

## Targeting Cell Division Cycle 7 Kinase: A New Approach for Cancer Therapy

Alessia Montagnoli, Jürgen Moll, and Francesco Colotta

### Abstract

The cell division cycle 7 (Cdc7) is a serine-threonine kinase, originally discovered in budding yeast, required to initiate DNA replication. Human Cdc7 phosphorylates the minichromosome maintenance protein 2 (Mcm2), a component of the DNA replicative helicase needed for genome duplication. Inhibition of Cdc7 in cancer cells impairs progression through S phase, inducing a p53-independent apoptotic cell death, whereas in normal cells, it does not affect cell viability. Small molecule compounds able to interfere with Cdc7 activity have been identified and shown to be effective in controlling tumor growth in animal models. Two Cdc7 inhibitors are currently in phase I clinical development. Inhibition of Cdc7 kinase activity in cancer cells restricts DNA replication and induces apoptotic cell death by an unprecedented molecular mechanism of action. *Clin Cancer Res*; 16(18); 4503–8. ©2010 AACR.

### Background

#### DNA Replication

Eukaryotic DNA replication is a tightly regulated process, which ensures that no origins fire more than once per cell cycle, in order to maintain genomic stability. It consists of two major steps: initiation of DNA replication followed by elongation of nascent DNA strands (1).

DNA replication initiation requires the assembly of the prereplicative complex (pre-RC) at sites in the genome called origins, the activation of the pre-RC, and the formation of the replisome, which is ultimately responsible for the duplication of DNA that occurs during the elongation step (2).

The origin recognition complex (ORC) marks the origin and initiates the sequential loading of the components of the pre-RC, including cell division cycle 6 (Cdc6; ref. 3), Cdt1, and the six minichromosome maintenance proteins (Mcm2-7) in late mitosis and early G1 (4). Mcm2-7 is the catalytic core of the replicative helicase that is specifically recruited by Cdc6 and Cdt1 through direct protein-protein interactions and ATP hydrolysis (5, 6). In cells, the formation of an active helicase at replication origins requires MCM phosphorylation and the loading of additional factors, including Cdc45 and the GINS complex. Once activated, Mcm2-7 unwinds double-stranded DNA at the origin to generate the single-

stranded DNA (ssDNA) template required to recruit the remaining DNA synthesis machinery (i.e., RPA, DNA polymerases) and to start chain elongation. The activation process is triggered by the S phase-promoting kinase Cdc7 that, together with cyclin-dependent kinase 2 (Cdk2), phosphorylates components of the MCM complex and stimulates the helicase activity by allowing the interaction between Cdc45, Mcm2-7, and Go-Ichi-Nii-San (GINS; Fig. 1; refs. 7, 8).

#### Cell division cycle 7 kinase

The gene encoding Cdc7 kinase was first isolated in budding yeast (9), whereas the human homolog was identified almost two decades later (10). In vertebrates, Cdc7 activation requires the interaction with either one of the regulatory subunits Dbf4 (11, 12) or Drf1 (13), whose binding to the kinase is mutually exclusive. In *Xenopus*, Drf1 seems to be the predominant activator of Cdc7 during early embryogenesis, whereas Dbf4 plays a more prominent role in somatic cells (14). In human cells, Dbf4 and Drf1 accumulate in S phase and regulate Cdc7 activity during the cell cycle, although the specific role of the two regulators has not yet been fully elucidated (15). Other proteins regulate formation of the complex at the transcriptional level; whereas *Dbf4* is an E2F-regulated gene, Cdc7 gene expression is directly regulated by the transcription factor Myb (16) and is repressed by Cdc7 expression repressor (CR)/periphilin (17).

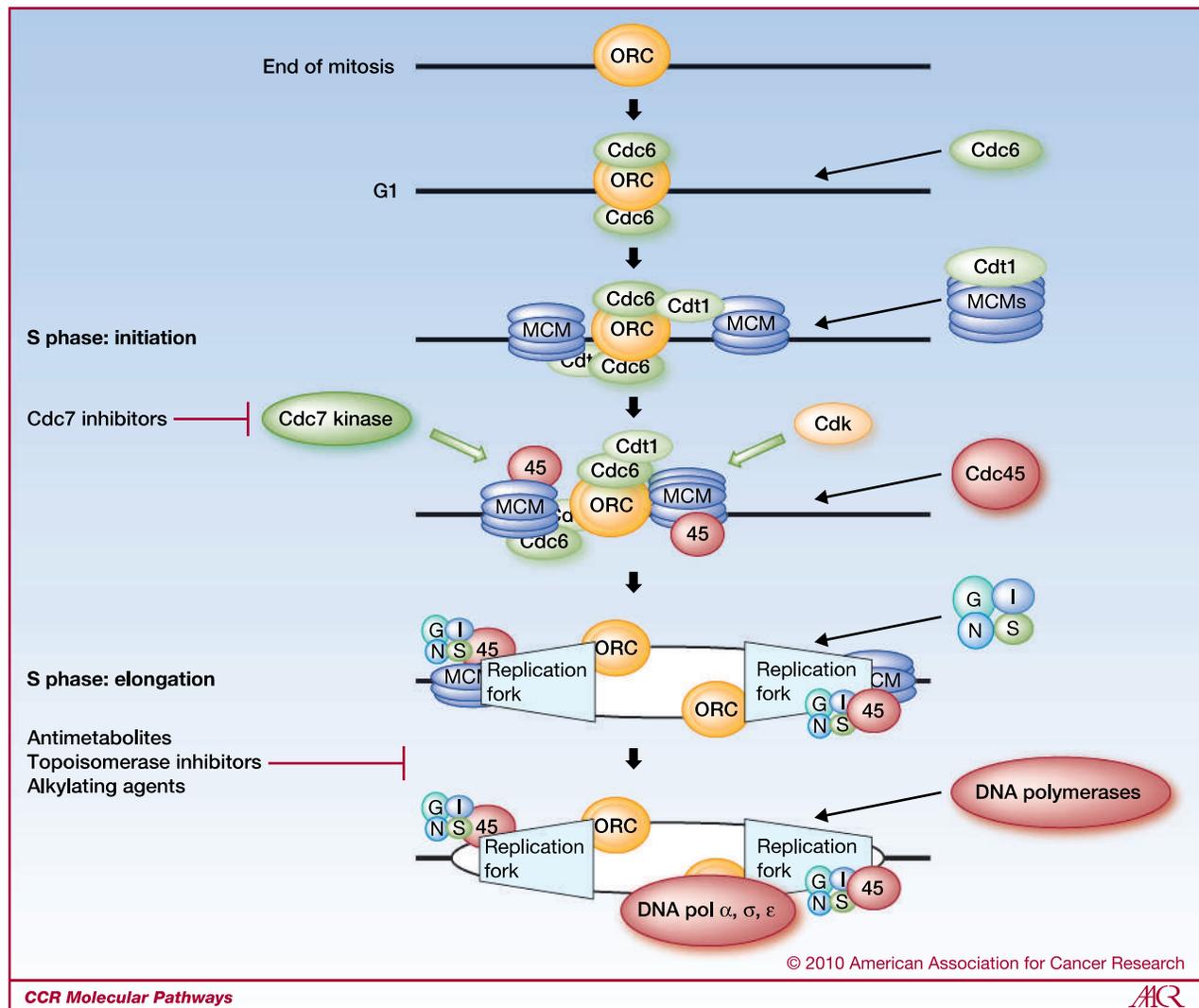
The major target of Cdc7 activity in mammalian cells is the MCM complex. Cdc7 phosphorylates chromatin-bound Mcm2 at the G1-S transition, and this phosphorylation is required for the initiation of DNA replication but not for Mcm2 loading on DNA (18–20). Another cellular target of Cdc7 activity is LEDGF, the immunodeficiency virus cofactor known to be functionally associated

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doi: 10.1158/1078-0432.CCR-10-0185

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**Fig. 1.** DNA replication in eukaryotic cells. Eukaryotic DNA replication is initiated by binding of ORC to replication origins. ORC marks the origins and initiates the sequential loading of Cdc6 and Cdt1 proteins during the G1 phase of the cell cycle. With the cooperation of Cdc6 and Cdt1, the six subunits of the replicative helicase MCM are also delivered at the origins of replication, generating a pre-RC. The MCM complex is loaded at origins in an inactive form and is then activated during S phase, in a manner that requires both Cdc7 and CDK activities. The formation of an active helicase is associated with the recruitment of additional factors, such as Cdc45 and the four subunits of the GINS complex, which is also dependent on Cdc7 kinase activity. The helicase activity allows the unwinding of the double strands and the establishment of the DNA replication forks. After initiation, with the help of DNA polymerases, replication proceeds bidirectionally away from origins until each fork encounters another fork coming from a neighboring origin. This step of the DNA replication is called "elongation." Anticancer agents, such as antimetabolites, topoisomerase inhibitors, and alkylating agents, block with different mechanisms this final step of DNA synthesis, whereas Cdc7 inhibitors act on the initiation step.

with transcriptionally active genomic loci (21). The interaction with LEDGF strongly stimulates Cdc7 activity and may provide a link between transcription and DNA replication.

In addition to its well-established role in S phase, Cdc7 controls chromosomal segregation in mitosis by phosphorylating the heterochromatin protein 1 (Hp1). Hp1 promotes cohesion of sister chromatids in mitosis specifically at centromeres (22). When sister chromatids are trapped into the cohesin ring, mitosis is blocked until DNA replication is completed (23).

### Cell division cycle 7 inhibition

The cellular effects of Cdc7 depletion have been studied by antibody microinjection (24), and more recently by RNA silencing (25), showing that in human cells, Cdc7 is essential for DNA replication. In cancer cells, lack of Cdc7 results in progression through a defective S phase that leads to p53-independent apoptotic cell death. It has been shown that the lack of Cdc7 affects neither exit from mitosis, nor G1-S progression, but produces cells bearing incompletely and/or abnormally replicated DNA, which can result in cell death. Some cancer cells undergo a

relatively rapid apoptosis, whereas others survive longer but ultimately die in mitosis with partially replicated chromosomes. The apoptotic response induced in cancer cells by Cdc7 depletion is not mediated by p53, but is activated by the stress-activated protein p38MAPK in an ATM- and Rad3-related (ATR)-dependent manner (26).

Generally, treatment with inhibitors of DNA replication, including antimetabolites (e.g., fludarabine, fluorouracil, cytarabine, gemcitabine), topoisomerase inhibitors, and intercalating (e.g., anthracyclins, topotecan, etoposide) and alkylating agents (e.g., cisplatin, temozolomide), results in DNA strand breakage and activation of the Chk1/Chk2-dependent checkpoint pathways that sense the damage and prevent cell-cycle progression and DNA synthesis. This cellular response, activated by aberrant DNA replication and DNA damage in S phase, is known as the S-phase replication checkpoint, which suppresses further DNA replication and stabilizes stalled replication forks to ensure proper restart after DNA repair (27). The pathway activated by replication blocks requires ATR and Chk1, which are important for determining cell fate upon such insults. Differently from other known DNA replication inhibitors, Cdc7 inhibition blocks DNA replication and induces apoptosis without eliciting the activation of the Chk1-dependent pathway. There is, in fact, growing evidence indicating that Cdc7 kinase activity is itself necessary for the activation of the S-phase checkpoint (28, 29). In particular, in human cells, we and other groups have shown that, in the presence of inhibitors of DNA replication such as topoisomerase inhibitors or hydroxyurea, Cdc7 is an active kinase that plays an essential role in mediating the ATR-Chk1 pathway by phosphorylating the Chk1 activator Claspin (30, 31). As a result of its double functions in DNA replication and DNA damage response, Cdc7 inhibition leads to apoptotic cell death, which does not occur with other inhibitors of DNA replication. It has been speculated that the lack of checkpoint activation in Cdc7-depleted cells may reflect an insufficient accumulation of ssDNA regions that are generally recognized by the sensor protein ATR. However, a study by Im and colleagues (26) shows that, upon Cdc7 depletion, ATR undergoes normal activation, whereas Chk1 does not, indicating that events resulting in lack of activation of the checkpoint pathway lie downstream of ATR.

A consequence of Cdc7's role in S-phase checkpoint is that Cdc7 inhibition, in the presence of genotoxic drugs, strongly increases the number of cells that enter the apoptotic pathway. This observation suggests that a combination of drugs that impair DNA replication, such as topoisomerase inhibitors or DNA-intercalating agents together with a Cdc7 inhibitor, may have a synergistic effect in killing tumor cells, opening possibilities for such combinations in future clinical studies.

What is the effect of Cdc7 inhibition on normal cells? Different studies show that Cdc7 depletion does not elicit apoptotic cell death in normal human fibroblasts, suggesting that in normal cells, a cell-cycle checkpoint mechanism that responds to Cdc7 depletion exists. Lack of Cdc7

causes a cell-cycle arrest at the G1-S boundary with unrepliated DNA, elevated p53 protein levels, and induction of the cyclin-dependent kinase inhibitor p21. As a consequence, Cdc7 depletion does not induce apoptotic cell death in normal human fibroblasts. Induction of p53 by Cdc7 depletion in normal cells has a key role in cell-cycle arrest and lack of cell death. By downregulating p53 in arrested cells, cell-cycle blockade can be completely bypassed, and the cells can progress through an aberrant S phase leading to apoptosis. The selective cytotoxic effect on cancer cells, which is a common and unique feature of inhibition of proteins involved in the initiation of DNA replication (32, 33), suggests that Cdc7 might be an attractive target for the development of drugs that kill proliferating malignant cells but spare actively replicating normal cells. The exact mechanisms accounting for the differential response on cancer cells have not yet been completely elucidated. We envisage that, in untransformed cells, inhibition of Cdc7 may trigger a "licensing checkpoint," leading to a block of DNA replication and reversible cell-cycle arrest that is lost in cancer cells. In particular, tumors that already harbor defects in G1-S checkpoint-related proteins might be more sensitive to the effects of a Cdc7 inhibitor. For example, tumors carrying defects in the p53 or Rb pathway, displaying low levels of tumor suppressors p27, p21, or p16 or overexpressing cyclins D or E, may be more sensitive to Cdc7 inhibition.

In summary, Cdc7 depletion impairs different cellular processes that cover regulation of DNA synthesis, chromosome segregation in mitosis, and the control of the DNA damage response.

### Cell division cycle 7 overexpression

As reported for other proteins involved in the initiation of DNA replication, Cdc7 and its protein regulator Dbf4 are overexpressed in human cancer cell lines and in many primary tumors compared with matched normal tissues (34). Overexpression of Cdc7 in ovarian cancer correlates with tumor anaplasia, aneuploidy, and advanced clinical stage. The greatest discriminatory effect of Cdc7 expression was observed for stage 3 and 4 ovarian tumors, which make up the vast majority of epithelial ovarian carcinoma cases (35). Cdc7 and Dbf4 are overexpressed in malignant melanoma compared with benign melanocytic nevi, and Dbf4 overexpression has been associated with lower relapse-free survival (36, 37). In diffuse large B-cell lymphoma, increased Cdc7 activity is associated with poor clinical outcome (38). High Cdc7 levels were also found in breast cancers analyzed by tissue microarray. Here, high expression levels are significantly related to a medullary histotype, high tumor grade, negative estrogen receptor status, and the amplification of a number of genes including c-myc and HER2 (39). A role of Cdc7 protein in promoting gene amplification has been described in other organisms, but the underlying mechanism for this phenomenon still needs to be explored. A strong staining intensity and high percentage of positive cells showing

Mcm2 phosphorylation, both indicators of high Cdc7 kinase activity, were found in particular in skin basaloma, laryngeal squamous cancer, serous ovarian cancer, in the majority of breast tumors, uterine tumors, urothelial bladder cancer, diffuse large B-cell non-Hodgkin's and Hodgkin's lymphomas, and in colon adenomas and adenocarcinomas.<sup>2</sup>

Because Cdc7 is required for DNA replication, higher Cdc7 levels might be linked to the proliferative capacity of tumor cells. However, increased Cdc7 expression levels do not always correlate with the proliferative status of cells, whereas they correlate with the percentage of cells in S phase.<sup>2</sup> Accordingly, Cdc7 and Dbf4 overexpression in cells causes cell-cycle arrest in S phase (40). An intriguing hypothesis is that, with Cdc7-Dbf4 being implicated in the DNA damage response, increased Cdc7 activity may aid recovery or repair of stalled replication forks to enhance survival of certain tumor cells.

Somatic mutations in CDC7 have been identified in colorectal and gastric carcinomas through comprehensive kinome screens of human tumors (41), although the biological consequences of these mutations have not yet been evaluated.

These findings suggest that alterations in Cdc7/Dbf4 protein abundance or activity during tumorigenesis may have important consequences for cell survival, underlining the potential of Cdc7 as an anticancer target with a new mechanism of action.

### Clinical-Translational Advances

The first Cdc7 kinase ATP-competitive small-molecule inhibitors, belonging to the 2-heteroaryl-pyrrolopyridones chemical class, were identified at Nerviano Medical Sciences Srl by high-throughput screening using a biochemical kinase assay (42–44).

Further lead optimization resulted in the selection of NMS-1116354, which entered phase I clinical trials in 2009 for solid tumors with a treatment schedule of 7 or 14 consecutive days every 2 or 3 weeks with oral administration (45). NMS-1116354 causes inhibition of DNA replication and p53-independent apoptosis during S phase in cancer cells. Additionally, the compound is able to reduce the expression of the anti-apoptotic proteins Mcl-1 and XIAP. This latter finding could be considered as an additional opportunity to increase the apoptotic response in particular tumor settings (46–48). The compound shows significant antitumor single-agent activity, with tumor regression in different preclinical models of solid tumors after oral administration without overt toxic effects. Mcm2 phosphorylation is also modulated *in vivo*, supporting its use as a pharmacodynamic biomarker in the tumors and/or surrogate tissues of patients.

In collaboration with Exelixis, Bristol Myers Squibb (BMS) is developing a benzofuroprimidinone Cdc7

inhibitor (XL-413/BMS-863233), which entered phase I-II clinical trials in 2009 in solid tumors and hematologic malignancies, with an oral treatment schedule of 7 or 14 days per 21- or 28-day cycle. BMS-863233 is a potent ATP-competitive inhibitor of Cdc7 kinase with some cross-reactivity against PIM-1 kinase. The mechanism of action of the compound was confirmed by following the effects on DNA replication and on Mcm2 phosphorylation. The compound is orally available and produces antitumor activity in a colon cancer xenograft model, when given daily for 2 weeks (49).

In addition to Nerviano and BMS, other companies, such as Novartis (50) and Sanofi-Aventis (51), have described low nanomolar Cdc7 inhibitors, but no further data are available for these compounds. A potent Cdc7 inhibitor with antitumor activity in a model of Philadelphia chromosome-positive acute leukemia was identified by high-throughput screening at the Memorial Sloan-Kettering Cancer Center and is reported to be in preclinical development (52).

Even if the toxicologic data for these compounds are not disclosed yet, the nature of the dose-limiting toxicity for many cell-cycle inhibitors is hematologic. However, preclinical data suggest that Cdc7 inhibition induces tumor-specific effects. These data might be explained by the combined effect of replication fork arrest and deficient checkpoint responses, which induce aberrant cell-cycle progression and death in tumor cells, whereas normal cells can survive through execution of back-up checkpoint mechanisms.

As a consequence, differently from other compounds that inhibit cell proliferation, a Cdc7 inhibitor is expected to have a wide therapeutic window. Data derived from animal models strongly support this hypothesis because an excellent antitumor efficacy as single agent is achieved without overt toxicity, using an optimized schedule of treatment.<sup>2</sup>

The fact that sustained inhibition of Cdc7, in the presence of compounds inducing DNA damage, increases cell death *in vitro* might suggest the use of Cdc7 inhibitors not only as single agents, but also in combination with other chemotherapeutics such as topoisomerase inhibitors and alkylating agents.

In conclusion, preclinical data show that Cdc7 is a novel and promising target for tumor-cell killing, as has been shown with different inhibitors. Ongoing clinical trials will reveal whether inhibition of this kinase represents a successful strategy that will bring benefits to cancer patients in terms of superior activity and/or better tolerability compared with the currently approved agents that affect DNA replication.

### Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to declare.

Received 05/03/2010; revised 06/23/2010; accepted 06/30/2010; published OnlineFirst 07/20/2010.

<sup>2</sup> A. Montagnoli and J. Moll, unpublished data.

## References

1. Bell SP, Dutta A. DNA replication in eukaryotic cells. *Annu Rev Biochem* 2002;71:333–74.
2. Takeda DY, Dutta A. DNA replication and progression through S phase. *Oncogene* 2005;24:2827–43.
3. Coleman TR, Carpenter PB, Dunphy WG. The *Xenopus* Cdc6 protein is essential for the initiation of a single round of DNA replication in cell-free extracts. *Cell* 1996;87:53–63.
4. Mendez J, Stillman B. Chromatin association of human origin recognition complex, Cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. *Mol Cell Biol* 2000;20:8602–12.
5. Tanaka T, Knapp D, Nasmith K. Loading of an mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. *Cell* 1997;90:649–60.
6. Randell JC, Bowers JL, Rodriguez HK, et al. Sequential hydrolysis by Cdc6 and ORC directs loading of the Mcm2-7 helicase. *Mol Cell* 2006;21:29–39.
7. Im JS, Ki S, Farina A, et al. Assembly of the Cdc45-Mcm2-7-GINS complex in human cells requires the Ctf4/And-1, RecQL4, and Mcm10 proteins. *Proc Natl Acad Sci U S A* 2009;106:15628–32.
8. Francis LI, Randell JC, Takara TJ, et al. Incorporation into the prereplicative complex activates the Mcm2-7 helicase for Cdc7/Dbf4 phosphorylation. *Genes Dev* 2009;23:643–54.
9. Hartwell LH, Culotti J, Pringle JR, et al. Genetic control of the cell division cycle in yeast. *Science* 1974;183:46–51.
10. Jiang W, Hunter T. Identification and characterization of a human protein kinase related to budding yeast Cdc7p. *Proc Natl Acad Sci U S A* 1997;94:14320–5.
11. Jackson AL, Pahl PM, Harrison K, et al. Cell cycle regulation of the yeast Cdc7 protein kinase by association with the Dbf4 protein. *Mol Cell Biol* 1993;13:2899–908.
12. Kumagai H, Sato N, Yamada M, et al. A novel growth- and cell cycle-regulated protein, ASK, activates human Cdc7-related kinase and is essential for G1/S transition in mammalian cells. *Mol Cell Biol* 1999;19:5083–95.
13. Montagnoli A, Bosotti R, Villa F, et al. Drf1, a novel regulatory subunit for human Cdc7 kinase. *EMBO J* 2002;21:3171–81.
14. Takahashi TS, Walter JC. Cdc7-Drf1 is a developmentally regulated protein kinase required for the initiation of vertebrate DNA replication. *Genes Dev* 2005;19:2295–300.
15. Yoshizawa-Sugata N, Ishii A, Taniyama C, et al. A second human Dbf4/ASK-related protein, Drf1/ASKL1, is required for efficient progression of S and M phases. *J Biol Chem* 2005;280:13062–70.
16. Thalheim LB, Shetzline SE, Cezary R, et al. Transcriptional regulation of the intra-S-phase regulator CDC7 in human myeloid leukemic cells: a new downstream target of the c-Myb protooncogene [abstract]. *Blood* 2005;106:1354.
17. Kurita M, Suzuki H, Masai H, et al. Overexpression of CR/periphilin downregulates CDC7 expression and induces S-phase arrest. *Biochem Biophys Res Commun* 2004;324:554–61.
18. Masai H, Matsui E, You Z, et al. Human Cdc7-related kinase complex. *In vitro* phosphorylation of MCM by concerted actions of Cdk5 and Cdc7 and that of a critical threonine residue of Cdc7 by Cdk5. *J Biol Chem* 2000;275:29042–52.
19. Montagnoli A, Valsasina B, Brotherton D, et al. Identification of Mcm2 phosphorylation sites by S-phase-regulating kinases. *J Biol Chem* 2006;281:10281–90.
20. Bruck I, Kaplan D. Dbf4-Cdc7 phosphorylation of Mcm2 is required for cell growth. *J Biol Chem* 2009;284:28823–31.
21. Hughes S, Jenkins V, Dar MJ. Transcriptional co-activator LEDGF interacts with cdc7-activator of S-phase kinase (ASK) and stimulates its enzymatic activity. *J Biol Chem* 2010;285:541–54.
22. Bailis JM, Bernard P, Antonelli R, et al. Hsk1-Dfp1 is required for heterochromatin-mediated cohesion at centromeres. *Nat Cell Biol* 2003;5:1111–6.
23. Takahashi TS, Basu A, Bernudez V, et al. Cdc7/Drf1 kinase links chromosome cohesion to the initiation of DNA replication in *Xenopus* egg extracts. *Genes Dev* 2008;22:1894–905.
24. Jiang W, McDonald D, Hope TJ, et al. Mammalian Cdc7-Dbf4 protein kinase complex is essential for initiation of DNA replication. *EMBO J* 1999;18:5703–13.
25. Montagnoli A, Tenca P, Sola F, et al. Cdc7 inhibition reveals a p53-dependent replication checkpoint that is defective in cancer cells. *Cancer Res* 2004;64:7110–6.
26. Im JS, Lee JK. ATR-dependent activation of p38 MAP kinase is responsible for apoptotic cell death in cells depleted of Cdc7. *J Biol Chem* 2008;283:25171–7.
27. Branzei D, Foiani M. The DNA damage response during DNA replication. *Curr Opin Cell Biol* 2005;17:568–75.
28. Costanzo V, Shechter D, Lupardus PJ, et al. An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication. *Mol Cell* 2003;11:203–13.
29. Tsuji T, Lau E, Chiang GG, et al. The role of Dbf4/Drf1-dependent kinase Cdc7 in DNA-damage checkpoint control. *Mol Cell* 2008;32:862–9.
30. Tenca P, Brotherton D, Montagnoli A, et al. Cdc7 is an active kinase in human cancer cells undergoing replication stress. *J Biol Chem* 2007;282:208–15.
31. Kim JM, Kakusho N, Yamada M, et al. Cdc7 kinase mediates Claspin phosphorylation in DNA replication checkpoint. *Oncogene* 2008;27:3475–82.
32. Feng D, Tu Z, Wu W, et al. Inhibiting the expression of DNA replication-initiation proteins induces apoptosis in human cancer cells. *Cancer Res* 2003;63:7356–64.
33. Shreeram S, Sparks A, Lane DP, et al. Cell type-specific responses of human cells to inhibition of replication licensing. *Oncogene* 2002;21:6624–32.
34. Bonte D, Lindvall C, Liu H, et al. Cdc7/Dbf4 kinase overexpression in multiple cancers and tumor cell lines is correlated with p53 inactivation. *Neoplasia* 2008;10:920–31.
35. Kulkarni AA, Kingsbury SR, Tudzarova S, et al. Cdc7 kinase is a predictor of survival and a novel therapeutic target in epithelial ovarian carcinoma. *Clin Cancer Res* 2009;15:2417–25.
36. Nambiar S, Mirmohammadsadegh A, Hassan M, et al. Identification and functional characterization of ASK/Dbf4, a novel survival gene in cutaneous melanoma with prognostic relevance. *Carcinogenesis* 2007;28:2501–10.
37. Clarke LE, Fountaine TJ, Hennessy J, et al. Cdc7 expression in melanomas, spitz tumors and melanocytic nevi. *J Cutan Pathol* 2009;36:433–8.
38. Krawczyk J, Egan C, Mulvihill M, et al. Increased activity of the S phase kinase Cdc7 is associated with poor outcome in diffuse large B cell lymphoma (DLBCL) [abstract]. *Blood (ASH Annual Meeting Abstracts)* 2009;114:Abstract nr 1914.
39. Choschzick M, Lebeau A, Marx AH, et al. Overexpression of cell division cycle 7 homolog is associated with gene amplification frequency in breast cancer. *Hum Pathol* 2010;41:358–65.
40. Guo B, Romero J, Kim BJ, et al. High levels of Cdc7 and Dbf4 proteins can arrest cell-cycle progression. *Eur J Cell Biol* 2005;84:927–38.
41. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. *Nature* 2007;446:153–8.
42. Vanotti E, Amici R, Bargiotti A, et al. Cdc7 kinase inhibitors: pyrrolopyridinones as potential antitumor agents. 1. Synthesis and structure-activity relationships. *J Med Chem* 2008;51:487–501.
43. Montagnoli A, Valsasina B, Croci V, et al. A Cdc7 kinase inhibitor restricts initiation of DNA replication and has antitumor activity. *Nat Chem Biol* 2008;4:357–65.
44. Jackson PK. Stopping replication, at the beginning. *Nat Chem Biol* 2008;4:331–2.
45. Montagnoli A, Santocanale C, Valsasina B, et al. A new potent orally available class of Cdc7 inhibitors with anti-tumor activity [abstract]. *Proceedings of the 99th Annual Meeting of the American Association*

- for Cancer Research; 2008 Apr 12-16; San Diego (CA). Philadelphia (PA): AACR; 2008, Abstract nr 755.
46. Cai D, Lantham VM, Zhang X, et al. Combined depletion of cell cycle and transcriptional cyclin-dependent kinase activities induces apoptosis in cancer cells. *Cancer Res* 2006;66:9270-80.
  47. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol* 2006;24:1770-83.
  48. Warr MR, Shore GC. Unique biology of Mcl-1: therapeutic opportunities in cancer. *Curr Mol Med* 2008;8:138-41.
  49. Robertson S. Identification of XL413, a selective Cdc7 kinase inhibitor which induces cell cycle arrest and exhibits potent antitumor activity. Proceedings of the 20th Annual Meeting of European Organisation for Research and Treatment of Cancer; 2008 21-24 Oct; Geneva. Brussels: EORTC; 2008, Abstract nr 339.
  50. Shafer CM, Lindvall M, Bellamacina C, et al. 4-(<sup>1</sup>H-Indazol-5-yl)-6-phenylpyrimid-2(<sup>1</sup>H)-one analogs as potent CDC7 inhibitors. *Bioorg Med Chem Lett* 2008;18:4482-5.
  51. Leroy V, Bacque E, Conseille E, et al. New imidazolone derivatives, preparation thereof as drugs, pharmaceutical composition, and use thereof as protein kinase inhibitors, in particular CDC7. French patent WO2008/046982. 2008 April 24, France.
  52. Frattini M, Shum D, O'Dwyer KM, et al. Discovery and validation of a novel class of small molecule inhibitors of the CDC7 kinase: modulation of tumor growth *in vitro* and *in vivo*. *Blood (ASH Annual Meeting Abstracts)* 2008;112:Abstract nr 2668.

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*Clin Cancer Res* 2010;16:4503-4508. Published OnlineFirst July 20, 2010.

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