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AN OVERVIEW ON THE CYCLIN-DEPENDENT KINASE 9-RELATED PATHWAY

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SUMMARY

The cyclin-dependent kinase 9 (CDK9)-related pathway is a key factor in the regulation of mammalian cell biology. Its main role consists of regulating transcription via phosphorylation of the RNA polymerase II carboxyl-terminus domain (CTD), which in turn stabilizes the elongation of nascent RNA. CDK9 is also involved in the replication program of different types of viruses, such as HIV-1 and HIV-2, human T-lymphotropic virus type 1 (HTLV-1), Epstein–Barr virus, human cytomegalovirus and herpes simplex virus 1 (HSV-1). A deregulated CDK9-related pathway is also associated with various types of human cancers and cardiac hypertrophy. On these grounds, the CDK9-related pathway may be considered a suitable therapeutic target for the treatment of cancer, cardiac hypertrophy and the aforementioned viral diseases. This review describes the complex biology of the CDK9-related pathway and the status of drug development for the targeting of CDK9 and other CDKs in cancer therapy.

INTRODUCTION

Cyclin-dependent kinase 9 (CDK9) was isolated from a human cDNA library and characterized in the mid-1990s and belongs to the family of the CDC2-like serine/threonine kinases (1). In most cases, CDKs, such as CDK1, CDK2, CDK3, CDK4, CDK6 and CDK11, regulate the cell cycle (2). CDK5 is involved in senescence (2), suppression of cell cycle re-entry in post-mitotic neurons (3), neuronal migration into the granule cell layer (3, 4), the formation of dendrite extensions and synapses (3, 4), neuronal survival (3) and neuronal cell death (3, 5). In contrast to other CDKs, CDK5 mainly relies on the non-cyclin partners p35 and p39 (3-5). CDK7 regulates RNA polymerase II (pol II)-mediated transcription and also functions as a CDK-activating kinase (CAK) (2), whereas CDK8 and CDK9 are mainly focused on RNA pol II transcription regulation (1, 2, 6-12). The mechanisms of CDK-mediated transcriptional control of RNA pol II will be described in greater detail in the next section, along with the list of factors that interact with the CDK9-related pathway (1, 7, 8, 13-39). CDK10 modulates its own transcriptional activity via association with the C-ets-2 transcription factor and regulates the G_2-M cell cycle phase (40, 41). In addition to cell cycle regulation, CDK11 is involved in the control of transcription and pre-mRNA splicing (2).

The biological activity of CDKs requires the noncovalent association with certain classes of cyclins. This association generates a heterodimer in which the CDK contains the catalytic domain while the cyclin partner operates as a regulatory subunit (1, 2, 6-12). CDKs and their cyclin partners are listed in Table I, and an example of a CDK9/cyclin T1 heterodimer is shown in Figure 1. Briefly, cyclins A, B, C, D and E and their corresponding CDK partners regulate the eukaryotic cell cycle, which consists of the following phases: Gap 0, or G_0 (this is the resting, or senescent, state); Gap 1, or G_1 (state of interphase; at this stage cells increase in size and are predisposed to synthesize DNA); Synthesis, or S (state of interphase; DNA synthesis takes place); Gap 2, or G_2 (state of interphase; this is the stage between DNA production and mitosis, in which the size of cells continues to increase); Mitosis, or M (state of cell division) (42, 43). The expression level of cyclins A, B and E varies with cell cycle progression (44). Conversely, cyclins C, H and T exhibit stable expression levels during the various phases of the eukaryotic cell cycle (45). This is consistent with the fact that the heterodimers CDK7/cyclin H, CDK8/cyclin C and CDK9/cyclin T regulate RNA pol II-mediated transcription instead of cell cycle progression (1, 2, 6-12).

The CDK9-related pathway is a key player in the regulation of mammalian cell biology (1, 2, 6-39). Its wide range of functions include the control of RNA pol II-mediated transcription, co-transcriptional mRNA processing, regulation of chromatin modification, and cell differentiation of muscle cells and B and T lymphocytes (1, 2, 6-11, 13-39). In addition, the CDK9/cyclin T1 complex plays an important role

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Table I. *Functions of cyclin-dependent kinases (CDKs) and their binding partners in mammalian cell biology.*

Class of CDK	Main binding partners (secondary binding factors are reported in parentheses)	Cellular factors that interact with the heterodimer	Functions of the heterodimer in mammalian cell biology	References
CDK1	A1; A2; B1; B2; (E; B3)	Cdc28-dependent kinase subunit (CKS)	G_2-M (cell cycle)	2, 11, 12
CDK2	A1; A2; E1; E2 (D1; D2; B1; B3)		G_1-S (cell cycle)	2, 11, 12
CDK3	E1; E2; A1; A2; C	E2F/dimerization partner (DP)	G_0-G_1-S (cell cycle)	2, 11, 12
CDK4	D1; D2; D3	MyoD	G_1-S (cell cycle)	2, 11, 12
CDK5	p35; p39 (cyclins D; E; G)		Senescence; inhibition of cell cycle re-entry in post-mitotic neurons; neuronal migration; formation of dendrite extensions and synapses; neuronal survival; neuronal death	2-5
CDK6	D1; D2; D3		G_1-S (cell cycle)	2, 11, 12
CDK7	H	RNA polymerase II	Transcription; CDK-activating kinase (CAK)	2
CDK8	C (K?)	RNA polymerase II; SMAD	Transcription	2, 6-11, 28
CDK9	T1; T2a; T2b; K	RNA polymerase II; MyoD; p53; pRb; hSPT5, c-Myc; SkiP; SMAD;STAT3; TRAF2; BRD4; NF- B; SUPT5H; NELF-E; UBE2A; E12/E47 (members of the basic helix-loop-helix family); HEXIM1; HEXIM2; 7SK snRNA; p300/GATA-4, HIV-1 and HIV-2 Tat protein; HTLV-1 Tax protein; EBV EBNA-2; hCMV UL69; HSV-1 ICP22 and ICP27; gp130*; HSP70*; HSP90*; Cdc37* (*these factors form a transient association with the monomeric CDK9)	Transcription; co-transcriptional mRNA processing; regulation of chromatin modification; cell differentiation (B and T lymphocytes, muscle cells); protection from apoptosis	1, 2, 6-11, 13-38, 39, 69
CDK10	Unidentified	C-ets-2	Transcription; G_2-M (cell cycle)	2, 13, 14
CDK11	L1; L2; (D)	RNA polymerase II; RanBPM; RNPS1; CK II; 14-3-3; 9G8; Elf-3; NOT2; MYST2	Transcription; pre-mRNA splicing; M (cell cycle)	2

in the replication of HIV-1 and HIV-2, human T-lymphotropic virus type 1 (HTLV-1), Epstein–Barr virus (EBV), human cytomegalovirus (hCMV) and herpes simplex virus 1 (HSV-1) (7, 15, 16, 21-26).

Interestingly, several studies have shown that dysfunctions in the CDK9-related pathway are associated with many types of human cancers and cardiac hypertrophy (2, 12, 45-55). A number of drugs have been developed to inhibit CDKs in the context of cancer therapy (2, 10-12, 56, 57). In this respect, alvocidib (flavopiridol) and seliciclib (CYC-202 or [*R*]-roscovitine) inhibit CDK9, along with other protein kinases, and have been used in clinical trials for the treatment of tumors for a number of years. Indeed, the CDK9-related pathway may be considered a proper target for the treatment of various types of cancer, cardiac hypertrophy, acquired immunodeficiency syndrome (AIDS) and other viral-induced diseases.

This review describes the biology of the CDK9-related signaling system, the effects associated with the deregulation of this pathway and the status of drug development for CDK inhibition in cancer therapy.

BASIC BIOLOGY OF THE CDK9-RELATED PATHWAY IN MAMMALIAN CELLS AND VIRAL REPLICATION

CDK9 is present in all types of human and murine tissues and reaches high levels of expression in terminally differentiated cells (6, 58, 59). There are two CDK9 isoforms in mammalian cells, which have been termed CDK9-42 and CDK9-55 (60-62). The numbers 42 and 55 are in reference to the apparent molecular weight of the two CDK9 isoforms, as visualized on Western blot assay and in immunoprecipitation experiments (1, 6, 58-62). The CDK9-55 isoform contains 117 additional amino acid residues at the amino-terminus region of CDK9-42 (60). The existence of two CDK9 isoforms derives from the structural organization of the human CDK9 promoter region, which contains two transcription starts (60, 61). The human CDK9 promoter domain that encodes for CDK9-42 mRNA lacks a functional TATA box and includes a GC-rich sequence. The region –352 to –1 comprises the necessary transcriptional components to ensure full promoter activity and the transcription start is situated 79 nucleotides upstream of the ATG initiation codon. On these grounds, the human CDK9-42 promoter achieves high levels of constitutive

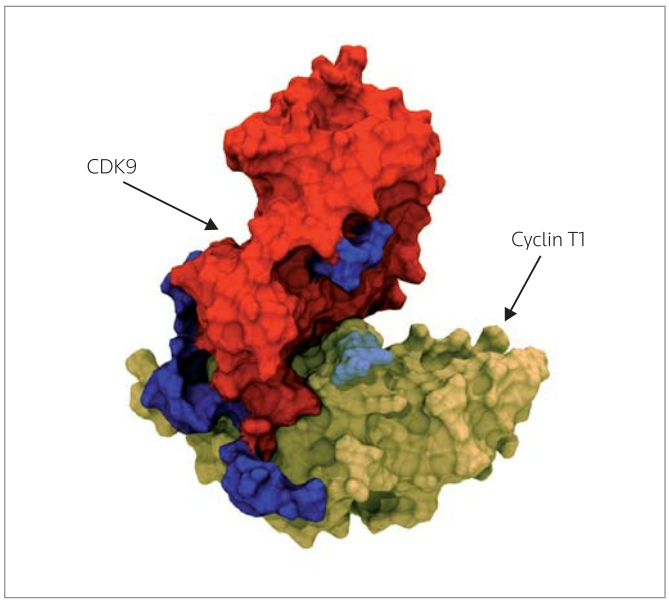

Figure 1. Crystal structure of the heterodimer CDK9/cyclin T1. CDK9 and cyclin T1 are not covalently bound to each other. This figure was obtained from the protein database (pdb).

activity and bears a resemblance to a housekeeping gene promoter (63). Conversely, the human CDK9-55 promoter has a TATA box, which is located roughly 500 bp upstream of the human CDK9-42 mRNA transcription start (60, 63).

CDK9-55 is predominantly present in the cell nucleus (61), whereas CDK9-42 is expressed both in the cell nucleus and cytoplasm (20). Furthermore, the two CDK9 isoforms exhibit substantial diversity in terms of tissue distribution in human and murine models. CDK9-42 is preferentially expressed in testis and spleen, whereas CDK9-55 is preferentially expressed in brain, lung and liver tissues (60, 61). The two CDK9 isoforms also show differential expression patterns in human and murine cell culture models. CDK9-42 is predominantly expressed in human primary undifferentiated monocytes (44), the human cervical carcinoma HeLa cell line (59) and murine NIH/3T3 fibroblasts (60). In contrast, CDK9-55 expression prevails following induced differentiation of human primary monocytes into macrophages (44), but the lipopolysaccharide (LPS)-mediated stimulation of human macrophages or HIV-1 infection changes the ratio in favor of CDK9-42 expression (60). Also, the activation of primary human lymphocytes causes a substantial decrease in CDK9-55 expression levels, and CDK9-42 becomes predominant. Interestingly, rat hepatocytes mainly express CDK9-55, but CDK9-42 overcomes CDK9-55 in primary cell cultures (44). Overall, the expression of the two CDK9 isoforms is differentially regulated according to the cell type and cell signaling system. In addition, CDK9-55 appears to be involved in the control of the cell differentiation program in a variety of tissues such as muscle (14, 54, 58), the hematopoietic compartment (44, 60, 61) and adipogenesis following in vitro differentiation of the murine 3T3-L1 cell line (64).

The two CDK9 isoforms associate either with type T cyclins or cyclin K to produce a heterodimer, which is the main component of the positive transcription elongation factor b (P-TEFb) (7, 30, 44, 46, 47, 60, 65). The cyclin T family includes three members: cyclin T1, T2a and T2b (2, 13). Type T cyclin expression is constant through the different phases of the cell cycle (44, 45). However, a considerable increase in cyclin T1 and CDK9 expression levels has been detected following the activation of quiescent human T lymphocytes by means of various stimuli, such as phytohemagglutinin (PHA) and/or phorbol myristate acetate (PMA) (16, 67, 68). An enhancement in cyclin T1 and CDK9 expression levels was also reported in antigen-challenged memory B cells (8, 37, 68, 69). Taken together, these observations indicate that tissue-specific signaling systems affect the regulation of cyclin T1 expression.

P-TEFb regulates transcription through the phosphorylation of the RNA pol II carboxyl-terminus domain (CTD) and its kinase activity is specifically inhibited by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (44, 60). The function of the P-TEFb complex consists of stabilizing the elongation of RNA pol II-mediated transcription (7, 46, 47, 65). RNA pol II per se has the ability to commence RNA transcription in the context of the early phases of transcription complex assemblage, but fails to promote elongation of the nascent RNA transcript (7, 46, 48). The inability of RNA pol II to stabilize nascent RNA transcript elongation is due to the interaction with the negative transcription elongation factor (N-TEF) (46, 48). Thus, the addition of P-TEFb to the transcription complex has a twofold purpose: the removal of N-TEF from the transcription complex and the phosphorylation of the CTD of RNA pol II, which, at this juncture, is capable of stabilizing elongation of the nascent RNA transcript (1, 6, 7, 46, 48). The human RNA pol II CTD has 52 replicates of the heptapeptide $Y_1S_2P_3T_4S_5P_6S_7$ (49-51). The unphosphorylated form of RNA pol II CTD is tightly associated with the components of the pre-initiation complex, which comprises the TATA-binding protein (TBP) and the mediator complex. The CDK7-mediated phosphorylation of Ser5 decreases the binding affinity between RNA pol II CTD and other factors of the pre-initiation complex. Subsequently, the capping enzyme binds to Ser5-phosphorylated CTD and the RNA capping structure is added to the nascent RNA transcript when it is roughly 25-30 nucleotides long. However, Ser5 phosphorylation of RNA pol II CTD is not sufficient for stabilization of the nascent RNA transcript elongation, which also requires CDK9-mediated phosphorylation of Ser2 of the CTD heptapeptide (49).

The chaperone proteins HSP70, HSP90 and Cdc37 play an important role in stabilizing the monomer CDK9 prior to its association with the cyclin partners T1, T2a, T2b and K (29). The heterodimers CDK9/cyclin T and CDK9/cyclin K are fairly stable, in contrast to the monomer CDK9, which tends to be degraded rather fast (20). For this reason, monomer CDK9 molecules must form a transient complex with chaperone proteins (29).

Besides phosphorylation of the RNA pol II CTD, the CDK9-related pathway is interrelated with several other cellular and viral factors (Table I), such as p53, MyoD, pRb, hSPT5, c-Myc, SkiP, SMAD, STAT3, TRAF2, BRD4, NK-κB, NELF-E, UBE2A, E12/E47, HEXIM1, HEXIM2, 7SK snRNA, HIV-1 and HIV-2 Tat protein, EBV EBNA-2, hCMV UL69, HSV-1 ICP22 and ICP27, gp130, HSP70, HSP90 and Cdc37 (1, 2, 6-11, 13-39). As anticipated, monomer CDK9 binds transiently to the chaperone proteins HSP70, HSP90 and Cdc37 (29), and it also binds to the cytoplasmic region of gp130, which is the receptor for the

interleukin-6 (IL-6) family of cytokines. This finding suggests a possible role for CDK9 in mediating IL-6-related signal transduction (20).

In addition to RNA pol II-mediated transcription regulation, the CDK9-related pathway is involved in the control of co-transcriptional mRNA processing, chromatin modification, cell differentiation, protection from apoptosis and activation of quiescent B and/or T lymphocytes (Table I) (1, 2, 6-11, 13-39, 70). Indeed, the CDK9-related pathway has emerged as a key component in the control of the differentiation program in the hematopoietic system (44, 60, 61), muscle tissue (13, 14, 54, 58), neurons (53) and adipogenesis (64). Interestingly, the inhibition of the CDK9-related pathway via retroviral-encoded dominant negative CDK9 (dn-CDK9) resulted in enhanced susceptibility to apoptosis both in human U-937 promonocytic and human Jurkat T cell lines (70). A similar finding was observed in another study, in which the overexpression of dn-CDK9 induced apoptosis in human monocytes, particularly after PMA-mediated activation (20).

THE CDK9-RELATED PATHWAY AND CARDIAC HYPERTROPHY

The increased size of terminally differentiated cardiomyocytes causes cardiac hypertrophy, which in turn results in several forms of cardiovascular disorders. Diminished heart function or hypertension may lead through different mechanisms to cardiac hypertrophy. For instance, diminished heart function is associated with hemodynamic stress, whereas hypertension typically induces biomechanical stress (55). In either case, there is an increased expression of those factors that promote transcription, translation and cell survival (71). Initially, cardiac hypertrophy has a beneficial response that allows the organism to adapt to the pathological stress that derives from the cardiovascular disorder (55, 72). However, cardiac hypertrophy may ultimately cause heart failure (72). In this scenario, necrotic cell death and apoptosis have a substantial role (71). Interestingly, the inhibition of cardiac hypertrophy resulted in decreased morbidity and mortality in patients with cardiovascular diseases (73).

The increase in both mRNA and protein expression levels may be considered a hallmark of cardiac hypertrophy. Therefore, the regulators of transcription P-TEFb and TFIIH play a substantial role in the growth of cardiomyocytes. CDK9 activity is silent both at the protein and transcriptional level in normal human cardiomyocytes (55, 71, 72). CDK9 transcriptional silencing is induced by muscle-specific microRNA-1 (miRNA-1) (74), whereas the activity of CDK9 protein is neutralized through the association with either HEXIM1-7SK or HEXIM2-7SK in the human model (55, 75, 76); the murine homologue for HEXIM1-7SK is also termed cardiac lineage protein 1 (77). Preclinical studies in the murine system clearly demonstrated that the activation of CDK9 and cyclin T1 expression resulted in cardiac hypertrophy. In addition, the persistent expression of CDK9 in murine cardiomyocytes caused mitochondrial anomalies and apoptosis, which in turn induced heart failure (78). On these grounds, the CDK9-related pathway may represent a suitable target for the treatment of cardiac hypertrophy-associated cardiovascular disorders.

DEREGULATION OF THE CDK9-RELATED PATHWAY IN MALIGNANT CELLS

As already pointed out, the CDK9-related pathway is an important regulator of mammalian cell biology (1, 2, 6-11, 13-39, 70). Not surprisingly, aberrations in the CDK9-related pathway may be involved in the establishment and/or maintenance of a malignant cell phenotype (9, 45, 52, 64, 79-81). In this context, particular emphasis may be placed on the antiapoptotic properties of CDK9 and its cyclin partners (9, 20, 45, 52, 64, 70, 79-81). For instance, a deregulated CDK9 pathway may stimulate the expression of myeloid leukemia cell differentiation protein (Mcl-1) (82-84). In addition, abnormal patterns of protein phosphorylation are often associated with a variety of pathological conditions, such as cancer, neurological diseases, diabetes, viral infections and inflammation (10-12, 85). Previous studies reported deregulation of the CDK9-related pathway in various human malignancies, such as lymphomas (9, 52, 86), rhabdomyosarcoma (54), primary neuroectodermal tumor (53, 80), neuroblastoma (53, 80) and prostate cancer (79). High levels of CDK9 and cyclin T1 protein expression were found in follicular lymphomas, B- and T-cell precursor-derived lymphomas and anaplastic lymphoma. Moreover, disparities in CDK9 and cyclin T1 mRNA levels were observed in Burkitt's lymphoma, Hodgkin's lymphoma, follicular lymphoma and diffuse large B-cell lymphoma (52). These findings underline the relevance of deregulation of the CDK9-related pathway in the establishment and/or maintenance of human malignancies. Thus, the deregulated CDK9-related pathway can be considered a suitable target for cancer therapy.

THE STATUS OF PROTEIN KINASE INHIBITOR DEVELOPMENT

The pharmacological inhibition of CDKs has emerged as a promising tool for the treatment of cancer in recent years (2, 10-12). Most of these inhibitors target the adenosine triphosphate (ATP) binding site of the CDK enzymatic domain (87-91). A number of CDK inhibitors have been evaluated in phase I and/or phase II clinical trials for a wide variety of tumors, which are listed in Table II. For instance, alvocidib and seliciclib have been tested in phase II clinical trials for the treatment of several malignancies and constitute the first generation of CDK inhibitors. However, alvocidib and seliciclib exhibited modest clinical efficacy in patients (2, 10-12).

First-generation CDK inhibitors are characterized by broad anti-CDK activity, simultaneously targeting CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 (2, 10-12). Besides alvocidib and seliciclib, the first generation of CDK inhibitors comprises SNS-032 (or BMS-387032), dinaciclib, R-547 (or RO-4584820) and AG-024322 (2, 10-12, 87). The second-generation CDK inhibitors are directed against a more restricted pool of CDKs, such as CDK4, CDK6 and/or CDK2 (2, 10-12), and include AT-7519, PD-0332991 and P-276-00 (2, 10-12, 92, 93). The third-generation CDK inhibitors target various CDKs and other protein kinases in order to optimize their efficacy against tumors. In this respect, ZK-304709 targets CDK1, CDK2, CDK4, CDK7, CDK9, vascular endothelial growth factor receptors VEGFR-1, VEGFR-2 and VEGFR-3, platelet-derived growth factor receptor β (PDGFR-β) and FLT3. JNJ-7706621 also belongs to the third-generation CDK inhibitors and is directed against CDK1, CDK2, CDK3 and Aurora-A/B, and GPC-286199 inhibits CDK1, CDK2, CDK3, CDK5, CDK7, CDK9 and CDK-related kinases (Table II) (2, 10-12, 87).

Table II. *List of cyclin-dependent kinase (CDK) inhibitors used in clinical trials for the treatment of cancer (http: www.clinicaltrials.gov).*

CDK inhibitor	Alias(es)	Generation	Main kinase activity	Clinical trials for cancer
Alvocidib	Flavopiridol	I	CDK1, 2, 4, 6, 7, 9; GSK-3β	Phase I/II for various types of cancers, such as multiple myeloma, leukemia, lymphomas, sarcoma and solid tumors
Seliciclib	CYC-202; (*R*)-roscovitine	I	CDK1, 2, 5, 7, 9; CK I; GSK-3α; MNBH; ERK-1	Phase I/II for non-small cell lung cancer (NSCLC) and other solid tumors
SNS-032	BMS-387032	I	CDK1, 2, 4, 7, 9	Phase I/II for B-cell malignancies, NSCLC, advanced breast cancer and melanoma
R547	RO-4584820	I	CDK1, 2, 4, 7	Phase I for advanced solid tumors
Dinaciclib	SCH-727965	I	CDK1, 2, 5, 9	Phase I/II for various solid tumors, acute myelogenous leukemia, acute lymphoblastic leukemia, mantle cell lymphoma and B-cell chronic lymphocytic leukemia
AG-024322		I	CDK1, 2, 4, 7	Phase I for non-Hodgkin's lymphoma and advanced solid tumors
AT-7519		II	CDK2, 4, 5, 9; GSK3β	Phase I/IIa for advanced and/or metastatic solid tumors and refractory non-Hodgkin's lymphoma
PD-0332991		II	CDK4, 6	Phase I for non-Hodgkin's lymphoma and other malignancies
P-276-00		II	CDK1, 4, 9	Phase I/II for multiple myeloma and various advanced refractory malignancies
RGB-286638		II	CDK1, 2, 4, 5, 7, 9	Entering a phase I clinical trial for the treatment of advanced solid tumors
PHA-690509		–	Pan-Aurora inhibitor	Preclinical testing for solid tumors
ZK 304709		III	CDK1, 2, 4, 7, 9; VEGFR-1, -2, -3; PDGFR-β; FLT3	Phase I trials for refractory and/or relapsed solid tumors
JNJ-7706621		III	CDK1, 2, 3; Aurora-A/B	Preclinical stage
GPC-286199	RGB-286199	III	CDK1, 2, 3, 5, 7, 9; CRKs	Preclinical stage

CONCLUSIONS

The CDK9-related pathway is a major regulator of mammalian cell biology and viral replication. It has been reported that deregulation of this pathway is associated with the establishment and/or maintenance of a malignant cell phenotype. In addition, the activation of CDK9 expression in cardiomyocytes is responsible for cardiac hypertrophy, which in turn may result in cardiovascular disorders. On these grounds, the development of more effective CDK9 inhibitors may indeed find useful application for the treatment of several types of cancers, cardiac hypertrophy-associated cardiovascular disorders, AIDS and other viral-related diseases.

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DISCLOSURES

The author states no conflicts of interest.

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