cancers. Activation of Ras gene leads to the expression of Ras proteins that are involved in cell signaling pathways, cell growth and apoptosis. They play role in signal transduction from tyrosine kinase receptor to several other intracellular effector pathways. Ras gene of a mammalian cell is made up of three functional subtypes namely, H-ras, K-ras and N-ras, that encodes for H-Ras, K-Ras and N-Ras proteins, respectively. These proteins help in controlling both the normal and the transformed cell growth. However, reports reveal that approximately 30% of human cancers bear mutated Ras genes. K-ras mutations are the most frequent and can be detected in nearly 90% of adenocarcinomas of colon, pancreas and lung. Alternatively, N-ras mutations are less frequent and mostly found in acute myeloid leukaemia as well as in myelodysplastic syndromes, whereas, H-ras mutations are rare but found abundantly in renal and bladder carcinomas.

Ras proteins have the ability to specifically bind to GTP in order to get activated and act as a signal transduction molecule. The conformational changes that occur then, allow interactions of Ras with other downstream signaling molecules. Further, the normal (non-mutated) proto-oncogene form of Ras acts as GTPase and thus, hydrolyses GTP to GDP, which is released along with the inactivated Ras. Mutations in this proto-oncogene decrease its ability to act as a GTPase and thus, continuous binding to GTP by mutated Ras facilitates it to act as a permanently activated signal transducer causing cell proliferation comparable to that of the normal Ras. Prior to activation of Ras as a signal transducer molecule, it has to undergo a series of post-translational modifications in the cytosol before its localization to inner surface of the cell membrane. Prenylation of Ras is the first and prime step in such modifications, involving enzyme mediated transfer of a C15 isoprenoid/farnesyli moiety from farnesyl diphasphate (FPP) to the sulphur atom of the cysteine locus of the carboxy terminal of tetrapeptide CAAX box ("C"- cysteine, "A"- leucine, isoleucine or valine, "X"- methionine or serine) of Ras. The enzyme necessary for this reaction is farnesyl transferase (FTase) and the process is termed as farnesylation. The role of activated Ras oncproteins and their farnesylation in the development and growth of various human tumors has been strongly associated with the process of initiation and maintenance of various unrestricted mitogenic signaling pathways mediated by tyrosine kinase receptor. In order to control the Ras mediated disturbances caused in cell signaling pathways, the enzyme FTase has emerged out as a useful anticancer drug target.

Various approaches to refrain the activation of Ras by inhibition of farnesylation have led to the development of different categories of farnesyl transferase inhibitors (FTi). In this direction, design of substrate inhibitors of FTase has appeared as one of the important approaches. Two substrates identified and studied extensively for this enzyme are, FPP and the CAAX tetrapeptides. Based on them, FTi are classified as FPP analogues, CAAX peptidomimetics and bisubstrate inhibitors.

The first reported active inhibitors of FTase were the FPP analogues. Compounds under this category show structural similarity with the FPP substrate. Manumycin/UCF1-C produced by Streptomyces species is one of the compounds from natural origin that has the capability to bind to FTase, but with lower affinity as compared to FPP itself. However, the synthetic derivative of FPP, (α-hydroxyfarnesyl) phosphonic acid has nearly 10 times stronger binding affinity as compared to that of FPP and also it partially inhibits H-Ras processing in cells. Benzo(f)perhydroisoindoles derivatives such as RPR 115135, RPR 130401 and PD 169451 designed mainly to target K-ras, selectively and potentially inhibit FTase.

The second category of inhibitors is the CAAX tetrapeptides and peptidomimetics that bear structural similarity to CAAX tetrapeptides and are further classified into peptide and non-peptide inhibitors. Nature of structural modifications at the AA amino acid site of CAAX determines whether, the compound is a substrate or an inhibitor. Further, modifications that bear an aromatic residue at the terminal A position makes the tetrapeptide a non-substrate inhibitor, whereas, any other modification makes CAAX tetrapeptides as an alternative substrate for FTase.

The first CAAX tetrapeptide inhibitor was CVFM and subsequently CIIM and CIFM were developed. A CVFM derivative, B581 inhibited farnesylation of H-Ras and lamin A and showed increased stability and cellular penetration. However, it failed to inhibit geranylgeranylation of other cellular proteins.
Other derivatives of CVFM that were evaluated include B956 and B1086. BZA-5B is also an extremely potent peptidomimetic methyl ester pro-drug inhibitor of FTase with a higher affinity for the same than for geranylgeranyl transferase (GGTase). L-731,735 and its methyl ester pro-drug L-731,734 are the structural derivatives of CIIM. Presence of a masked carboxylate charge makes them exhibit superior cellular penetration; however, they have shown modest potency and less chemical stability as compared to their parent compounds. Further, structural modifications on L-731,735 have led to the development of almost 1000-fold more potent L-739,750. Other peptidomimetics, developed as FTi are L-744,832, isopropyl ester pro-drug of L-739,750 and L-778,123.

Among the non-peptide CAAX peptidomimetics, 8-chlorobenzocycloheptapyridines are the non-sulphhydryl tricyclic selective inhibitors that were identified from random screening of certain antihistamines. SCH 44342, the first compound had a high specificity for FTase over GGTase I and prevented Ras induced morphological changes in malignant cells. However, it failed to show any effective antitumor activity in vivo. Other inhibitors worth mentioning in this category includes lonafarnib/SCH66336 (Sarasar), exhibiting good oral bioavailability, antitumor activity in terms of tumor regression and has shown additive effect with other cytotoxic agents such as 5-flourouracil, vincristine, cytoxan and paclitaxel. Tipifarnib/R115777 (Zarnestra) is an imidazole based oral antifungal that exhibits high specificity to FTase and has also shown effective enzyme inhibition.

The third category of FTi is bisubstrate inhibitors that have combined features of both FPP analogues and non-peptide CAAX peptidomimetics. Presence of a phosphoryl or phosphinyl linkage brings together the FPP moiety with the non-sulphhydryl CAAX tetrapeptide. BMS 186511, the methyl carboxyl pro-drug of a phosphinate inhibitor BMS 185878 has exhibited a 2000-fold higher affinity for FTase over GGTase along with good activity. BMS 184467, a phosphonate bisubstrate is another such inhibitor under this category worth mentioning.

Scope—Most of the FTi bear a cytostatic effect rather than cytotoxic effect as evident from some preclinical studies. Although FPP analogues such as manumycin and (α-hydroxyfarnesy1) phosphonic acid inhibit FTase more effectively than GGTase, it is unclear, whether the cytotoxic effects observed are due to cellular inhibition or as a result of inhibition of other enzymes such as squalene synthase and farnesyl diphosphate synthase. It has also been reported that J-104, 871, a modified squalene synthetase inhibitor selectively and effectively inhibits FTase by FPP competition mechanism. Until recently, only limited FTi have reached clinical trials such as tipifarnib, lonafarnib, BMS-214662, and L778123 (Fig. 3). However, their antitumor activity in clinical trials has been far less than anticipated. FTi fail to demonstrate specific antitumor activity in mutated Ras cancers presumably because of bypass of FTase blockade through cross-prenylation by the related enzyme such as GGTase I. In addition, certain genetic studies indicate that FTase may not be the sole enzyme responsible for oncogenic Ras activities and therefore, efforts to exploit other Ras related targets may be considered important to assess the maximum potential of FTi in clinical application. Emergence of CAAX tetrapeptides and peptidomimetics has led to potential inhibitors, but they also suffer certain shortcoming such as chemical stability and poor cellular permeability. Since then, a number of modifications of the AA amino acid location have been attempted, that have led to the development of intrinsically active agents and their corresponding pro-drugs. BMS
214662, is one of the recent non-peptide imidazole FTi showing high affinity for FTase over GGTase with complete tumor regressions in various tumor xenograft models after oral as well as intraperitoneal administration

Further, early phase II/III clinical studies have suggested a modest activity of FTi as single agent and often exhibit lower activity than that of standard cytotoxic drugs. However, certain FTi have shown to increase the effectiveness when given as adjuvant with other cytotoxic agents and thus, their concomitant use with other cytotoxic agents have revealed their immense potential in clinical development.

**Epigenetic targets**

**Histone deacetylase**

Cancer epigenetics is of growing interest and arises from the fact that, epigenetic changes are seen in virtually every stage of tumor development and progression. The nuclear epigenetic mechanisms control various important enzyme homeostasis that regulate the normal cell cycle. There are several conventional epigenetic mechanisms that are involved in the regulation of gene expression. The modifications of DNA and/or histones, without any alteration of nucleotide sequence of the genes include DNA methylation, histone acetylation, methylation, phosphorylation, ubiquitination, etc. Chromatin remodelling offers a wide range of information on gene function and disruption that leads to tumor initiation and progression. DNA methyl transferase, histone methyl transferase, histone deacetylase and histone demethylase are involved in transcriptional repression of tumor suppressor genes in cancer cells. However, restoring their balanced physiological levels in cells is quite essential for the normal activity.

Both, histone acetyl transferases (HAT) and histone deacetylases (HDAC) are important determinant for chromatin organization and gene expression. HDAC enzyme deacetylate the histone thereby, decreases the gap between the nucleosome and the DNA wrapped around the histone, leading to reduced transcription factor accessibility that produces transcriptional repression. The acetyl groups removed from histone by HDAC thereby regulates the inactive or close chromatin structure. HDAC enzyme overexpression is found in majority of liquid and solid tumors. HAT plays a role in the transfer of acetyl group to lysine N-terminal tail of histone leading to active or open chromatin structure and gene expressions. Furthermore, HAT and HDAC are also involved in biological processes such as organ development, cells proliferation, differentiation, cell cycle regulation and cell death/apoptosis. In post-translational modification of histone proteins, HAT and HDAC have opposing roles in acetylating and deacetylating highly conserved lysine residues on the N-terminal tail of histones, altering chromatin assembly and transcriptional activity. Thus, the balance between the HAT and HDAC monitors the normal cellular activity. In malignant cells, the balance between these two enzymes is disturbed towards HDAC overexpression.

The HDAC family has been classified into different subclasses such as Class I, HDAC-1, -2, -3 and -8; Class II A, HDAC-4, -5, -7 and -9; Class II B, HDAC-6 and -10 and Class IV comprising of HDAC-11 and each member performs specific biological functions. Histone deacetylase inhibitors (HDACi) are potential anticancer agents that have shown maximum cancer cell death through apoptosis, cell cycle arrest and reduction in proliferation by epigenetic or non-epigenetic regulatory mechanism. Recently, two drugs such as vorinostat (Suberoylanilide hydroxamic acid/SAHA) and romidepsin (Depsipetide) (Fig. 4), have been clinically validated in cancer patients and approved by USFDA for cutaneous T-cell lymphoma and peripheral T-cell lymphoma. Also, several clinical trials are underway for HDACi as anticancer therapeutics for use as single drug or in combination with other anticancer drugs.

HDACi have also been classified based on their mechanism of action, such as reversible inhibitors [trichostatin A (TSA) and SAHA] and irreversible inhibitors [trapoxin and depudecin] (Fig. 4). Alternatively, they are also classified based on their structure, such as short chain carboxylic acids.

![Inhibitors of histone deacetylase](image-url)
(butyrate, phenylbutyrate, phenylacetate and valproic acid); hydroxamic acids (TSA, SAHA and oxamflatin); cyclic tetrapeptides containing a 2-amino-8-oxo-9, 10-epoxydecanoyl (AOE) moiety (Trapoxin A/B); cyclic peptides without AOE moiety (FR- 901228 and apicidin); and lastly, synthetic benzamide derivates (MS-275, MGCD0103, CI-994 and N-acetyldinaline)

Scope—Presently, HDACi are under in preclinical as well as clinical trials. However, they bear certain shortcomings such as thrombocytopenia, cardiac problem and poor efficacy in solid tumors, owing to their non-selective inhibition. Vorinostat is the most advanced HDACi approved by USFDA for the treatment of advanced form of cutaneous T cell lymphoma (CTCL). Further, romidepsin is also USFDA approved for the treatment of advanced form of CTCL and peripheral T-cell lymphoma. Some of the major adverse effects associated with these inhibitors include pulmonary embolism, thrombocytopenia, granulocytopenia, prolongation of the QT-interval, asymptomatic T-wave flattening and ST-segment depression. Non-histone protein acetylation is another off-target effect observed owing to their non-selective inhibition. Though the HDAC is the most promising target for drug intervention, there is a need to design and develop selective HDACi to reduce their side effects and improve their therapeutic profile.

HDACi were initially developed as antitumor agents for cancer treatment, but many are now being explored for treating CNS, immunologic, metabolic, inflammatory and cardiovascular disorders.

DNA and histone modifications in tumor cells are responsible for the aberrant chromatin structure formation. More insight on these structures may benefit in differentiating the tumor cells from the normal cells and thereby enhance the scope for development of new drugs.

DNA methylase and cancer

DNA methylation is an epigenetic process that involves addition of a methyl group on the 5-position of cytosine termed as methylation of CpG islands by DNA methylase (DNMT1). It has several functions such as, chromatin structure modification, silencing of genes required for tumor suppression and transposable elements suppression implicated in tumor formation. In cancer, pattern of methylation changes resulting in DNA-hypermethylation that leads to genetic instability.

New epigenetic therapy has significantly improved the selectivity towards the target cancer cells as compared to that of the normal cells (broad therapeutic index) in cancer patients. Combination of DNA demethylating agents and agents that maintain histone acetylation levels with the conventional anticancer agents bear a potential for improved treatment of haematological malignancies as well as solid tumors. Further, aza-nucleotides such as azacitidine and decitabine (Fig. 5) degrade the DNMT by incorporation into the DNA during replication leading to the trapping of DNMT in the process. The RG108 (Fig. 5), blocks the catalytic pocket of free DNMT.

Histone methyl transferase and cancer

Histone methyl transferases (HMT) are histone-modifying enzymes that transfer methyl groups to specific lysine and arginine residues of histone (H3 and H4) proteins. Methylation of histones by HMT (lysine N-methyltransferase and arginine N-methyltransferase) is an epigenetic modification of chromatin that regulates gene expression, genomic stability, stem cell maturation and cell mitosis. In recent years, epigenetic modification including methylation and demethylation in both DNA and histone proteins, mainly at methylating positions on histones is an emerging research area in anticancer drug development. Abnormal expression or activity of HMT is associated with various human cancers such as colorectal, ovarian and lung cancer, signifying the association between histone methylation and tumors development. One such inhibitor, 3-deazaneplanocin A (Fig. 6), under this class is in preclinical investigations.
Histone demethylase and cancer

Histone demethylases is another important enzyme in epigenetic modifications, as they are responsible for the removal of methyl groups from histone\(^87\). They play an important role in transcriptional regulation of the genome\(^88\). Recently, it has been found that histone demethylase also has key role in tumor development\(^89,90\). The successful development of DNA methylation and HDACi as anticancer therapeutics for specific cancer has paved the way for the development of newer epigenetic agents. Further, histone lysine demethylase enzyme inhibitors are in the trend in anticancer drug development. Tranylcypromine (Fig. 7), a non-selective irreversible inhibitor of monoamine oxidase (MAO) clinically used for mood and anxiety related disorders, is one of the few preclinical drugs from this class under investigations as anticancer agent. It inhibits the histone demethylase BHC110/LSD1, resulting in the suppression of transcriptional activity of BHC110/LSD1 target genes and thereby, prevents tumor cell proliferation\(^91\).

Tyrosine kinase

The growth factor signaling modulation has emerged out as one of the crucial pathways for targeting deregulated cellular processes\(^92\). Protein tyrosine kinases (PTK) are the ATP-consuming enzymes that regulate cellular process such as cell differentiation, proliferation and apoptosis under normal as well as diseased conditions\(^93,94\). The process of phosphorylation of tyrosine molecules is a critical step involved in cell signaling pathway that leads to activation of various growth factors. Approximately, 80% of oncogenes and proto-oncogenes responsible for human cancers code for PTK leading to their malfunction and overexpression\(^95\). An abnormal PTK activity is reported to be associated with different malignancies as well as with some of the non-malignant disease conditions such as psoriasis, papilloma, restenosis, and pulmonary fibrosis and inflammation\(^95\). The Human Genome project identified approximately 600 protein kinases that were subdivided based on their catalytic enzyme specificity as tyrosine specific (Tyr), serine/threonine specific (Ser/Thr) and specific for both Tyr and Ser/Thr\(^96,97\).

Tyrosine kinase, a class of enzyme has been classified into receptor protein kinases and non-receptor protein kinases\(^92\). Receptor tyrosine kinases are membrane-spanning cell surface proteins that bear tyrosine kinase catalytic site and perform transduction of extracellular signals. More than 50% of these mutated or overexpressed kinases are found to be associated with human malignancies\(^93,98-100\). On the contrary, non-receptor tyrosine kinases regulate intracellular signaling pathways. The process of cellular activation of tyrosine kinase is initiated with ligand binding that induces dimerization of receptor tyrosine kinases, followed by autophosphorylation of their cytoplasmic domains and activation of tyrosine kinase activity. Further, activation of numerous other cytoplasmic signaling pathways along with intracellular mediators perform signal transduction from membrane receptors through the cytosol into the nucleus that results in altered DNA synthesis and other biological processes like cell division, growth, migration, differentiation and death\(^92\).

The drug discovery program in the late 1980’s led to the emergence of the first competitive inhibitors against these kinases called as the tyrphostins or the inhibitors of tyrosine phosphorylation (TKi)\(^101\). At present, all TKi bind to the ATP binding sites of the target PTK. Thereafter, two main classes of small molecular weight non-ATP inhibitors were generated namely, substrate competitive inhibitors and mixed competitive inhibitors. Non-competitive ATP inhibitors are another class of TKi that include allosteric inhibitors developed against drug resistance BCR-ABL (a point mutation within the ATP binding domain)\(^94\).

The search for inhibitors against tyrosine kinases began with the epidermal growth factor receptor (EGFR) as the prototype TKi target. Research
suggests that EGFR protein mutations resulting in their overexpression have been observed in many tumors such as that of breast, ovaries and kidneys. However, their overexpression is more conspicuous in non-small cell lung cancer (NSCLC)\textsuperscript{92,94}. Furthermore, overexpression of EGFR kinase is considered as a hallmark in most of the epithelial cancers\textsuperscript{95}. An activated EGFR also acts as a stimulator for vascular endothelial growth factor (VEGF), the primary inducer of angiogenesis\textsuperscript{102}. EGFR kinase family consists of four trans-membrane growth factor receptors HER1 (c-ErbB-1/EGFR); HER2 (c-ErbB2/neu); HER3 (c-ErbB3); and HER4 (c-ErbB4) that bear structural similarities in terms of an extracellular ligand-binding domain and a cytoplasmic tyrosine kinase domain\textsuperscript{103}. Some of the approaches to inhibit EGFR activity are directed towards either blocking the extracellular ligand binding domain of the receptor using anti-EGFR antibodies or by inhibition of downstream EGFR cellular pathway using small molecules inhibitors of EGFR tyrosine kinase\textsuperscript{104}. A few prominent inhibitors against this target includes gefitinib (Iressa) and erlotinib, FDA-approved EGFR kinases inhibitors (Fig. 8); lapatinib, a EGFR/Her-2 dual kinase inhibitor; neratinib, an irreversible EGFR kinase inhibitor; two monoclonal antibodies against EGFR such as cetuximab and panitumumab; and two anti-Her-2 monoclonal antibodies, trastuzumab and pertuzumab (all FDA-approved)\textsuperscript{94}. The second tyrosine kinase target is vascular endothelial growth factor (VEGF) that plays a vital role in neo-vascularization process such as angiogenesis and metastasis\textsuperscript{93}. VEGF regulates the vascular proliferation and permeability and is secreted by almost all solid tumors and also by tumors associated with hypoxia and macrophages\textsuperscript{92}. Overexpression of this growth factor has been reported to be closely associated with increased microvascular density, cancer recurrence and decreased survival\textsuperscript{105}. Therefore, inhibition of VEGF induced angiogenic signals has become an approach to selectively target tumor-associated vessels and also tumor-induced edema\textsuperscript{106}. So far, there are six established ligands for VEGF receptor (VEGFR) namely, VEGF A-E and placenta growth factor. The angiogenic signals are transmitted through cell surface receptors by means of ligand binding to the specific receptors located on the host vascular endothelium mostly, VEGFR-1 (Flt-1), VEGFR-2 (FLK-1/KDR) and -3 that possess intracellular tyrosine kinase activity. Response to VEGFR-2 induces endothelial cell proliferation, permeability and survival, whereas, VEGFR-3 is one of the most probable pathway to mediate lymphangiogenesis. Other important ligand-receptor interactions include binding of VEGF-A to VEGFR-1 leading to endothelial cell migration and the binding of VEGF to VEGFR-2 receptors resulting in activation of intracellular tyrosine kinase domains and triggering of intracellular signaling cascade\textsuperscript{92}. Some of the inhibitors designed and investigated against this class include semaxinib, a non-selective receptor TKi of VEGFR-2, c-KIT, and FLT-3 and the first VEGFR inhibitor to be tested clinically; vatalanib, a selective inhibitor VEGFR-1 (Flt-1) and VEGFR-2 (FLK-1/KDR); sorafenib, a dual inhibitor targeting Raf kinase and VEGFR; and sutent, a multikinase inhibitor of VEGFR, PDGFR, c-KIT, and FLT-3\textsuperscript{92}. The third target in the tyrosine kinase family is platelet-derived growth factor (PDGF). It is reported to play a role in the cellular process of growth, proliferation and differentiation. PDGF works via signaling of PDGF tyrosine kinase receptor (PDGFR) located on the cell surface. Activation of this receptor and its overexpression are one of the factors that trigger unregulated mitotic signals. Two types of PDGFR that have been identified are, -α and -β\textsuperscript{107}. Overexpression of PDGFR and PDGF has been reported in glioblastoma and other solid tumors\textsuperscript{106}. Research reveals that some of the selective PDGFR kinase inhibitors namely, AG 1295, AG 1296 and AGL 2043 possess strong anti-restenosis effects when tested in in vivo model for restenosis\textsuperscript{94,95}. Leflunomide, an immunomodulatory agent mostly used to treat active rheumatoid arthritis, is also a small molecule inhibitor of PDGFR, inhibiting PDGF-mediated cell signaling\textsuperscript{108}.
One among the most prominent inhibitors developed as TKi is imatinib (STI571, Glivec/Gleevec) shown in Fig. 8, that paved the way for discovery of target specific inhibitor based on rational design\(^{109}\). In early 2000, this molecule was approved by USFDA in USA and Europe for the treatment of various stages of chronic myelogenous leukemia (CML), a hematological disorder resulting in excessive myeloid proliferation\(^{10}\). CML condition is the consequence of oncogenic events that leads to the expression of BCR-ABL fusion proteins. The reciprocal translocation of the shortened version of chromosome 22 (Philadelphia chromosome/Ph) bearing the \(BCR\) gene and chromosome 9 bearing \(ABL\) tyrosine kinase gene leads to the fusion of \(BCR\) and \(ABL\) genes that gives rise to the \(BCR-ABL\) oncogene\(^{110}\). Further, the molecular consequence of this event led to the expression of two forms of protein tyrosine kinases namely, p190 (BCR-ABL) and p210 (BCR-ABL). As a result, the increased tyrosine kinase activity is observed in the form of deregulation of intracellular signaling, increased proliferation and resistance to apoptosis of hematopoietic stem or progenitor cells resulting in the substantial increase in myeloid cells\(^{102}\). The presence of this cytogenic abnormality is considered as a marker for the identification of patients suffering from myeloid leukemia owing to its presence in approximately 95\% of patients suffering from CML and nearly 15-30\% of adults suffering from acute lymphoblastic leukemia (ALL), as reported\(^{106,110}\).

Identification and understanding of cancer etiology specifically in CML cells as compared to normal cells, has led to the selection of BCR-ABL tyrosine kinase as the most suitable drug target for designing drugs against this disease.

Imatinib, was initially identified from the random screening as a protein kinase C-\(\alpha\) inhibitor (PKC-\(\alpha\)). However, as a result of continuous efforts in the lead optimization of this phenylamino pyrimidine derivative, a selective inhibition of BCR-ABL tyrosine kinase was observed by this compound. Docking and crystallography revealed that it inhibits ABL kinase by binding to its inactive form with high specificity. Further, studies also revealed the binding of this inhibitor to the ATP-binding domain in kinase. The simplicity of the lead structure, non-complex chemical synthetic route provided a platform to perform varied structural modifications on it that led to the clear understanding of the effect of substitutions at different positions of the lead structure. The consolidation of these studies generated the structure-activity relationship that made important revelations that the 3′-pyridyl group at 3′-position of the pyrimidine led to strong PKC inhibition; the presence of an amide group on the phenyl ring led to inhibitory activity against other kinase such as BCR–ABL tyrosine kinase; and the key finding that the substitution at 6-position of the diaminophenyl ring in form of a “flag-methyl” group led to the complete abolishment of PKC activity. On the contrary, it led to the retention and enhancement of activities of other tyrosine kinases. The outcome of further lead modifications on this system initiated towards the improvement of safety and efficacy resulted in the structure of most promising compound STI571/imatinib, selected as the investigational compound against BCR-ABL tyrosine kinase. Later, results from \textit{in vitro} screening demonstrated inhibition of autophosphorylation of some of the additional protein kinases by this compound such as receptor for stem-cell factor (SCF)-c-KIT, the PDGF receptor and more recently inhibition of Abelson-related gene (\(ARG\) or \(ABL2\)) kinase activity\(^{106,109}\).

Alternatively, the resistance towards imatinib therapy has been documented in nearly 33\% of patient population that mediated through various resistance mechanisms and in approximately 20\% of patients who did not achieve complete cytogenic response (CCyR) with the imatinib treatment\(^{110}\). Two possible pathways for imanitib resistance identified include the BCR-ABL dependent and BCR-ABL independent mechanisms. BCR-ABL-dependent mechanism comprise of BCR-ABL point mutations occurring frequently in more than 50\% of relapse cases, resulting in altered binding affinity of imatinib to BCR-ABL tyrosine kinase target and oncogene amplification. On the other hand, BCR-ABL independent mechanism comprises of kinetics and dynamic factors such as drug efflux/import/binding. Some of the other mechanisms to explain resistance include deregulations in alternative signaling pathways such as Ras/Raf/MEK kinase, STAT, Erk2 and SRC family kinase (SFK)\(^{109,112}\). The limitations of imatinib therapy have led to the emergence of second generation TKi such as dasatinib and nilotinib that are more potent than imatinib. These drugs have been approved as first line treatment for CML-chronic phase (CML-CL) and also for imatinib-resistant/-intolerant CML in both chronic as well as acute.
phase. Dasatinib is a dual SRC and ABL kinase inhibitor, binds to the active and inactive conformation of the ABL kinase domain. Other drugs noteworthy to be mentioned include nilotinib, which is active against many imatinib-resistant BCR-ABL mutations and also it inhibits c-Kit and PDGFR. Bosutinib is a dual SRC/ABL TKi with minimal inhibition of PDGFR and c-KIT. Ponatinib, is also a powerful pan-BCR-ABL TKi undergoing investigation as treatment for patients who fail therapy with imatinib, dasatinib and nilotinib.\(^{(110,113)}\)

Scope—The clinical success and effectiveness of TKi therapy in the treatment of different malignancies provide direction to the designing of target specific therapy in oncology. Imatinib is one of the prototype inhibitor, introduced for the first time in clinical oncology from this class of inhibitors. TKi are also reported to have beneficial effects in inflammatory diseases and non-cancerous proliferative conditions, therefore, their combination with immunotherapy is also a promising therapeutic area.\(^{(95)}\) Though, targeted therapies with TKi proved efficacious in treating different malignancies, yet they suffer in achieving sufficient therapeutic benefits in patients. Certain limitations of TKi therapy that need to be addressed include mutation induced drug resistance, lack of tumor response and off-target effects.\(^{(110,113)}\) For TKi to occupy a major role in clinical anticancer therapy, studies could focus upon recognition of the molecular phenotype of tumors and also, monitoring of target inhibition in tumor response during treatment.\(^{(92)}\)

**Cyclin-dependent kinase**

One of the interesting developments in cancer research during the 90’s has been elucidation of molecular mechanisms involved in cell cycle i.e., the control progression of cells through different cell cycle phases. The cell cycle mechanisms through which cells divide is a sequential and tightly regulated process involving four phases namely, G1, S, G2 and M.\(^{(114,115)}\) Cyclin-dependent kinases (CDK) are a set of kinases that act as one the major cell cycle regulators. As deregulated cell cycle progression is considered to be a hallmark of cancer cell,\(^{(116-117)}\) consequent to these observations, CDK occupied a prime position as anticancer target.

CDK are the serine/threonine protein kinases that regulate the cell cycle progression through different cell cycle phases and are dependent on cyclins (regulatory subunits) for their activation.\(^{(118)}\) Unlike the cyclin levels in a cell that vary according to the cell cycle phase, the levels of CDK remain constant in a cell.\(^{(119)}\) Further, binding to cyclin is a limiting step in their activation and phosphorylation at active site of threonine residue is an essential requirement for their activity.\(^{(120)}\) The formation of cyclin-CDK complex activates the CDK active site. The phosphorylation at threonine-14 or tyrosine-15 site leads to its inactivation/deregulation while phosphorylation at threonine-161 around the T-loop leads to its activation.\(^{(121)}\) In response to different stimuli, the heterodimeric complexes phosphorylate various substrates involved in the transcription control and cell cycle progression.\(^{(118)}\) Formation of the cyclin-CDK complex is itself regulated by phosphate and kinase molecules like CDK-activating kinase (CAK) such as, Cdc25 and Wee-1 kinase. However, CDK can also get activated by certain non-cyclin CDK activators such as, CDK5 activators, viral cyclins and RINGO/Speedy.\(^{(119,122)}\) Frequent overexpression of positive regulators (cyclins) and subsequent inactivation of CDK inhibitors might be a contributing factor for the increased CDK activity as reported in cancer cells.\(^{(123)}\) So far, 9 CDKs (CDK1–CDK9) and various cyclins (cyclin A–cyclin T) have been reported.\(^{(119,124)}\) The CDK3, CDK4 and CDK6 are implicated in regulation of G1-S phase transition; CDK2 is associated with the entry into S phase and replication of DNA; while CDK1 is crucial for mitosis phase.\(^{(119)}\) CDK activity in cells is governed by four mechanisms namely, binding of cyclin proteins for activation, inhibition of activity by cyclin-dependent kinase inhibitors (CKI), conserved residues phosphorylation at ATP-binding pocket of CDK (for its inhibitory activity) and phosphorylation at a conserved residue of CDK T-loop (for its activation).\(^{(122)}\) Mechanisms of CDK inhibition in mammalian cells reveals that, the CKI either binds to the CDK itself or to the CDK-cyclin complex. Such inhibitors are small polypeptides that regulate and control the activity of CDK-cyclin complexes and are categorized into two families namely, INK4 and Cip/Kip. The former includes p15 (INK4b), p16 (INK4a), p18 (INK4c), p19 (INK4d) that inactivates G1 phase CDK (CDK4 and CDK6) by binding strongly with it and prevents further complex formation with Cyclin D. The latter category includes p21 (Waf1, Cip1), p27 (Cip2), p57 (Kip2) that exhibits broad spectrum inhibitory effect on cyclin-CDK complexes. They inhibit G1 phase of CDK-cyclin complexes and to a lesser extent the CDK1-Cyclin B complexes.\(^{(126-128)}\)
Earlier report also reveals the association of an altered CDK activity with the development of viral infections, neurodegenerative disorders such as Alzheimer’s disease and proliferative diseases such as renal diseases and cancers\textsuperscript{129}. Continuous pursuits for drugs that inhibit CDK have been a strong area of research for last four decades, and so far, numerous CDK inhibitors (CDKi) have been identified as pan-CDKi or selective CDKi\textsuperscript{130}. Pan-CDKi such as Flavopiridol and (R)-roscovitine /CY-202 (Fig. 9), are among the first-few compounds that have undergone numerous phase II and III clinical trials. Flavopiridol (Alvocidib)/HMR-1275/L-868275/NSC-649890 is a novel semi-synthetic flavone analogue of rohitukine, a plant based alkaloid from \textit{Dysoxylum binectariferum}\textsuperscript{131}. It inhibits CDK1,-2, -3 and -4, causes both G1 and G2 cell cycle arrest and induce apoptosis in some cases\textsuperscript{124}. It is reported to have cytotoxic activity against a wide range of cancer cell lines and was also the first CDKi in early clinical trials for the potential treatment of renal, prostate, gastric and colon cancer as well as non-Hodgkin's lymphoma\textsuperscript{132}. (R)-roscovitine/CYC202 (Seliciclib) is another potent oral inhibitor of CDK2/cyclin E, CDK 1/cyclin B, CDK7/cyclin H, and CDK9/cyclin T1. It acts as a suppressor to genes that inhibit apoptosis and has shown \textit{in vitro} activity against a range of tumors as single drug\textsuperscript{124,133,134}. Second generation CDKi are being used in clinical trials and advanced preclinical phases. They have been found to be consistently more potent and druggable as compared to the first generation molecules (Flavopiridol and CY-202). They are categorized as inhibitors with a broad CDK activity profile (against CDK-1, -2, -4, -6, -7 and -9) such as, SNS-032, AG-024322 and R-547, inhibitors with exclusive/preferential CDK4/6 or CDK2 activity such as, AT-7519 (CDK2) and PD-0332991/Palbociclib. Lastly, inhibitors with activity against CDK as well as additional kinases include ZK-304709\textsuperscript{129}.

Other CDKi undergoing clinical trials include bryostatin-1, a macrocyclic lactone that induces p21 and inactivates CDK2, undergoing clinical trials in combination with other chemotherapeutic agents\textsuperscript{135,136}, AZD5438, a novel CDKi with preclinical activity against a range of human tumor xenografts\textsuperscript{124}; indisulam (E7070), a synthetic sulphonamide targeting the G1 phase by depleting cyclin E, inducing p53 and p21 and inhibiting cdc2 phosphorylation\textsuperscript{137,138} and LY2835219 and LEE011, a CDK4/6 inhibitor that inhibit phosphorylation of the retinoblastoma protein in the early G1 phase and block progression to S phase\textsuperscript{139}.

Scope—CDKi bear the potential to become successful anticancer therapeutics owing to the strong mechanistic supporting their use. However, they are yet to get commercially exploited\textsuperscript{139}. Inhibitors including flavopiridol and CYC-202 are undergoing numerous clinical trials to prove their efficacy and safety in cancer patients as single agents and in combination with other chemotherapeutic agents. However, their efficacy is quite modest when administered as mono-drug therapy whereas, in combination with other cytotoxic agents, they have shown the potential to improve cytotoxic efficacy and overcome drug resistance\textsuperscript{124,132,140}. Despite their effectiveness as cytotoxic agents, still there remains much scope for development of third generation analogues of the existing CDKi in order to improve their pharmacokinetic profile addressing adverse effects and non-selectivity\textsuperscript{132}. Further, the pharmacodynamic approaches show that optimisation of their dose and dosing regimen is also crucial for exhibiting the maximum therapeutic effect\textsuperscript{124}. Studies have also shown that increased activation of certain regulatory cellular proteins and CAKs may contribute in understanding the cancer pathogenesis in a better way. The ATP and the non-ATP competitive inhibitors are the newer emerging areas in anticancer therapy\textsuperscript{117,119}. Moreover, to improve the efficacy of treatments in different tumors, there is a need to understand the type of CDK expression that play role in such oncogenic process and therefore, this strategy could be of use in selecting suitable CDKi against specific types of cancers\textsuperscript{139}.  

Fig. 9.—Inhibitors of cyclin-dependent kinase
Monoclonal antibodies

Monoclonal antibodies (mAb) have been developed in laboratory and engineered as such to attach to specific antigen or protein markers on cancer cells. They mimic antibodies that are produced naturally as part of immune system's response to foreign antigen. Modern recombinant techniques have made it possible to rapidly produce chimeric antibodies, humanized antibodies and total human antibodies\(^{141}\).

Three decades ago, cancer therapy though successful, did not meet with tumor cell specificity and suffered treatment related difficulties\(^{142}\). It was then anticipated that, agents that possess better tumor cell specificity would pave the way for new generation therapeutics, a breakthrough in cancer research. At the same time, evidences from several animal and clinical studies revealed the potential use of monoclonal antibodies as one of the major treatment modalities in cancer.

It was in 1997, a biological therapeutic agent, rituximab appeared for the first time as the first monoclonal antibody approved for treatment in different types of lymphomas and chronic lymphocytic leukaemia. Currently, USFDA has approved several mAb products for cancer therapy\(^{143}\). At present, many of the mAb–based biologic drugs are under different phases of clinical trials\(^{144,145}\).

The mAb are relatively a newer class of therapeutic agents used for treating cancer and have gained much attention in innovative drugs market over the past decade. Several of such drugs are currently available for treating various types of cancers (Table 2)\(^{146,147}\).

### Cancer stem cells (CSCs)

Cancer stem cells (CSC) were first identified in leukaemia in 1997\(^{148}\). Presently, attention has been drawn towards cancer initiation and progression based on CSC. This is supported by the evidence that all cancer cells are clonal in origin and originates from a single cell. Their presence has been further strengthened by their existence in tumors of various organs\(^{149}\). These cells have the ability of self-renewal and multi-lineage differentiation that leads to initiation and maintenance of tumor\(^{150}\). Other cancer cells, except CSC, do not have tumorigenic property. Chemotherapy causes tumor regression and leaves behind the rare CSC. Further, these cells bearing the tumorigenic property lead to relapse in cancer patients. Hence, the present therapy needs to focus upon eradicating the tumorigenic CSC\(^{151}\). Various targets have been proposed based on the properties of stem cells such as, self-renewal pathways, receptors for tumorogenesis (aryl hydrocarbon receptor, growth factors receptors and co-receptors), signaling

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