Inhibition of the checkpoint kinase Chk1 induces DNA damage and cell death in human Leukemia and Lymphoma cells

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Abstract

Background: Chk1 forms a core component of the DNA damage response and small molecule inhibitors are currently being investigated in the clinic as cytotoxic chemotherapy potentiators. Recent evidence suggests that Chk1 inhibitors may demonstrate significant single agent activity in tumors with specific DNA repair defects, a constitutively activated DNA damage response or oncogene induced replicative stress.

Methods: Growth inhibition induced by the small molecule Chk1 inhibitor V158411 was assessed in a panel of human leukemia and lymphoma cell lines and compared to cancer cell lines derived from solid tumors. The effects on cell cycle and DNA damage response markers were further evaluated.

Results: Leukemia and lymphoma cell lines were identified as particularly sensitive to the Chk1 inhibitor V158411 (mean GI50 0.17 μM) compared to colon (2.8 μM) or lung (6.9 μM) cancer cell lines. Chk1 inhibition by V158411 in the leukemia and lymphoma cell lines induced DNA fragmentation and cell death that was both caspase dependent and independent, and prevented cells undergoing mitosis. An analysis of in vitro pharmacodynamic markers identified a dose dependent decrease in Chk1 and cyclin B1 protein levels and Cdc2 Thr15 phosphorylation along with a concomitant increase in H2AX phosphorylation at Ser139 following V158411 treatment.

Conclusions: These data support the further evaluation of Chk1 inhibitors in hematopoietic cancers as single agents as well as in combination with standard of care cytotoxic drugs.

Keywords: Chk1, Leukemia, Lymphoma, Kinase inhibitor, V158411

Background

The serine-threonine checkpoint kinases Chk1 and Chk2 form central key components in the DNA damage signaling response (DDR) [1]. Activation of the DDR results in a number of cellular responses including checkpoint activation and cell cycle arrest, initiation of DNA repair, regulation of transcription and apoptosis. The DDR can be activated by a range of endogenous and external insults including therapies currently used for the treatment of cancer such as ionizing radiation and cytotoxic chemotherapeutic agents such as gemcitabine, irinotecan and cisplatin [2,3]. Despite their similarity in name, Chk1 and Chk2 differ substantially in the structure of their kinase pocket [4,5] and in their cellular function with Chk1 suggested to be the major component responsible for responses to DNA damage [3,6,7]. Inhibiting Chk1 following genotoxic stress (such as that induced by cytotoxic chemotherapy) results in checkpoint abrogation, inhibition of DNA repair and induction of cell death in cells with a defective p53 response [8,9]. Small molecule inhibitors of predominantly the Chk1 kinase have been readily sought as a mechanism through which the anti-tumor activity of cytotoxic chemotherapeutics may be increased whilst sparing the normal cells [10-12]. This approach is currently being tested in the clinic with a variety of agents including LY2603618 [13], MK-8776 [14], GDC-0425 and GDC-0575 in combination with a range of standard of care chemotherapy drugs.

Evidence has begun to emerge that small molecule Chk1 inhibitors may have significant single agent activity in cancer cells with specific underlying genetic defects. This is often defined as a synthetic lethal relationship
These can so far be defined as having specific defects in DNA-damage repair or response components, or are constitutively dependent on the DDR to complete an unperturbed round of DNA replication. The Fanconi Anemia (FA) pathway is a DNA repair pathway that is responsible for repairing crosslinked DNA [17]. Components of the FA pathway have been found to be lost or defective in a range of human cancers and are characterized by hypersensitivity to DNA crosslinking agents, chromosomal instability and reliance on DNA repair mediated by ATM. FA deficient cell lines were found to be sensitive to Chk1 silencing by siRNA compared to FA proficient cells [18]. Patients with complex karyotype acute myeloid leukemia (AML) had high levels of constitutive DNA damage (including high levels of pH2AX) and checkpoint activation. AML blast cells derived from these patients were sensitive to Chk1 siRNA or the kinase inhibitor UCN-01 compared to normal granulocyte progenitors [19]. Sensitivity to Chk1 inhibition has also been linked to replicative stress in a number of cancer cell types. In neuroblastoma cell lines, an siRNA screen identified siRNAs against Chk1 as the most potent inducers of cytotoxicity [20]. Chk1 mRNA expression was higher in MYC-Neuroblastoma-related (MYCN) amplified cancers and Chk1 was found to be phosphorylated on the auto-phosphorylation site Ser296 and the ATM activation site Ser345 in the absence of exogenous DNA damage insults. Neuroblastoma cell lines were found to be more sensitive to two Chk1 inhibitors SB21807 and TCS2312 compared to three non-neuroblastoma cancer cell lines. Sensitivity to SB21807 correlated with MYCN protein levels. Inhibition of Chk1 with the small molecule inhibitor AR678 inhibited the proliferation of a range of melanoma cell lines with low nM efficiency in vitro. The cytotoxicity of AR678 was suggested to be due to inhibition of S-phase Chk1 and failure of cytotokinesis or induction of apoptotic death and sensitivity correlated with levels of endogenous DNA damage most likely induced by replicative stress [21].

We utilized our own novel, potent, selective small molecule inhibitor of Chk1, V158411, to screen cell lines from a range of cancer types in an effort to identify additional tumor types for which single agent Chk1 inhibitor therapy may prove a rational treatment option.

Results

Pharmacological inhibition of Chk1 is cytotoxic in leukemia and lymphoma cell lines

Emerging evidence suggests that inhibiting the checkpoint kinase Chk1, in addition to potentiating cytotoxic chemotherapeutic agents, may exhibit single agent activity in cancers with underlying DNA repair, DNA damage response or DNA replication defects. We used the highly selective, potent checkpoint kinase inhibitor V158411 as a tool to identify cancer types where checkpoint inhibition may be a rationale therapeutic option.

V158411 is a novel, potent, selective inhibitor of recombinant Chk1 and Chk2 kinases in vitro with IC50s of 3.5 and 2.5 nM respectively [22]. Against a panel of 386 kinases in a wide panel binding assay, V158411 inhibited the activity of one kinase (Chk1) in the range 99 – 100%, three kinases 90 – 99% and 19 kinases 65 – 90% at 50 nM (Figure 1A). In p53 defective HT29 cells, V158411 inhibited the etoposide induced auto-phosphorylation of Chk1 on Ser296 with an IC50 of 48 nM and Chk2 on Ser516 with an IC50 of 904 nM indicating a 19-fold cellular selectivity for Chk1 over Chk2. V158411 potentiated cytotoxic chemotherapy in p53 defective cancer cells in vitro and in vivo.

In a screen of cell lines, V158411 inhibited the proliferation of five out of six of the leukemia and lymphoma cell lines tested with an average GI50 of 0.17 μM (Figure 1B and 1C, and Table 1) following 72 hour exposure to the drug. In comparison, the average GI50 for the seven colon cancer cell lines and for the seven lung cancer cell lines were 2.8 μM and 6.9 μM respectively. To confirm this, a second Chk1 inhibitor PF-477736 [23] was also profiled. As was observed for V158411, PF-477736 selectively inhibited the proliferation of the same five leukemia and lymphoma cell lines with an average GI50 of 0.28 μM (Table 1) compared to 1.7 μM for one lung and six colon cancer cell lines (Figure 1C). There was a close correlation between the sensitivity of a given cell line to V158411 and PF-477736 (R2 = 0.829, Figure 1D). Inhibition of cell proliferation was accompanied by a rapid and sustained reduction of apoptotic death and sensitivity correlated with levels of endogenous DNA damage most likely induced by replicative stress [21].

Cell death induced by Chk1 inhibition could, however, occur independently of caspase-3/7 activity. The caspase-3/7 inhibitor zVAD-FMK effectively blocked V158411 induced caspase-3/7 activation (Figure 2B). Jurkat, Raji or U937 cells treated with V158411 still underwent cell death in the presence of the general caspase inhibitor zVAD-FMK (Figure 2C). In both Jurkat and U937 but not Raji cells, more cell death was observed in the absence of zVAD-FMK but was not blocked completely by zVAD-FMK. This therefore suggests that Chk1 inhibition in leukemia and lymphoma cells can induce cell death through a variety of cell death pathways. To further understand the effects of Chk1 inhibition on cell proliferation, two different drug exposure regimes were compared in U937 cells. A single 24 hour pulse of 0.7 μM V158411 reduced the fraction of viable U937 cells by 96% 24 hours after the end of treatment (Figure 2D). However, the viable population rebounded rapidly with
### A

| Inhibition at 50nM (%) | Kinases Inhibited                                      |
|------------------------|--------------------------------------------------------|
| 99 - 100               | Chk1 (100% inhibition at this concentration)          |
| 90 - <99               | AAK1, RIOK1, RIOK3                                     |
| 65 - <90               | BIKE, FLT3, GRK4, LKB1, LOK, MEK5, MELK, PDGFRB, PIP5K1A, PIP5K2B, PKN1, PRKG2, PRKX, SLK, SNARK, SRPK1, SRPK3, STK16, YSK4 |

V158411 was screened against a panel of 386 wild-type kinases using the DiscoveRx Kinome scanMAX technology.

### B

![Graph of inhibition at 50nM (%) for different cell lines](image)

### C

![Bar chart showing average GI50 ± SD (µM) for different cell lines](image)

### D

![Graph showing correlation between GI50 and inhibition](image)

\[ R^2 = 0.829 \]

**Figure 1** (See legend on next page.)
the number of viable U937 cells increased 5.5-fold and 11-fold compared to the 24 hour cell count 48 and 72 hours after the end of treatment. Continual exposure to 0.7 μM V158411 for 72 hours had a more marked and permanent effect on U937 survival reducing the fraction of viable U937 cells by 99.9%.

**Inhibition of Chk1 induces DNA fragmentation and prevents entry into mitosis**

Pharmacological inhibition of Chk1 with V158411 did not induce a definitive cell cycle arrest in the five sensitive hematopoietic cancer cell lines (Figure 3A and B). However, changes in the ratio of cells in G1:S:G2/M was observed in 3 of the 5 cell lines. In Jurkat cells, V158411 treatment reduced the fraction of cells in S and G2/M relative to G1. In Raji and MV4;11, the fraction of cells in S and G2/M was increased by V158411 treatment. This was most noticeable for the MV4;11 cells (Figure 3C). V158411 induced a dose dependent increase in the fraction of cells with a sub-G1 DNA content. In Jurkat, HL60 and U937 cells, this accounted for nearly 90% of the cell population at the higher concentrations (Figure 3D). This is highly indicative of DNA fragmentation and cell death via apoptosis. To evaluate if cells were progressing into mitosis and undergoing death via mitotic catastrophe, we utilized nocodazole to trap cells in mitosis. Treatment of Jurkat, Raji or U937 cells with nocodazole led to an increase in the fraction of cells in mitosis as evidenced by an increase in the levels of phH3 (S10). Treatment with V158411 prevented cells progressing through the cell cycle and becoming arrested in mitosis by nocodazole (Figure 4). The addition of nocodazole did not prevent the V158411 induced degradation of Chk1.

**Chk1 inhibition induces Chk1 degradation and H2AX phosphorylation**

The effects of V158411 on biomarker changes in Jurkat, Raji and U937 cells was evaluated. Treatment of all three cell lines with V158411 for 24 hours lead to a dose dependent decrease in Chk1 protein levels and a concomitant increase in the amount of H2AX phosphorylated at Ser139 (Figure 5A). In addition, the levels of Cdc2 phosphorylated at Tyr15 and total cyclin B1 were also reduced albeit at higher doses of V158411 than those needed to reduce Chk1 and induced pH2AX. In U937 cells, a reduction in the amount of Histone H3 phosphorylation on Ser10 could be observed. A time course of V158411 treatment in Jurkat and Raji cells indicated that maximal Chk1, cyclin B1 and pCdc2 (Y15) reduction occurred after 24 hours (Figure 5B). The kinetics of pH2AX induction differed between the two cell lines with an increase in pH2AX observed after 6 hours in Jurkat but not until 24 hours in Raji cells. The duration of pH2AX induction in Jurkat cells was maintained for at least 24 hours. In both cells lines, the degradation of Chk1 was dependent on the presence of a functioning proteasome. The proteasome inhibitor MG132 inhibited the V158411 induced degradation of Chk1 in Jurkat and Raji cells (Figure 5C).

**Western blot analysis of leukemia and lymphoma cell lines**

In order to further understand the underlying mechanism for sensitivity of Leukemia and Lymphoma cells to the Chk1 inhibitors and identify biomarkers that may be potentially useful for identifying sensitive patients in clinical studies, we examined the expression levels and phosphorylation status of Chk1 in these cell lines by western blotting and compared it to a panel of six lung lines (Figure 6). Chk1 expression levels varied across the cell lines with the highest expression levels identified in NCI-H520 and K562 cells and very low levels in U937 and Raji cells. Phosphorylation of Chk1 on either serine 296, 317 or 345 was highly variable across the cell lines investigated. No correlation between Chk1 expression levels or phosphorylation on serine 296, 317 or 345 and

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**Table 1 Growth inhibition of leukemia and lymphoma cell lines by V158411 and PF-477736**

| Cell line | p53 status | GI50 (μM) ± SD | Tumor type |
|-----------|------------|----------------|------------|
|           |            | V158411 | PF-477736 |
| HL60      | Mut        | 0.21 ± 0.083 | 0.73 ± 0.033 | Promyelocytic Leukemia |
| Jurkat    | Mut        | 0.12 ± 0.027 | 0.12 ± 0.046 | T-cell Lymphoma |
| K562      | Mut        | 2.8 ± 1.6   | 1.9 ± 1.4   | Chronic Myelogenous Leukemia |
| MV4-11    | WT         | 0.063 ± 0.033 | 0.13 ± 0.051 | Acute Monocytic Leukemia |
| Raji      | Mut        | 0.42 ± 0.14 | 0.33 ± 0.11 | Burkett’s Lymphoma |
| U937      | Mut        | 0.034 ± 0.018 | 0.099 ± 0.006 | Histiocytic Lymphoma |

Mut, mutant; WT, wild type. GI50 values are the mean of n ≥ 4 ± SD.
Figure 2 (See legend on next page.)
sensitivity to the Chk1 inhibitors could be identified. There was an apparent increased basal expression level of pChk1 (S345) in lung cancer cell lines especially A549, NCI-H23 and NCI-H520 but this was not significantly higher than the leukemia and lymphoma cell lines ($P = 0.02$). There was no correlation between pChk1 (S345) expression levels and sensitivity to V158411 (Figure 6B, $R^2 = 0.186$). In three of the Chk1 inhibitor sensitive leukemia/lymphoma cell lines, U937, HL-60 and MV4-11, the endogenous levels of H2AX phosphorylated on Ser139 was much higher compared to all other cell lines. Across the whole panel of cell lines analyzed, there was a weak correlation ($R^2 = 0.404$) between pH2AX (S139) expression and V158411 sensitivity but no correlation ($R^2 = 0.250$) when just the leukemia and lymphoma subset of cell lines were analyzed (Figure 6B).

**Discussion**

Small molecule inhibitors of the checkpoint kinase Chk1 are currently undergoing early stage clinical evaluation in combination with DNA damaging cytotoxic chemotherapeutic drugs such as irinotecan and gemcitabine. Recent studies have started to identify cancer types sensitive to Chk1 inhibition as single agents; that is, in the absence of a cytotoxic chemotherapeutic drug. RNAi sensitive to Chk1 inhibition as single agents is applicable to a wider range of blood-derived cancers. The observation that Chk1-A inhibits additional kinases important for proliferation and survival of solid cancer-derived cell lines, that Chk1-A exhibits potent single agent activity in solid cancer cell lines as well as hematopoietic cancer cell lines (in contrast to V158411 and PF-477736) suggests that Chk1-A may inhibit additional kinases important for proliferation and survival of solid cancer-derived cell lines. The mechanism by which Chk1 inhibition leads to the death of hematopoietic cells is yet to be fully elucidated and understood. The molecular defects in these cell lines most likely occur in pathways for which Chk1 can...
Figure 3 (See legend on next page.)
mutually compensate to protect genomic integrity and therefore Chk1 inhibition is synthetically lethal. Studies in other cancer models provide possible mechanisms which may leave these cell lines more Chk1 dependent than other solid cancer cell types such as lung or colon cancer. Two possible mechanisms have so far been suggested for Chk1 inhibitor sensitivity: increased oncogenic replicative stress or reduced DNA repair capacity due to defects in specific DNA repair pathways especially those responsible for processing and repairing DNA double strand breaks (DSBs) [29,30].

Two previous studies, one in neuroblastoma cells [20] and another in a mouse derived Eμ-myc driven lymphoma cell model [25], identified increased oncogenic replicative stress due to amplification of the Myc oncogene as a potential underlying mechanism for sensitivity to Chk1 inhibition. In the Eμ-myc lymphoma model, sensitivity to the Chk1 inhibitor PF-477736 was dependent on a p53 wild type background. Apoptosis induced by oncogenic replicative stress can be suppressed by ATR and Chk1 [29,31]. All the cell lines used in this study, with the exception of MV4-11, are known to harbor amplifications of the c-myc oncogene [32,33] and therefore increased replicative stress due to amplified Myc driven proliferation [34] may underlie the sensitivity of some of these cell lines. However, in contrast to the Eμ-myc lymphoma model, all of the four c-myc amplified sensitive cell lines harbor mutations in p53 suggesting that sensitivity to Chk1 inhibitors may not be dependent on a p53 wild type background. The CML cell line K562 has amplifications in the c-myc and l-myc oncogenes but is resistant, compared to all the other leukemia and lymphoma cell lines so far tested, to Chk1 inhibitors as single agents. Therefore additional factors along with Myc induced oncogenic stress potentially contribute to Chk1 inhibitor sensitivity.

MV4-11 cells harbor an internal tandem duplication (ITD) in the juxtamembrane domain of FLT3 leading to deregulated FLT3 kinase signaling that drives the proliferation of this cell line [35]. Like deregulation of the c-Myc oncogene, the FLT3-ITD mutation induces oncogenic replicative stress [36,37] and may account for the sensitivity of this cell line to Chk1 inhibition. Along with U937 and HL-60 cells, MV4-11 cells exhibited a high level of expression of H2AX phosphorylated on serine 139 under normal cell growth conditions. Increased expression of pH2AX (S139) is associated with increased DNA damage especially double strand breaks [38] and in MV4-11 cells is consistent with increased oncogenic replicative stress induced by FLT3 mutation.

Molecular defects in pathways responsible for processing DNA breaks, especially DNA double strand breaks,
have been postulated to be potentially synthetically lethal with Chk1 inhibition. One example so far discovered is in the Fanconi Anemia (FA) DNA repair pathway. The Fanconi Anemia (FA) repair pathway is responsible for repairing crosslinked DNA and maintaining chromosomal stability [17]. FA deficient cell lines were found to be sensitive to Chk1 silencing by siRNA and the small molecule Go6975 compared to FA proficient cells due to an accumulation of unreparable DNA double strand breaks [18]. Similarly, AML with a complex karyotype demonstrate high levels of constitutive DNA damage and checkpoint activation. siRNA against Chk1 or the small molecule kinase inhibitor UCN-01 reduced the clonogenic survival of patient derived AML blast cells [19]. UCN-01 is a non-specific pan-kinase inhibitor derived from staurosporine and effects induced by this molecule cannot be reliably attributed to Chk1 inhibition. Reduced or defective DNA strand break repair capacity could underlie the sensitivity of leukemia and lymphoma cell lines to Chk1 inhibition. The sensitivity of leukemia and lymphoma cell lines to Chk1 inhibition may be due to reduced DNA repair capacity, oncogenic replication stress or a combination of both mechanisms.

All the studies so far conducted have been undertaken on established cell lines that grow indefinitely under

![Figure 5](image-url)

**Figure 5** Chk1 inhibition by V158411 induces Chk1 degradation and H2AX phosphorylation. (A) Cells were treated with the indicated concentrations of V158411 for 24 hours. (B) Jurkat and Raji cells were treated with 1 μM V158411 for the indicated times. (C) Jurkat and Raji cells were treated with 1 μM V158411 in the presence or absence of 10 μM MG132 for 24 hours. Protein expression was characterized by western blotting with GAPDH used as a loading control.
optimal culture conditions. Selection of these cell lines for growth in culture may have resulted in the selection for factors that drive cell proliferation in culture rather than tumor proliferation in situ. Replicative stress due to deregulated oncogenes, and hence sensitivity to Chk1 inhibitors, may be amplified due to selection of cells that proliferate rapidly in culture and may not truly reflect the oncogenic replicative stress observed in human disease. Further work is needed on leukemia and lymphoma samples derived from patients that have undergone limited ex vivo culture to confirm and understand these observations.

From these studies, Chk1 inhibitors may be a useful addition to the arsenal of drugs suitable for use in the clinic against hematopoietic cancers. The ability to stratify patients based on genetic markers predictive of sensitivity will be necessary to achieve optimal clinical benefit. Studies so far suggest that deregulated Myc oncogene expression may be one such marker.

Figure 6 Leukemia and lymphoma cell lines do not exhibit high endogenous expression levels of phosphorylated Chk1. (A) Untreated whole cell protein extracts were prepared from the indicated cell lines and the expression levels of various protein markers determined by western blotting. (B) Protein expression was quantified by densitometry and correlated with the cell line sensitivity to V158411. Circles, lung cell lines; squares, leukemia and lymphoma cell lines.
Conclusions
Cell lines derived from human leukemias and lymphomas exhibited greater sensitive to the Chk1 inhibitor V158411 than cell lines derived from solid tumors. Replication stress, due to oncogene activation, may account for the sensitivity of these cell lines to Chk1 inhibition. This data supports the further evaluation of Chk1 inhibitors in hematopoietic cancers as single agents as well as in combination with standard of care cytotoxic drugs.

Methods

Cell culture and cytotoxicity assay
All cells were obtained from the American Type Culture Collection (ATCC) or Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ) and cultured in DMEM or RPMI containing 10% FCS (Invitrogen). The cytotoxicity of V158411 was determined following exposure of cells in 96 well plates to a 10-point titration for 72 hours. Cell proliferation was determined using sulphorhodamine B staining following protein precipitation with 10% TCA for adherent cell lines or cell titer glo (Promega) for suspension cell lines. For cell counts, cells were seeded in 24 well plates and counted daily using a haemocytometer following trypan blue staining. Cells were diluted to maintain log phase cultures.

Determination of caspase-3/7 dependent apoptosis
Cells were seeded in 96 well plates and treated with 5- or 10-times the GI50 of V158411 for 24 or 48 hours. Caspase-3/7 activity was determined using a homogenous ware (BD).

Competing interests
CB, KS and AJM undertook this work as employees of Vemalais R&D Ltd. AJM is a stock option holder of Vemalais R&D Ltd.

Author contributions
AJM designed and coordinated the studies and is the author responsible for writing the paper. CB, KS and AJM carried out the studies. All authors read and approved the final manuscript.

Author information
All authors are either current or past employees of Vemalais R&D Ltd and undertook this study as part of their employment. AJM is a stock option holder of Vemalais R&D Ltd.

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References
1. Zhou BB, Elledge SJ: The DNA damage response: putting checkpoints in perspective. Nature 2000, 408:433–439.
2. Dai Y, Grant S: Targeting Chk1 in the replicative stress response. Cell Cycle 2010, 9:1025.
3. Smith J, Tho LM, Xu N, Gillepsie DA: The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. Adv Cancer Res 2010, 108:73–112.
4. Chen P, Luo C, Deng Y, Ryan K, Register J, Margosiak S, Tempczyk-Russell A, Nguyen B, Myers P, Lundgren K, Kan CC, O’Connor PM: The 1.7 A crystal structure of human cell cycle checkpoint kinase Chk1: implications for Chk1 regulation. Cell 2000, 100:851–692.
5. Cai Z, Chehab NH, Pavletich NP: Structure and activation mechanism of the CHK2 DNA damage checkpoint kinase. Mol Cell 2009, 35:818–829.
6. Lu Q, Guntuku S, Cui XS, Matsuoka S, Cortez D, Tarni K, Luo G, Garantti-Rivera S, DeMayo F, Bradley A, Donehower LA, Elledge SJ: Chk1 is an essential kinase that is regulated by AtT and required for the G2/M DNA damage checkpoint. Genes Dev 2000, 14:1448–1459.
7. Xiao Z, Chen Z, Gunasekera AH, Sowin TJ, Rosenberg SH, Fesik S, Zhang H: Chk1 mediates G2 and G2 arrests through Cdc25A degradation in response to DNA-damaging agents. J Biol Chem 2003, 278:21767–21773.
8. Buchar N, Britten CD: G2 checkpoint abrogation and checkpoint kinase-1 targeting in the treatment of cancer. Br J Cancer 2008, 98:523–528.
9. Cho SH, Tousli CD, Fuji GH, Grai C, Pary D: Chk1 is essential for tumor cell viability following activation of the replication checkpoint. Cell Cycle 2005, 4:131–139.
10. Garrett MD, Collins I: Anticancer therapy with checkpoint inhibitors: where, when and why? Trends Pharmacol Sci 2011, 32:308–316.
11. Ashwell S, Janetka JW, Zabludoff S: Keeping checkpoint kinases in line: new selective inhibitors in clinical trials. Expert Opin Investig Drugs 2008, 17:1331–1340.
12. Ma CX, Janetka JW, Pienica-Worms H: Death by releasing the breaks: Chk1 inhibitors as cancer therapeutics. Trends Mol Med 2011, 17:88–96.
13. King C, Diaz H, Bernard B, Barda D, Clackson D, Blosser W, Cox K, Guo S, Marshall M: Characterization and preclinical development of LY2603618: a selective and potent Chk1 inhibitor. Invest New Drugs 2013, 31:213–226.
14. Guzi TJ, Paruch K, Dwyer MP, Labroli M, Shanahan F, Davis N, Tarciani L, Wiswell D, Seghazzo W, Penaffier D, Bhagwat B, Wang W, Gu D, Hsieh Y, Lee S, Liu M, Pary D: Targeting the replication checkpoint using SCH 900776, a potent and functionally selective CHK1 inhibitor identified via high content screening. Mol Cancer Ther 2011, 10:591–602.
15. Shaheen M, Allen C, Nickoloff JA, Horns R: Synthetic lethality: exploiting the addiction of cancer to DNA repair. Blood 2011, 117:6074–6082.
16. Brough R, Franklin JK, Costa-Cabral S, Lord CJ, Ashworth A: Searching for synthetic lethality in cancer. Curr Opin Genet Dev 2011, 21:324–41.
17. Wang W: Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat Rev Genet 2007, 8:735–748.
18. Chen CC, Kennedy RD, Sidi S, Look AT, D’Andrea A: Chk1 inhibition as a strategy for targeting Fanconi Anemia (FA) DNA repair pathway deficient tumors. Mol Cancer 2009, 8:24.
33. Nishikura K, Erikson J, ar-Rushdi A, Huebner K, Croce CM: Internal tandem duplication of FLT3 (FLT3/ITD) induces increased ROS yielding cancer. *Nature* 2002, 120:1011–1018.

34. Vafa O, Wade M, Kern S, Beeche M, Pandita TK, Hampton GM, Wahl GM: A potent Chek1 inhibitor is selectively cytotoxic in melanomas with high levels of replicative stress. *Oncogene* 2013, 32:788–796.

35. Cole RA, Huggins J, Laquaglia M, Hudlerman CE, Russell MR, Bosse K, Diskin SI, Attiyeh EF, Sennett R, Norris G, Lautenbarger M, Wood AC, Mesec FA, Jagannathan J, Winter C, Moses YP: RNAi screen of the protein kinome identifies checkpoint kinase 1 (CHK1) as a therapeutic target in AML. *Mol Cancer* 2011, 10:333–341.

36. Sallmyr A, Fan J, Datta K, Kim KT, Grosu D, Shapiro P, Small D, Rasool F: Constitutive activation of the DNA damage signaling pathway in acute myeloid leukemia with complex karyotype: potential importance for checkpoint targeting therapy. *Cancer Res* 2009, 69:8652–8661.

37. Fan J, Li L, Small D, Rasool F: Cells expressing FLT3/ITD mutations exhibit elevated repair errors generated through alternative NHEJ pathways: implications for genomic instability and therapy. *Blood* 2010, 116:5298–5305.

38. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, Pommier Y: GammaH2AX and cancer. *Nat Rev Cancer* 2008, 8:957–967.

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