Targeting the S and G2 checkpoint to treat cancer

Tao Chen, Peter A. Stephens, Fiona K. Middleton and Nicola J. Curtin

Newcastle University, Northern Institute for Cancer Research, Paul O'Gorman Building, Framlington Place, Newcastle-upon-Tyne NE2 4HH, UK

Cell survival following DNA damage depends on activating checkpoints to arrest proliferation. Most cancer cells have dysregulated G1 checkpoints making them dependent on their S and G2 checkpoints, which are activated by ATR/Chk1 signalling. Thus, inhibiting ATR or Chk1 should selectively sensitise cancer cells to DNA damage. Genetic inactivation of ATR and Chk1 abrogates cell cycle arrest and enhances cytotoxicity following exposure to DNA-damaging agents. Similar effects were seen with small-molecule Chk1 inhibitors in preclinical studies, and clinical trial data are starting to emerge. Recently, potent ATR inhibitors have been identified that also sensitise cancer cells *in vitro*. ATR and Chk1 inhibitors might also cause 'synthetic lethality' in tumour cells defective in defined DNA repair pathways.

The role of DNA damage checkpoints

All cells face a battery of insults to their DNA from endogenous sources, for example spontaneous cytosine deamination, replication errors and base damage from oxygen radicals generated as a product of normal metabolism. Together with exogenous sources: UV, dietary toxins, aromatic hydrocarbons, among others, this adds up to a significant value that has been estimated as 10 000–40 000 single strand breaks (SSBs) and up to 50 double strand breaks (DSBs) per cell per day [1]. To prevent this damage being carried as mutations to the next generation, living organisms have evolved a complex and carefully orchestrated network of proteins known as the DNA damage response (DDR), which comprises sensors that detect and signal DNA damage to downstream proteins that either arrest the cell cycle providing time for repair or initiate cell death pathways [2].

DNA-damage checkpoints occur throughout the cell cycle. The G1 checkpoint, activated byATM signalling to Chk2 and p53, among other targets, prevents the replication of damaged DNA by blocking entry into S-phase [3]. If damage occurs during S-phase or has evaded the G1 checkpoint, the intra-S-phase checkpoint is activated blocking further replication. Remaining damage is detected by the G2 checkpoint which prevents cells entering mitosis. These latter checkpoints are triggered by ATR/Chk1 signalling. ATM and ATR are both members of the PI-3K-like kinase family (PIKK) of protein kinases that also includes DNA-dependent protein kinase (DNA-PK) and mammalian target of rapamycin (mTOR). There is crosstalk between the ATM/Chk2 and ATR/Chk1 pathways and they share many substrates [4].

Although normal cells have intact checkpoint signalling pathways, defective G1 checkpoint control is a common feature of cancer cells, for example owing to mutations in the p53 or pRb tumour suppressor genes or imbalance in cyclins, cyclin-dependent kinases (CDKs) and their inhibitors [5,6]. As a result, cancer cells tend to rely on their S and G2 checkpoint to avoid DNA damage being translated into cell death. The S/G2 checkpoint is therefore an attractive target for cancer-specific sensitisation to DNA-damaging chemotherapy and radiotherapy (Fig. 1).

ATR and Chk1 function

ATR and Chk1 are crucial kinases for genome maintenance even in the absence of exogenous sources of DNA damage. Homozygous deletion of either ATR or Chk1 leads to peri-implantation embryonic lethality at E7 [7,8] or between E3.5 and E7.5 [9], respectively. Inactivation of ATR and Chk1 results in profound G2 defects after DNA damage or replication arrest [4]. Chk1 deficiency in embryonic stem cells gives rise to a proliferation defect and subsequent cell death [10]. Chk1-deficient tumour cells are viable, but exhibit

Corresponding author:. Curtin, N.J. (nicola.curtin@newcastle.ac.uk), (n.j.curtin@newcastle.ac.uk)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2011.12.009

GLOSSARY

ATM Ataxia Telangiectasia Mutated protein
ATR Ataxia Telangiectasia Mutated and Rad3 related protein
ATRIP ATR interacting protein
BLM Blooms Syndrome helicase
BRCA1 BreastCancer1 protein
CHK1 Cell cycle Checkpoint Kinase 1
FANCD2 Fanconi anaemia group D2 protein
FANCE Fanconi anaemia group E protein
H2AX Histone variant 2AX
Rad9 Cell cycle checkpoint control protein RAD9
Rad17 Cell cycle checkpoint control protein RAD17
Rad51 RAD51 recombinase homologous to the bacterial RecA
TopBP1 DNA topoisomerase 2-binding protein
WRN Werner syndrome helicase

multiple checkpoint and survival defects [11]. No living humans have been identified as completely lacking either ATR or Chk1 function, but a hypomorphic mutation in ATR is linked to Seckel syndrome which is characterised by growth retardation and microcephaly similar to other DNA repair and checkpoint syndromes [12].

ATR is primarily activated by single stranded (ss)–double stranded (ds) DNA junctions, these most commonly arise when the replication fork encounters a DNA lesion owing to arrest of one polymerase while the other continues [4]. A ssDNA–dsDNA structure can also be generated by nucleotide excision repair (NER) or from resection of a DSB [13] (Fig. 2). Stalled replication forks occur following single base damage and antimetabolite exposure, DSBs can be induced by ionising radiation and topoisomerase II poisons and common targets for NER are cisplatin adducts and UV damage. The ssDNA is immediately coated by replication protein A (RPA), which recruits ATR *via* ATRIP, a regulatory protein in complex with ATR that enhances ATR binding and activity. ATR is further activated by DNA topoisomerase II binding protein 1 (TopBP1) [14] and Claspin, co-mediators of Chk1 activation [15], and the G2 degradation of these proteins facilitates checkpoint recovery and passage into mitosis [16].

The primary target of activated ATR is phosphorylation of Chk1 at Ser^{317} and Ser^{345}, but ATR also phosphorylates a number of proteins including those involved in the stabilisation of stalled replication forks and DNA repair such as ATRIP, Rad17, Rad9, TopBP1, Claspin, H2AX, WRN, BLM, BRCA1 and FANCD2 (some of which also activate the ATR/Chk1 pathway) thus linking ATR to homologous recombination repair (HRR) and DNA crosslink repair (Fig. 2).

ATR-induced phosphorylation at Ser^{345} and Ser^{317} directly leads to Chk1 activation. Ser^{345} is essential for Chk1 kinase activation and function, whereas Ser^{317} only has a contributory role [17,18]. Phosphorylation of Chk1 by ATR stimulates kinase activity promoting autophosphorylation at Ser^{296} and dissociation of Chk1 from chromatin. Activated Chk1 then phosphorylates Cdc25A and Cdc25 C, leading to their inactivation [19]. Cdc25A and Cdc25 C are phosphatases that activate S-phase progression and entry into mitosis, respectively. For example, Cdc25 C removes the inactivating phosphates at Thr^{14}/Tyr^{15} on Cdc2 (CDK1), and the activity of Cdc2/cyclin B promotes G2 to M transition [19]. Therefore Chk1-mediated inactivation of these proteins results in cell cycle arrest [20] and suppression of mitotic entry [21], which is

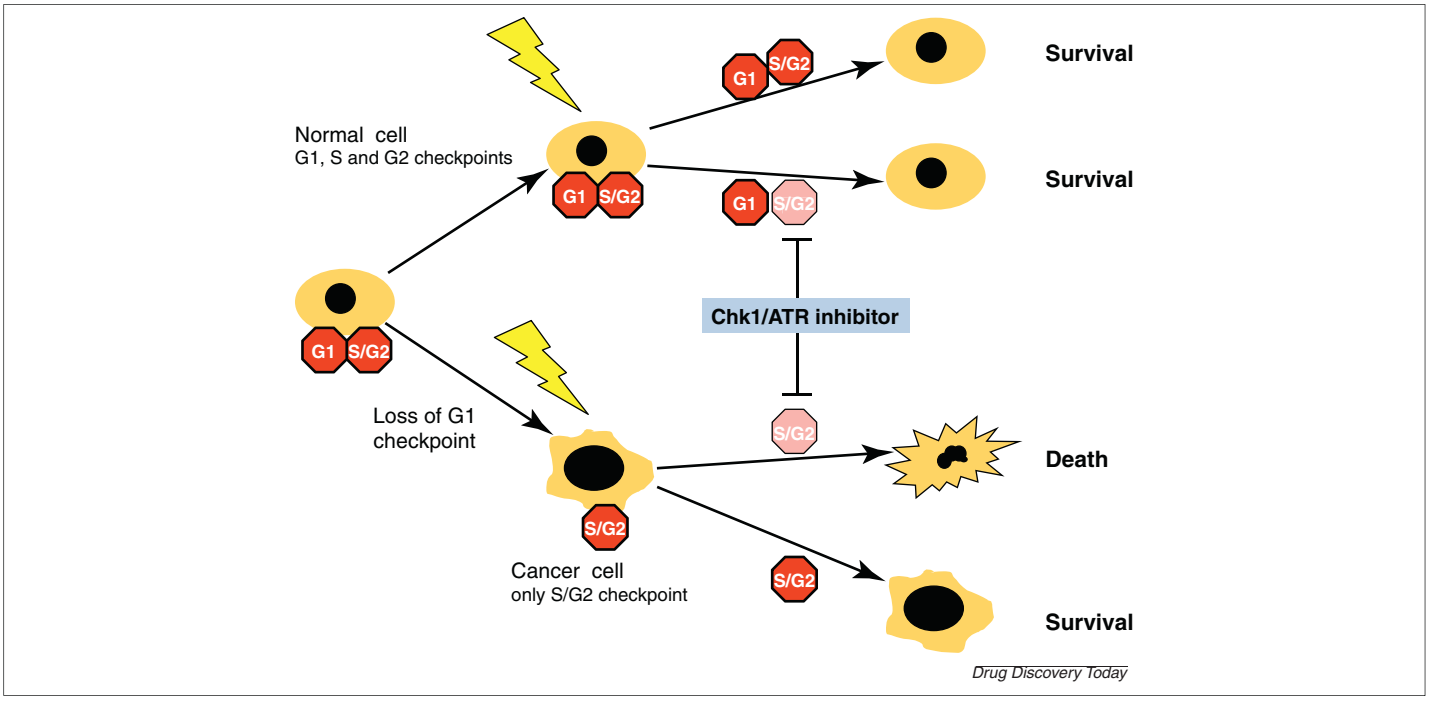

FIGURE 1

Selective sensitisation of cells lacking G1 checkpoint control. Normal cells have G1 and S/G2 checkpoints intact but cancer cells lose their G1 checkpoint through, for example, p53 pathway disruption or dysregulation of the G1 cyclins, CDKs or their inhibitors. Following DNA damage normal cells activate G1, S and G2 checkpoints such that if the S/G2 checkpoint is inhibited through either ATR or Chk1 inactivation cells can still arrest by activation of the G1 checkpoint, promoting survival. By contrast, cancer cells rely on their S/G2 checkpoints following DNA damage and when this is inhibited they enter premature lethal mitosis.

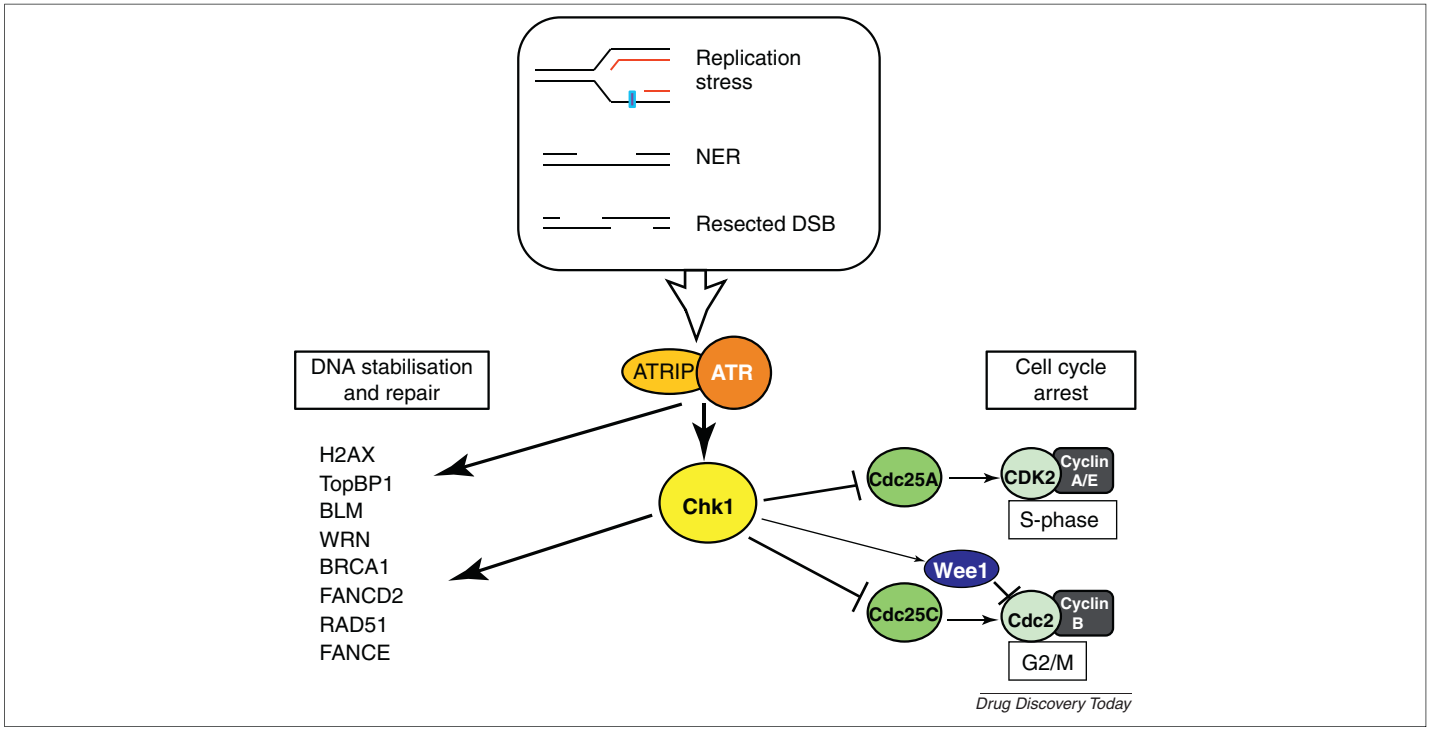

FIGURE 2

ATR and Chk1 signalling. ATR and ATR interacting protein (ATRIP) bind to ssDNA that arise owing to stalled replication forks (replication stress), following excision of a single stranded oligonucleotide during nucleotide excision repair and following resection of a double strand break (DSB). Thus, activated ATR phosphorylates Chk1 and a number of proteins involved with DNA stabilisation and repair. Chk1 also phosphorylates some of these proteins and also Cdc25A and Cdc25 C leading to their inactivation. Cdc25A dephosphorylates and activates CDK2/Cyclin A or E eliciting S-phase entry and Cdc25C dephosphorylates and activates Cdc2/Cyclin B promoting G2/M transition. Therefore inactivation of Cdc25A and C results in S and G2/M arrest, respectively.

further reinforced by Chk1-mediated stabilisation of Wee, the kinase that phosphorylates Cdc2 at Thr^{14}/Tyr^{15}. Chk1 also targets Rad51, FANCD2 and FANCE regulating HRR and stabilising stalled replication forks, induces the mitotic spindle checkpoint and inhibits caspase-3-mediated apoptosis [19].

ATR and CHK1 inactivation results in chemo- and radiosensitisation

Loss of ATR or Chk1 function is predicted to inhibit cell cycle arrest and increase cytotoxicity following exposure to DNA-damaging agents, as has been demonstrated by genetic studies. Dominant-negative inhibition of ATR by overexpressing a kinase-dead mutant ATR (ATR-kd) leads to abrogation of ionising radiation (IR) and topotecan-induced G2/M arrest, and sensitisation of human fibroblasts to multiple classes of DNA-damaging agents, including IR, alkylating agents (methyl-methanesulfonate), DNA crosslinking agents (cisplatin), topoisomerase I and II poisons (topotecan, etoposide, doxorubicin) and antimetabolites (hydroxyurea) [22,23]. The relative role of the ATR/Chk1 versus the ATM/Chk2 pathway has been explored: Chk1 but not Chk2 knockdown by siRNA abrogated G2 arrest and increased cytotoxicity induced by irradiation and cisplatin in HCT116 cells (human colon cancer) [24]. Further experiments showed that ATR or Chk1 siRNA, but not ATM siRNA or deletion of Chk2, sensitised HeLa cells (human cervical cancer) to topoisomerase I poisons [25]. This work has been extended to demonstrate that Chk1 knockdown sensitises HCT116 cells to 5-fluorouracil, doxorubicin and etoposide [26].

It has been postulated that targeting ATR or Chk1 is only relevant in cells with defective G1 control through loss of the tumour suppressor gene p53. The most convincing data regarding p53 dependence come from experiments using paired isogenic cell lines that differ only in their p53 status. For example, studies have shown that Chk1 knockdown sensitised p53-mutant HCT116 more than p53-wild-type HCT116 cells [26,27]. Similarly, inactivation of ATR selectively sensitised HCT116 cells lacking p53 function to cisplatin cytotoxicity [28]. However, HCT116 cells are also DNA mismatch repair deficient and have a defect in their ATM signalling [13,29]; and data with other cell lines can be conflicting, suggesting that p53 status is not the sole determinant. For example, Chk1 knockdown sensitised wild type (A549; human NSCLC) and p53 dysfunctional (HeLa) cell lines to gemcitabine and cytarabine [30]. Recent work in paired MCF7 (human breast cancer) and U2OS (human osteosarcoma) cells showed that although abrogation of the DNA-damage-induced cell cycle arrest by Chk1 inactivation was p53-dependent chemosensitisation (of cisplatin and irinotecan) was not [31]. Similarly, apoptosis in cells with silenced Chk1 exposed to hydroxyurea or cytosine arabinoside was not found to be dependent on p53 [32]. By the same token, the selectivity of ATR inactivation might not be restricted to p53-defective cells. ATR silencing sensitised HeLa (p53-defective) and U2OS (p53-wild-type) to topoisomerase I poisons [25]. Moreover, dominant-negative inactivation of ATR sensitised U2OS cells rendered defective in G1 arrest for a variety of reasons (e.g. overexpression of cyclin D1, cyclin E, CDK2 or MDM2) to a variety of DNA-damaging agents [23].

TABLE 1

Chk1 inhibitors in development.

Compounds	Preclinical data	Development phase	Trial Combination	Dose-limiting toxicity	Future development
UCN01	[36,39,89]	Phase I–II trials (short and long infusions) [58,59]	Single agent in AST (1/47 PR, 1/47 SD)	Hypotension and hyperglycaemia	Stopped–no further development planned
		Phase I [60,90]	Combination with cisplatin in AST (no response data)	Neutropenic sepsis and SVT	Stopped–no further development planned
		Phase I [61]	Combination with topotecan in AST (1/22 PR, 12/22 SD, 8/22 PD)	Neutropenia, thrombocytopenia and hyperglycaemia	Stopped–no further development planned
		Phase II (not published)	Combination with topotecan in ovarian cancer	Hyperglycaemia	Stopped–no further development planned
AZD7762 (Astra Zeneca)	[43,47,54,80]	Phase I [65]	Combination with gem in AST (2/42 PR)	Myocardial ischaemia and neutropenia	Development programme stopped
		Phase I [66]	Combination with irinotecan in AST (1/68 CR, 1/68 PR)	Myocardial ischaemia	Development programme stopped
		Phase I (not published)	Combination with gem in AST	Unknown	Trial terminated
PF00477736 (Pfizer)	[42]	Phase I [63]	Combination with gem in AST (3/36 PR)	Thrombocytopenia and neutropenia	Development programme stopped
SCH900776 (Schering Plough)	[44]	Phase I [64]	Combination with gem in AST (1/26 PR, 3/26 SD)	SVT and thrombocytopenia	Unknown
		Phase I	Combination with cytarabine in leukaemia		Closed–awaiting publication
XL9844 (Exelixis)	[46]	Phase I	Combination with gem in AST and lymphoma		Trial terminated
LY2606368 (Eli Lilly)	No	Phase I	Single agent in AST		Phase I–finishing Feb 2012
PD321852 (Pfizer)	[41]	Preclinical	No clinical trials		Development programme stopped
V158411 (Vernalis)	No	Preclinical	No clinical trials		
ARRY575 (Array Pharma)	No	Preclinical			
CEP3891 (Cephalon)	[37,55]	Preclinical			
SAR020106 (Sareum)	[45]	Preclinical			
GDC0245 (Genentech/Roche)	No	Preclinical			

Note: Data taken from ClinicalTrials.gov (http://clinicaltrials.gov/) compounds that are thought to be in late preclinical development are also included for completeness. All compounds are intravenous.

Abbreviations: AST; advanced solid tumours; gem; gemcitabine; SVT; supraventricular tachycardia; CR; complete response; PR; partial response; SD; static disease; PD; progressive disease.

These data, suggesting that ATR and Chk1 inhibitors would be potent chemosensitisers and radiosensitisers for cancer therapy, provided the impetus for drug development in this area. The development of Chk1 inhibitors is more advanced than ATR inhibitors and will be reviewed first. Readers might also wish to consult some recent excellent reviews in this area [7,19,22,33–35] as well as the primary publications cited in this review. A summary of Chk1 inhibitors in preclinical and clinical development is outlined in Table 1.

Preclinical development of Chk1 inhibitors

The first Chk1 inhibitor was UCN01, a staurosporine analogue, which inhibits protein kinase C [36], but also inhibits several other kinases including Chk1. Early studies showed that UCN01 reduced radiation-induced degradation of Cdc25A in U2OS cells [37] and abrogated doxorubicin-induced G2/M arrest [38]. UCN01 has been shown to potentiate cisplatin cytotoxicity in NSCLC cell lines (A549 and H596) [39], as well as camptothecin toxicity in colon cell lines (HCT116) [40].

Development of inhibitors

Subsequently, more-potent and -specific Chk1 inhibitors have been developed, most are ATP-competitive but with varying potencies and specificities. These include the UCN01 analogues, such as PD321852 (Chk1 IC_{50} = 5 nM) [41], and other structural types such as: the diazepinoindolone PF00477736 (Pfizer), with a K_i of 0.49 nM and 100-fold selectivity for Chk1 versus Chk2 and a cellular EC_{50} of 45 nM (assessed by measuring mitotic entry by histone H3 phosphorylation) [42]; AZD7762, a thiophene carboxamide urea (AstraZeneca), which inhibits Chk1 (K_i = 3.6 nM, IC_{50} = 5 nM) and Chk2 (IC_{50} < 10 nM) with similar potency [43]; and CEP3891 (Cephalon, structure not disclosed), a selective Chk1 inhibitor (IC_{50} = 5 nM) [37].

Recently developed potent and selective Chk1 inhibitors include SCH900776, a pyrazolo[1,5-a]pyrimidine (K_d = 2 nM,

IC_{50} = 60 nM), which causes a concentration-dependent inhibition of hydroxyurea-induced Chk1 Ser^{296} autophosphorylation in intact U2OS cells [44], and SAR020106 (Sareum; IC_{50} = 13.3 nM), which abrogates etoposide-induced arrest of HT29 cells with an IC_{50} of 55 nM [45]. XL9844 (EXEL9844) is an aminopyrazine inhibitor of Chk1 and Chk2 (K_i = 2.2 nM and 0.07 nM, respectively) that increases Chk1 phosphorylation at Ser^{317} and H2AX phosphorylation and inhibits Cdc25A degradation [46].

Inhibition of cell cycle checkpoints

Most Chk1 inhibitors do not substantially affect cell cycle progression or viability *per se* but do block cell cycle arrest and enhance cytotoxicity after DNA damage. In general, studies show the greatest activity with agents that cause replication stress, such as antimetabolites (particularly fraudulent nucleosides that cause replication arrest, such as gemcitabine), topoisomerase I poisons and DNA crosslinking agents, such as cisplatin, suggesting S-phase checkpoint is crucial. For example, AZD7762 alone does not affect cell proliferation [47] but it abrogates the camptothecin-induced G2 arrest [43] and potentiates the cytotoxicity of gemcitabine and topotecan [47]. Similarly, monoagent PF00477736 did not change the cell cycle, but abrogated gemcitabine- and camptothecin-induced S-phase and G2/M arrest in CA46 cells (p53-mutated lymphoma) and HeLa cells and potentiated the activity of a variety of DNA-damaging agents in several human cancer cell lines [42]. Likewise, XL844 alone did not affect the cell cycle distribution of PANC-1 (human pancreatic cancer) cells but abrogated gemcitabine-induced S-phase arrest, resulting in premature entry into mitosis [46]. In contrast to these observations, administration of SCH900776 as a single-agent administered to U2OS cells caused a concentration-dependent accumulation in G2 and an increase in the sub-G1 apoptotic population [44].

Synthetic lethality

Interestingly, Chk1 is upregulated in Myc-overexpressing murine and human lymphomas and its inactivation was cytotoxic in Myc-amplified cells and prolonged the survival of Myc-lymphoma-bearing mice [48,49]. In addition, recent studies have shown the combination of Chk1 inhibitors (i.e. UCN01, AZD7762) was synthetically lethal with various inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), including those currently in clinical trials (ABT888 and AZD2281), in a panel of human breast and pancreatic cell lines [50]. Synthetic lethality is an exciting new concept in cancer therapy and it describes the phenomenon where inactivation of two pathways independently does not affect viability, but inactivation of both together causes cell death.

In vivo *chemosensitisation*

Chemosensitisation by Chk1 inhibitors has also been demonstrated in *in vivo* models of human cancer, for example PF00477736 enhanced the antitumour activity of gemcitabine in mice bearing CoLo205 (human colon cancer) xenografts [42] and, by abrogating the mitotic checkpoint, sensitised cell lines and xenografts to docetaxel [51]. AZD7762 increased the antitumour activity of gemcitabine and of irinotecan against human cancer xenografts in mice and rats [43] and XL844 increased the efficacy of gemcitabine against PANC-1 xenografts [46]. Concomitant administration of a Chk1 inhibitor with cytotoxic therapy appears to be more efficacious than delayed administration of the Chk1 inhibitor. For example, simultaneous administration of gemcitabine and SAR020106 delayed SW620 xenograft growth better than if SAR020106 was administered 24 hours after gemcitabine [45].

Inhibition of DNA repair

Inhibition of Chk1 not only affects cell cycle arrest but also DNA repair. The histone H2A variant, H2AX, can be phosphorylated on Ser^{139} (γ-H2AX) by ATM, DNA-PK and ATR to form nuclear foci in response to stalled replication forks and DSBs [52]. γ-H2AX is measured by immunofluorescence, flow cytometry or western blotting and serves as a sensitive indicator of these DNA lesions [53]. The combination of PF00477736 and gemcitabine led to the formation of γ-H2AX foci (compared with none following exposure to either drug alone) [42]. Similarly, AZD7762 alone did not stimulate H2AX phosphorylation but the combination with gemcitabine led to increased γ-H2AX foci [47]. Because ATR is one of the kinases that phosphorylate H2AX, this is somewhat surprising leading to the suggestion that Chk1 inhibition with AZD7762 converts gemcitabine-induced stalled replication forks into DSBs triggering ATM and DNA-PK-mediated H2AX phosphorylation independent of ATR [47]. *In vivo* studies in mice bearing A2780 (human ovarian cancer) and MiaPaca2 (human pancreatic cancer) xenografts showed that SCH900776, administered concomitantly with gemcitabine, increased gemcitabine-induced γ-H2AX accumulation and increased the anticancer activity of gemcitabine without exacerbating gemcitabine-induced myelosuppression [44].

Radiosensitisation

Chk1 inhibitors have been postulated to be potential radiosensitisers. In DU145 (prostate) A549 and H460 (non-small-cell lung cancer) and HT29 (colon) cells radiation exposure caused an upregulation of Chk1 and AZD7762 markedly increased IR cytotoxicity [54]. The effects were greatest in p53-mutated cell lines but remained significant in p53-wild-type cell lines. Similarly, CEP3891 prevented IR-induced S and G2 arrest in U2OS cells and increased nuclear fragmentation and decreased survival compared with untreated controls [55]. AZD7762 has also been shown to be a radiosensitiser in lung cancer cell lines (H23 and PC14PE6) and in xenograft models of lung cancer with brain metastasis resulting in prolonged survival [56]. As with the knockdown studies there seem to be cell-line-specific differences in the dependence on p53; radiosensitisation by UCN01 and CEP3891 was dependent on p53 status in HCT116 cells but independent in U2OS cells [57]. This suggests that preclinical studies and clinical trials should not be restricted to p53-mutated cells or tumours; but that careful note should be made of the p53 status of cells and tumours so that results can be stratified by p53 status.

Chk1 clinical trials

The first-generation Chk1 inhibitor UCN01 underwent two single agent Phase I trials. The first trial, using a 72-hour infusion administration, resulted in significant dose-limiting toxicity (DLT) with symptomatic hypotension and hyperglycaemia at 53 mg/m^2/day [58]. There was avid plasma binding and a long variable half-life. In a second trial using a short 3-hour infusion

tolerability was improved but DLT of symptomatic hypotension at a potentially efficacious dose (95 mg/m^2) of UCN01 stopped the trial [59].

Phase I trials of the short infusion schedule of UCN01 in combination with cisplatin and topotecan [60,61] showed that UCN01 prevented cisplatin-induced S and G2 arrest (as determined by geminin staining) [60] and possible efficacy of the topotecan combination in ovarian carcinoma, which was unfortunately not confirmed in Phase II studies. In a recently reported Phase I trial of UCN01 in combination with irinotecan in 25 patients there was an increase in γ-H2AX staining and two patients with p53-mutated triple-negative breast cancer responded [62]. However, clinical trials with this drug have been discontinued.

A number of second-generation inhibitors have been through Phase I trials. The only agent used as a single-agent is LY2606368; the trial is ongoing. Most Phase I trials have been in combination with gemcitabine after a single-agent run in and have only been reported in abstract form. Haematological toxicity (neutropenia and thrombocytopenia) was commonly seen with PF00477736 and SCH900776 in combination with gemcitabine [63,64] and with AZD7762 in combination with gemcitabine and irinotecan [65,66]. Reversible myocardial ischemia with AZD7762 was seen as a DLT in combination with irinotecan and gemcitabine. Pharmacodynamic (PD) studies with SCH900776, using an *ex vivo* assay of γ-H2AX intensity in K562 cells exposed to samples of patient plasma, showed a dose-dependent prolongation of γ-H2AX [64].

ATR inhibitors

Development of inhibitors

Unlike Chk1, there has been a shortage of small-molecule ATR inhibitors, which might reflect the difficulty of assaying an enzyme that requires a complex of co-activators and regulators (described above). Caffeine was one of the earliest ATR inhibitors, but it is weak (IC_{50} = 1.1 mM) [67] and non-specific as it is a more potent inhibitor of ATM (IC_{50} = 0.2 mM) [68]. The fungal metabolite wortmannin has also been used in studies involving inhibition of ATR (IC_{50} = 1.8 μM). Like caffeine, wortmannin inhibits multiple PIKKs including ATM (IC_{50} = 150 nM). More recently, the PI-3K inhibitor PI103 was found to inhibit ATR (IC_{50} = 850 nM) as well as other PIKKs including ATM (IC_{50} = 920 nM) and DNA-PK (IC_{50} = 2 nM) and this compound does not appear to have been further investigated as an ATR inhibitor [69].

The natural product, schisandrin B is slightly more specific for ATR with an *in vitro* IC_{50} of 7.25 μM [70]. Caffeine abrogated IR-induced G2/M checkpoint arrest in A549 and MCF7 cells [68,71], and schisandrin B sensitised A549 cells to UV radiation and abrogated the UV-induced G2/M and S-phase checkpoint [70].

Recently, there has been some progress in the development of small-molecule ATR inhibitors. Vertex Pharmaceuticals has discovered a series of potent 3-amino-6-arylpyrazines that inhibit the phosphorylation of a target peptide by purified ATR with K_is in the nanomolar range including VE821 (IC_{50} = 420 nM) and cellular IC_{50} values in the sub-to-low micromolar range [72,73]. NU6027 (2,6-diamino-4-cyclohexyl-methyloxy-5-nitroso-pyrimidine), originally designed as a CDK2 inhibitor, was found to be more efficient at inhibiting cellular ATR activity (as determined by Chk1 Ser^{345} phosphorylation) than CDK2 activity (IC_{50} = 6.7 μM for ATR and >10 μM for CDK2), with no effect on ATM and DNA-PK [74]. Recently, a new 96-well plate assay based on stimulation of ATR activity by a TopBP1–estrogen-receptor fusion protein has been developed that could lead to the identification of further ATR inhibitors. Using this assay to screen 623 compounds the PI-3 K inhibitor ETP46464 was identified as a potent ATR inhibitor (IC_{50} = 25 nM) [75].

Chemo- and radio-sensitisation

Chemosensitisation and radiosensitisation have been reported for these new inhibitors. NU6027, at concentrations that were not cytotoxic *per se*, sensitised MCF7 cells to IR, temozolomide, cisplatin, camptothecin, doxorubicin and hydroxyurea, but not the antitubulin agent paclitaxel. Most interestingly, NU6027 inhibited Rad51 focus formation and was synthetically lethal with PARP inhibition or XRCC1 defects [74]. VE821 sensitised HCT116 cancer cells to gemcitabine, camptothecin, etoposide, IR and especially cisplatin by over tenfold [73]. In these studies the greatest chemosensitisation by VE821 was observed in cells deficient in either ATM or p53 [73]. Interestingly, VE821 has little effect in MCF7 cells treated with cisplatin, which is different from the effect of NU6027 [73,74].

Pharmacodynamic biomarkers

For targeted agents without intrinsic toxicity a PD biomarker is needed to demonstrate a pharmacological effect and to direct dose escalation. Assessment of PD biomarkers on serially collected tumour and surrogate tissues such as skin, blood and urine is an important way to provide molecular confirmation of target inhibition. Below is a brief introduction of the main ATR and Chk1 biomarkers, a summary of which can be found in Table 2.

TABLE 2

The main ATR and Chk1 PD biomarkers.

	Biomarkers	Function	Expected outcome	Detection methods	Reliability and/or specificity
ATR	pS345 Chk1	ATR activity	Decrease	WB, IF, IHC	+++
	pS317 Chk1	ATR/ATM? activity	Decrease	WB	++
	γ-H2AX	DNA DSBs	Decrease	WB, IF, IHC, FC	+
	pT1989 ATR	ATR autophosphorylation?	Decrease	IP/WB	++
Chk1	pS345 Chk1	ATR activity	Increase	WB, IF, IHC	+++
	pS296 Chk1	Chk1 autophosphorylation	Decrease	WB	+++
	γ-H2AX	DNA DSBs	Increase	WB, IF, FC	+
	pY15 CDK1	G2 arrest	Decrease	WB, IF	+

Abbreviations: WB, western blot; IF, immunofluorescence; IHC, immunohistochemistry; FC, flow cytometry; IP/WB, immunoprecipitation/western blot.

ATR biomarkers

Antibodies to phosphorylated ATR at Ser^{428} and Thr^{1989} have been developed but it is not clear if these are dependent on DNA-damage activation of ATR or regulate ATR activity [76,77]. ATR phosphorylates H2AX in response to replication stress and UV irradiation, but H2AX can also be phosphorylated by ATM and DNA-PK in response to IR [78] and, as described above, inhibition of Chk1 can lead to an increase in γ-H2AX and this marker is therefore not specific for ATR.

More specifically, activated ATR phosphorylates Chk1 at Ser^{317} and Ser^{345}, and both of these phosphorylation sites have commonly been used as indicators for ATR activity. Studies using a variety of DNA-damaging agents in ATR functional and kinase-dead cells indicate that pChk1 Ser^{345} was specific for ATR activity, whereas Ser^{317} was not [74]. On the basis of current evidence pChk1 Ser^{345} is the most specific cellular marker of ATR activity. Whether this or any of the other potential biomarkers can be adapted for clinical use remains to be demonstrated. As yet there are no reports of *in vivo* studies with ATR inhibitors that use PD biomarkers.

Chk1 biomarkers

Chk1 can be autophosphorylated at Ser^{296} which is closely linked to its activity. Immunoblotting of Chk1 Ser^{296} is therefore widely used as a biomarker in preclinical studies of Chk1 inhibitors [45]. However, phospho-Ser^{296} Chk1 immunohistochemistry produced high background staining and results inconsistent with immunoblotting in response to gemcitabine and the Chk1 inhibitor AZD7762 in pancreatic tumours [79]. Development of a better antibody could solve this problem.

A number of studies showed that phospho-Ser^{345} Chk1 was increased in response to Chk1 inhibition even in the absence of DNA-damaging agents [79–81]. This might be owing to inhibition of the role Chk1 has in DNA repair, leading to an accumulation of DNA DSBs that further amplifies ATR/ATM-mediated Chk1 phosphorylation [81]. The inhibitory feedback of Chk1 itself to protein phosphatase PP2A also contributes to the high level of Chk1 Ser^{345} [79,81]. Increase of Chk1 Ser^{345} was also found to be a consistent event in tumours and normal tissues (hair follicles) in mice treated with gemcitabine and Chk1 inhibitors [81].

In addition, γ-H2AX (a marker of DSB), phosphorylated histone H3 (Ser^{10}, a marker of mitotic entry), geminin (a marker of cells in S- and G2-phases), caspase-3 (a marker of apoptosis), CDK1 (pY15) and Cdc25 C (phopho-Ser^{216}) have all been used as potential PD biomarkers for Chk1 activity [19,45], but they are either indirect or inconsistent.

Comparison of ATR and Chk1 inactivation

Chemosensitisation

ATR inhibitors might not be entirely synonymous with Chk1 inhibitors, although ATR signals largely through Chk1 it phosphorylates many other targets. ATR has a greater role in DNA crosslink repair, as demonstrated by the observation that ATR siRNA but not Chk1 siRNA sensitises cells to platinum drugs [82]. The limited data available suggest that ATR inhibitors show the greatest sensitisation of platinum agents. By contrast, Chk1 inhibitors show the greatest activity in combination with the antimetabolite gemcitabine, and also enhance antitubulin agents by blocking the mitotic checkpoint [51]. Conversely, ATR inactivation does not enhance antitubulin agents [73,74,22]. Thus, inhibitors of ATR are predicted to have a different spectrum of activity clinically.

Synthetic lethality

An exciting prospect is the synthetic lethality between ATR and Chk1 inhibitors with another class of DNA repair inhibitors–the PARP inhibitors [50,74]. Such combinations need to be assessed *in vivo* to ensure that the combination does not cause synergistic toxicity as well as antitumour activity. Synthetic lethality with single-agent Chk1 and ATR inhibitors could be a viable approach in tumours associated with amplified Myc [48,49] or with specific defects, for example, those with defects in the Fanconi Anaemia (FA) pathway are hypersensitive to Chk1 siRNA and UCN01 [83]. Recently, the weak ATR inhibitor caffeine has been shown to radiosensitise selectively cells with DNA Polβ defects [84] and defects in XRCC1 confer sensitivity to single-agent NU6027 [74]. These data are significant because Myc amplification is found in several cancer types and Polβ and XRCC1 polymorphisms associated with reduced function are also associated with cancer [85,86,87].

Concluding remarks and future prospective

The preclinical data generated using ATR or Chk1 inactivation by genetic means or with chemical inhibitors are encouraging, with abrogation of the cell cycle arrest and increased tumour cell kill following exposure to a variety of DNA-damaging agents *in vitro* and *in vivo*. Data suggest that although defects in p53 are associated with greater sensitisation by either ATR or Chk1 inactivation they are not the sole determinants and defects in ATM signalling and/or other aspects of G1 control are also associated with sensitisation. This is particularly attractive because, whereas ~50% of cancers have defects in p53 signalling, virtually all are thought to have a defect in G1 control [6] and this feature distinguishes them from normal cells. It is therefore disappointing that the Chk1 inhibitors have been associated with significant toxicities in the clinical setting. However, the toxicities appear to be drug-specific rather than class-specific and might reflect off-target effects. For example, hyperglycaemia is associated with UCN01 exposure, whereas AZD7762 is associated with cardiac toxicities that have also been noted with other kinase inhibitors [88]. Despite the toxicities seen in the first clinical trials of Chk1 inhibitors, it is hoped that more specific inhibitors could be free of such toxicities. In addition, careful PD monitoring of Chk1 inhibition so that patients are not overdosed could also help reduce toxicities.

Interestingly, phosphorylation of Chk1 Ser^{345} appears to be a good biomarker for ATR and Chk1 inhibitors, with ATR inhibitors causing a reduction and inhibition of Chk1 generating an increase. We are now entering an exciting era where manipulation of the DNA-damage response can have selective antitumour activity; it will be interesting to follow the parallel development of ATR and Chk1 inhibitors through the clinic.

Conflicts of interest

FKM and NJC are in receipt of research funding from Vertex Pharmaceuticals (MRC Industrial CASE studentship). PAS is in receipt of research materials from Vernalis (Chk1 inhibitor).

References

1 Vilenchik, M.M. and Knudson, A. (2003) Endogenous DNA double-strand breaks: production, fidelity of repair, and induction of cancer. *Proc. Natl Acad. Sci. U. S. A.* 100, 12871–12876
2 Poehlmann, A. and Roessner, A. (2010) Importance of DNA damage checkpoints in the pathogenesis of human cancers. *Pathol. Res. Pract.* 206, 591–601
3 Shiloh, Y. (2006) The ATM-mediated DNA-damage response: taking shape. *Trends Biochem. Sci.* 31, 402–410
4 Smith, J. *et al.* (2010) The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. *Adv. Cancer Res.* 108, 73–112
5 Sherr, C.J. (1996) Cancer cell cycles. *Science* 274, 1672–1674
6 Massague, J. (2004) G1 cell-cycle control and cancer. *Nature* 432, 298–306
7 Garrett, M.D. and Collins, I. (2011) Anticancer therapy with checkpoint inhibitors: what, where and when? *Trends Pharm. Sci.* 32, 308–316
8 Brown, E.J. and Baltimore, D. (2000) ATR disruption leads to chromosomal fragmentation and early embryonic lethality. *Genes Dev.* 14, 397–402
9 Takai, H. *et al.* (2000) Aberrant cell cycle checkpoint function and early embryonic death in Chk1(−/−) mice. *Genes Dev.* 14, 1439–1447
10 Liu, Q. *et al.* (2000) Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes Dev.* 14, 1448–1459
11 Zachos, G. *et al.* (2003) Lethal errors in checkpoint control – life without Chk1. *Cell Cycle* 2, 14–16
12 O'Driscoll, M. *et al.* (2003) A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. *Nat. Genet.* 33, 497–501
13 Takemura, H. *et al.* (2006) Defective Mre11-dependent activation of Chk2 by ataxia telangiectasia mutated in colorectal carcinoma cells in response to replication-dependent DNA double strand breaks. *J. Biol. Chem.* 281, 30814–30823
14 Flynn, R.L. and Zou, L. (2011) ATR: A master conductor of cellular responses to DNA replication stress. *Trends Biochem. Sci.* 36, 133–140
15 Zhang, J. *et al.* (2009) Prevalence and functional analysis of sequence variants in the ATR checkpoint mediator Claspin. *Mol. Cancer Res.* 7, 1510–1516
16 Mamely, I. *et al.* (2006) Polo-like kinase-1 controls proteasome-dependent degradation of Claspin during checkpoint recovery. *Curr. Biol.* 16, 1950–1955
17 Walker, M. *et al.* (2009) Chk1 C-terminal regulatory phosphorylation mediates checkpoint activation by de-repression of Chk1 catalytic activity. *Oncogene* 28, 2314–2323
18 Wilsker, D. *et al.* (2008) Essential function of Chk1 can be uncoupled from DNA damage checkpoint and replication control. *Proc. Natl Acad. Sci. U. S. A.* 105, 20752–20757
19 Dai, Y. and Grant, S. (2010) New insights into checkpoint kinase 1 in the DNA damage response signaling network. *Clin. Cancer Res.* 16, 376–383
20 Shapiro, G.I. and Harper, J.W. (1999) Anticancer drug targets: cell cycle and checkpoint control. *J. Clin. Invest.* 104, 1645–1653
21 Duensing, A. *et al.* (2006) A role of the mitotic spindle checkpoint in the cellular response to DNA replication stress. *J. Cell Biochem.* 99, 759–769
22 Cliby, W.A. *et al.* (1998) Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints. *EMBO J.* 17, 159–169
23 Nghiem, P. *et al.* (2001) ATR inhibition selectively sensitizes G1 checkpoint-deficient cells to lethal premature chromatin condensation. *Proc. Natl Acad. Sci. U. S. A.* 98, 9092–9097
24 Carrassa, L. *et al.* (2004) Chk1, but not Chk2, is involved in the cellular response to DNA damaging agents: differential activity in cells expressing or not p53. *Cell Cycle* 3, 1177–1181
25 Flatten, K. *et al.* (2005) The role of checkpoint kinase 1 in sensitivity to topoisomerase I poisons. *J. Biol. Chem.* 280, 14349–14355
26 Ganzinelli, M. *et al.* (2008) Checkpoint kinase 1 down-regulation by an inducible small interfering RNA expression system sensitized in vivo tumors to treatment with 5-fluorouracil. *Clin. Cancer Res.* 14, 5131–5141
27 Pan, Y. *et al.* (2009) Knockdown of Chk1 sensitizes human colon carcinoma HCT116 cells in a p53-dependent manner to lidamycin through abrogation of a G2/M checkpoint and induction of apoptosis. *Cancer Biol. Ther.* 8, 1559–1566
28 Sangster-Guity, N. *et al.* (2011) ATR mediates cisplatin resistance in a p53 genotype-specific manner. *Oncogene* 30, 2526–2533
29 Kim, W.J. *et al.* (2002) Aberrant methylation of the ATM promoter correlates with increased radiosensitivity in a human colorectal tumor cell line. *Oncogene* 21, 3864–3871
30 Karnitz, L.M. *et al.* (2005) Gemcitabine-induced activation of checkpoint signaling pathways that affect tumor cell survival. *Mol. Pharmacol.* 68, 1636–1644
31 Zenvirt, S. *et al.* (2010) Status of p53 in human cancer cells does not predict efficacy of CHK1 kinase inhibitors combined with chemotherapeutic agents. *Oncogene* 29, 6149–6159
32 Cho, S.H. *et al.* (2005) Chk1 is essential for tumor cell viability following activation of the replication checkpoint. *Cell Cycle* 4, 131–139
33 Ma, C.X. *et al.* (2011) Death by releasing the breaks: CHK1 inhibitors as cancer therapeutics. *Trends Mol. Med.* 17, 88–96
34 Ashwell, S. and Zabludoff, S. (2008) DNA damage detection and repair pathways – recent advances with inhibitors of checkpoint kinases in cancer therapy. *Clin. Cancer Res.* 14, 4032–4037
35 Wagner, J.M. and Kaufmann, S.H. (2010) Prospects for the use of ATR inhibitors to treat cancer. *Pharmaceuticals* 3, 1311–1334
36 Courage, C. *et al.* (1995) Comparison of ability of protein kinase C inhibitors to arrest cell growth and to alter cellular protein kinase C localisation. *Br. J. Cancer* 71, 697–704
37 Sorensen, C.S. *et al.* (2003) Chk1 regulates the S phase checkpoint by coupling the physiological turnover and ionizing radiation-induced accelerated proteolysis of Cdc25A. *Cancer Cell* 3, 247–258
38 Luo, Y. *et al.* (2001) Blocking Chk1 expression induces apoptosis and abrogates the G2 checkpoint mechanism. *Neoplasia* 3, 411–419
39 Mack, P.C. *et al.* (2003) Cell cycle-dependent potentiation of cisplatin by UCN-01 in non-small-cell lung carcinoma. *Cancer Chemother. Pharmacol.* 51, 337–348
40 Furuta, T. *et al.* (2006) p21CDKN1A allows the repair of replication-mediated DNA double-strand breaks induced by topoisomerase I and is inactivated by the checkpoint kinase inhibitor 7-hydroxystaurosporine. *Oncogene* 25, 2839–2849
41 Parsels, L.A. *et al.* (2009) Gemcitabine sensitization by checkpoint kinase 1 inhibition correlates with inhibition of a Rad51 DNA damage response in pancreatic cancer cells. *Mol. Cancer Ther.* 8, 45–54
42 Blasina, A. *et al.* (2008) Breaching the DNA damage checkpoint via PF-00477736, a novel small-molecule inhibitor of checkpoint kinase 1. *Mol. Cancer Ther.* 7, 2394–2404
43 Zabludoff, S.D. *et al.* (2008) AZD7762, a novel checkpoint kinase inhibitor, drives checkpoint abrogation and potentiates DNA-targeted therapies. *Mol. Cancer Ther.* 7, 2955–2966
44 Guzi, T.J. *et al.* (2011) Targeting the replication checkpoint using SCH 900776, a potent and functionally selective CHK1 inhibitor identified via high content screening. *Mol. Cancer Ther.* 10, 591–602
45 Walton, M.I. *et al.* (2010) The preclinical pharmacology and therapeutic activity of the novel CHK1 inhibitor SAR-020106. *Mol. Cancer Ther.* 9, 89–100
46 Matthews, D.J. *et al.* (2007) Pharmacological abrogation of S-phase checkpoint enhances the anti-tumor activity of gemcitabine *in vivo*. *Cell Cycle* 6, 104–110
47 McNeely, S. *et al.* (2010) Chk1 inhibition after replicative stress activates a double strand break response mediated by ATM and DNA-dependent protein kinase. *Cell Cycle* 9, 995–1004
48 Ferrao, P.T. *et al.* (2011) Efficacy of CHK inhibitors as single agents in MYC-driven lymphoma cells. *Oncogene* 10.1038/onc.2011.358
49 Hoglund, A. *et al.* (2011) Therapeutic implications for the induced levels of chk1 in myc-expressing cancer cells. *Clin. Cancer Res.* 17, 7067–7079
50 Mitchell, C. *et al.* (2010) Poly(ADP-ribose) polymerase 1 modulates the lethality of CHK1 inhibitors in carcinoma cells. *Mol. Pharmacol.* 78, 909–917
51 Zhang, C. *et al.* (2009) PF-0077736 mediates checkpoint kinase 1 signaling pathway and potentiates docetaxel-induced efficacy in xenografts. *Clin. Cancer Res.* 15, 4460–4630
52 Redon, C.E. *et al.* (2010) Histone {gamma}H2AX and Poly(ADP-Ribose) as clinical pharmacodynamic biomarkers. *Clin. Cancer Res.* 16, 4532–4542
53 Dickey, J.S. *et al.* (2009) H2AX: functional roles and potential applications. *Chromosoma* 118, 683–692
54 Mitchell, J.B. *et al.* (2010) In vitro and *in vivo* radiation sensitization of human tumor cells by a novel checkpoint kinase inhibitor, AZD7762. *Clin. Cancer Res.* 16, 2076–2084
55 Syljuasen, R.G. *et al.* (2004) Inhibition of Chk1 by CEP-3891 accelerates mitotic nuclear fragmentation in response to ionizing radiation. *Cancer Res.* 64, 9035–9040
56 Yang, H. *et al.* (2011) Inhibition of checkpoint kinase 1 sensitizes lung cancer brain metastases to radiotherapy. *Biochem. Biophys. Res. Commun.* 406, 53–58
57 Petersen, L. *et al.* (2010) p53-dependent G(1) arrest in 1st or 2nd cell cycle may protect human cancer cells from cell death after treatment with ionizing radiation and Chk1 inhibitors. *Cell Prolif.* 43, 365–371
58 Sausville, E.A. *et al.* (2001) Phase I trial of 72-hour continuous infusion UCN-01 in patients with refractory neoplasms. *J. Clin. Oncol.* 19, 2319–2333
59 Dees, E.C. *et al.* (2005) A phase I and pharmacokinetic study of short infusions of UCN-01 in patients with refractory solid tumors. *Clin. Cancer Res.* 11, 664–671

60 Perez, R.P. *et al.* (2006) Modulation of cell cycle progression in human tumors: a pharmacokinetic and tumor molecular pharmacodynamic study of cisplatin plus the Chk1 inhibitor UCN-01 (NSC 638850). *Clin. Cancer Res.* 12, 7079–7085

61 Hotte, S.J. *et al.* (2006) Phase I trial of UCN-01 in combination with topotecan in patients with advanced solid cancers: a Princess Margaret Hospital Phase II Consortium study. *Ann. Oncol.* 17, 334–340

62 Fracasso, P.M. *et al.* (2011) A Phase 1 study of UCN-01 in combination with irinotecan in patients with resistant solid tumor malignancies. *Cancer Chemoth. Pharm.* 67, 1225–1237

63 Brega, N. *et al.* (2010) Phase 1 clinical trial of gemcitablne (GEM) in combination with PF-00477736 (PF-736), a selective inhibitor of CHK1 kinase. *ASCO Annual Meeting*, Chicago

64 Daud, A. *et al.* (2010) A Phase I dose-escalation study of SCH-900776, a selective inhibitor of checkpoint 1 (CHK1), in combination with gemcitabine (Gem) in subjects with advanced solid tumours. *ASCO Annual Meeting*, Chicago

65 Sausville, E. *et al.* (2011) Phase 1 dose-escalation study of AZD7762 in combination with gemcitabine (gem) in patients (pts) with advanced solid tumours. *ASCO Annual Meeting*

66 Ho, A.L. *et al.* (2011) Phase 1, open-label, dose-escalation study of AZD7762 in combination with irinotecan (irino) in patients (pts) with advanced solid tumours. *ASCO Annual Meeting*

67 Cortez, D. (2003) Caffeine inhibits checkpoint responses without inhibiting the ataxia-telangiectasia-mutated (ATM) and ATM- and Rad3-related (ATR) protein kinases. *J. Biol. Chem.* 278, 37139–37145

68 Sarkaria, J.N. *et al.* (1999) Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. *Cancer Res.* 59, 4375–4382

69 Knight, Z.A. *et al.* (2006) A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. *Cell* 125, 733–747

70 Nishida, H. *et al.* (2009) Inhibition of ATR protein kinase activity by schisandrin B in DNA damage response. *Nucleic Acids Res.* 37, 5678–5689

71 Essmann, F. *et al.* (2004) Apoptosis resistance of MCF-7 breast carcinoma cells to ionizing radiation is independent of p53 and cell cycle control but caused by the lack of caspase-3 and a caffeine-inhibitable event. *Cancer Res.* 64, 7065–7072

72 Charrier, J.D. *et al.* (2011) Discovery of potent and selective inhibitors of ataxia telangiectasia mutated and Rad3 related (ATR) protein kinase as potential anticancer agents. *J. Med. Chem.* 54, 2320–2330

73 Reaper, P.M. *et al.* (2011) Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. *Nat. Chem. Biol.* 7, 428–430

74 Peasland, A. *et al.* (2011) Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. *Br. J. Cancer* 105, 372–381

75 Toledo, L.I. *et al.* (2011) A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. *Nat. Struct. Mol. Biol.* 18, 721–727

76 Nam, E.A. *et al.* (2011) T1989 phosphorylation is a marker of active ataxia telangiectasia-mutated and rad3-related (ATR) kinase. *J. Biol. Chem.* 286, 28707–28714

77 Liu, S. *et al.* (2011) ATR autophosphorylation as a molecular switch for checkpoint activation. *Mol. Cell* 43, 192–202

78 Rossetto, D. *et al.* (2010) Epigenetic modifications in double-strand break DNA damage signaling and repair. *Clin. Cancer Res.* 16, 4543–4552

79 Parsels, L.A. *et al.* (2011) Assessment of chk1 phosphorylation as a pharmacodynamic biomarker of chk1 inhibition. *Clin. Cancer Res.* 17, 3706–3715

80 Morgan, M.A. *et al.* (2010) Mechanism of radiosensitization by the Chk1/2 inhibitor AZD7762 involves abrogation of the G2 checkpoint and inhibition of homologous recombinational DNA repair. *Cancer Res.* 70, 4972–4981

81 Leung-Pineda, V. *et al.* (2006) Phosphorylation of Chk1 by ATR is antagonized by a Chk1-regulated protein phosphatase 2A circuit. *Mol. Cell Biol.* 26, 7529–7538

82 Wagner, J.M. and Karnitz, L.M. (2009) Cisplatin-induced DNA damage activates replication checkpoint signaling components that differentially affect tumor cell survival. *Mol. Pharmacol.* 76, 208–214

83 Chen, C.C. *et al.* (2009) CHK1 inhibition as a strategy for targeting Fanconi Anemia (FA) DNA repair pathway deficient tumors. *Mol. Cancer* 8, 24

84 Neijenhuis, S. *et al.* (2010) Targeted radiosensitization of cells expressing truncated DNA polymerase {beta}. *Cancer Res.* 70, 8706–8714

85 Kiyohara, C. *et al.* (2006) Association of genetic polymorphisms in the base excision repair pathway with lung cancer risk: a meta-analysis. *Lung cancer* 54, 267–283

86 Starcevic, D. *et al.* (2004) Is there a link between DNA polymerase beta and cancer? *Cell Cycle* 3, 998–1001

87 Dang, C.V. *et al.* (1999) Function of the c-Myc oncogenic transcription factor. *Exp. Cell Res.* 253, 63–77

88 Orphanos, G.S. *et al.* (2009) Cardiotoxicity induced by tyrosine kinase inhibitors. *Acta Oncol.* 48, 964–970

89 Dai, Y. *et al.* (2002) Combined treatment with the checkpoint abrogator UCN-01 and MEK1/2 inhibitors potently induces apoptosis in drug-sensitive and -resistant myeloma cells through an IL-6-independent mechanism. *Blood* 100, 3333–3343

90 Lara, P.N. *et al.* (2005) The cyclin-dependent kinase inhibitor UCN-01 plus cisplatin in advanced solid tumors: a California cancer consortium phase I pharmacokinetic and molecular correlative trial. *Clin. Cancer Res.* 11, 4444–4450