Cell cycle control in cancer

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Abstract | **Cancer is a group of diseases in which cells divide continuously and excessively. Cell division is tightly regulated via multiple evolutionary-conserved cell cycle control mechanisms to ensure the production of two genetically identical cells. Cell cycle checkpoints operate as DNA surveillance mechanisms that prevent the accumulation and propagation of genetic errors during cell division. Checkpoints can delay cell cycle progression or, in response to irreparable DNA damage, induce cell cycle exit or cell death. Cancer-associated mutations that perturb cell cycle control allow continuous cell division chiefly by compromising the ability of cells to exit the cell cycle. Continuous rounds of division however, creates increased reliance on other cell cycle control mechanisms to prevent catastrophic levels of damage and maintain viability . New detailed insights into cell cycle control mechanisms and their role in cancer reveal how these dependencies can be best exploited in cancer treatment.**

[H1] Introduction

Cell cycle control is mainly focused on two events, the replication of genomic DNA and its subsequent segregation between daughter cells, which in eukaryotic cells occur during distinct cell cycle phases. It is often assumed that cancer cells undergo uncontrolled cell cycle progression and that most, if not all, cell cycle checkpoints need to be defective for a cell to become cancerous. However, a large body of recent work has provided strong evidence that only specific aspects of cell cycle control need to be disrupted for cancer cells to continue to divide. This work indicates that cancer cells are mainly compromised in their ability to exit the cell cycle, and consequently continue to divide, rather than undergoing uncontrolled cell division. Importantly, it also suggests that most cell cycle control functions are essential for cancer cell viability. These findings underscore the fundamental difference between the DNA damage checkpoint and DNA replication stress checkpoint responses: to prevent the accumulation and propagation of DNA damage and to prevent replication stress-induced DNA damage, respectively. Understanding these differences is essential in the context of cancer. The DNA damage checkpoint is often compromised in cancer cells , allowing continuous cell division despite the accumulation of genetic errors. In contrast, genes involved in the replication stress checkpoint are rarely mutated in cancer cells, as many cancers become increasingly dependent on checkpoint function to tolerate high levels of replication stress. Similarly cancer cells also rely on a functional mitotic checkpoint to prevent catastrophic chromosome mis-segregation[1] **[Ref 132: Kops 2004]** . This shift in the paradigm of the role of checkpoints in cancer provides a better understanding of cell cycle control in cancer and reveals particular dependency on specific checkpoint functions, creating new therapeutic opportunities. In this Review, we discuss our current understanding of cell cycle control mechanisms and outline the specific functions of the various cell cycle checkpoints that prevent or correct errors to avoid propagation of genetic alterations. We briefly describe cell cycle control pathways, such as cyclins and Cyclin Dependent Kinases (CDKs), G1–S transcriptional regulation, checkpoint signaling and the ubiquitin ligase regulatory pathways, but refer readers to more comprehensive reviews on these specific topics (see references for G1-S transcription[2,3], replication stress[4] , DNA damage[5] , CDK[6] , mitotic checkpoint[7]). We point out which aspects of cell cycle control are frequently compromised in cancer cells to allow continued cell division, and on which cancer cells become increasingly dependent for their viability. Finally, we share our views on how these dependencies on cell cycle control and checkpoint mechanisms can be targeted therapeutically.

[H1] The cell division cycle

In unicellular and multicellular eurokaryotes, cell division is controlled by a complex network of regulatory mechanisms, checks and balances to ensure that no mistakes are made before a cell is allowed to enter and progress through the cell cycle in order to divide.

[H2] Cell cycle phases. The mitotic cell cycle is divided into two distinct stages, interphase and M phase (Figure 1). This allows for the temporal separation of the duplication of cellular content during interphase and its separation into two genetically identical daughter cells in mitosis. The complex network of regulatory elements that form the cell cycle has one goal: the timely and accurate duplication and segregation of the genomic DNA. DNA replication takes place in interphase during S phase (Synthesis phase),defined as the time in interphase during which DNA replication has been initiated but not completed. The periods of interphase that separate S phase from M-phase have historically been named Gap phases, or G1 before S phase and G2 after S phase, based on the evident observation that these are gaps inbetween the two main events, duplication and segragation of the DNA. However, these phases are key periods for cell cycle regulation and include the crucial decision to enter the cell cycle during G1, and to initiate the process that leads to chromosome segregation during G2.

[H2] Cell cycle entry and progression. Before S-phase, in the pre-replicative G1 phase, there is a decision window during which cells can commit to initiate DNA replication and enter the cell cycle or stay in G1 phase (Figure 1). During G1 cells can also exit the cell cycle into a non-proliferative state known as quiescence, or G0. The vast majority of cells in an adult body are in a non-proliferative state and would need to transit into G1 before being able to initiate DNA replication and enter the cell cycle. Upon completion of DNA replication, there is another decision window during the post-replicative G2 phase. During this window, cells can commit to enter M phase by initiating chromatin condensation and the central alignment of chromosomes. M phase serves the dual function of both accurately separating the duplicated DNA (mitosis) and dividing the entire cellular content into two new daughter cells (cytokinesis). During M phase, cells commit to the segregation of the genetic material and reset the cell cycle to return to interphase.

[H2] CDKs and cell cycle regulated transcription drive cell cycle progression. The key regulator of cell cycle processes is Cyclin-Dependent-kinase (CDK) activity (Box 1). Specific cyclins accumulate during different stages of the cell cycle, driven by cell cycle regulated transcription and the inhibition of protein degradation. In turn, cell cycle regulated transcription depends on CDK activity for activation. This inter-dependence creates a regulatory network that ensures that cell cycle progression is sequential and unidirectional[8,9]. Cell cycle-dependent changes in CDK activity drive cell cycle entry, progression and completion. CDK activity in the pre-replicative G1 phase is required for initiation of DNA replication and thereby commitment to cell cycle entry. Subsequently, during the post-replicative G2 phase, CDK activity plays a key role in preparing for chromosome segregation, via initiating condensation, permeabilization of the nuclear envelope and central alignment of the replicated chromosomal DNA. Inactivation of CDK activity,

via the Anaphase Promoting Complex/Cyclosome **[G]** (APC/C^{cdc20})-dependent targeted destruction of cyclins, coincides with chromosome segregation and return to interphase[10].

[H2] Choice to enter the cell cycle. There are several stages during the pre-replicative G1 phase. In early G1 phase, the cell faces a 'choice' to either remain in a cell cycle state or exit the cell cycle into quiescence. A large body of research has shown that CDK activity plays a central role in this decision[2,3]. Growth-dependent CDK activity (D-type cyclin CDK) creates a decision window during which the cell can commit to initiate replication and enter a new cell cycle (Box 1). Inhibition or absence of this CDK activity can take the cell out of the G1 state and into the quiescence **[G]** state.

Commitment to replication initiation and S phase entry is closely linked to activation of the E2F-dependent transcriptional network (reviewed in[11]). The transcriptional network is activated in the G1 phase, drives S phase entry and is subsequently inactivated during S phase. The E2F-transcriptional network includes many genes that encode key proteins in cell cycle and DNA replication control, but also genome protection mechanisms and growth. E2F-dependent transcriptional regulation depends on a family of transcription factors (E2F1-8) and their co-regulators the pocket proteins (retinoblastoma protein (RB), p107 and p130). Activation of E2F transcription is initiated by growth signals and mitogens that stimulate CDK activity, through antagonizing cyclin-dependent kinase inhibitors (CKIs) activity and by the expression of D-type cyclins. The upstream signalling largely depends on the MAPK pathway (Ras-Raf-Mek-Erk) feeding into the transcriptional regulator c-Myc, which controls the expression of critical positive cell cycle regulators.

[H2] Decision to enter the cell cycle. The E2F inhibitor RB plays a central role in the 'decision' to enter a new cell cycle[2,3]. During the pre-replicative G1 phase, RB keeps E2F-dependent transcription inactive. However, RB is inactivated by CDK-dependent phosphorylation as these enzymes become more active, resulting in E2F-dependent transcription. This results in the expression of E-type cyclins, which further increases the overall activity of CDKs. The increased CDK activity induces further phosphorylation of RB, which fully inactivates this E2F inhibitor and allows the expression of E2F-dependent genes. This positive feedback loop creates a decision window to enter S phase by driving the accumulation of E-type and A-type cyclin CDK activity required to initiate DNA replication and cell cycle entry[12].

Initially, RB inactivation was thought to be driven by an increase in CDK activity associated with D-type cyclins/CDK4-6, and subsequently with E-type cyclin/CDK2 during the G1 phase[13]. However, recent data suggests that D-type cyclin/CDK might not be involved in the initial inactivation of RB, but rather 'primes' a cell for entry into the cell cycle by preventing its exit from, or promoting its entry into, the G1 phase potentially by controlling the metabolic state of the cell [14–16]. In this model, mono-phosphorylation of RB by

D-type cyclin/CDK activity does not activate E2F-dependent transcription, but creates a state between cell cycle exit (quiescence) and the cell cycle entry decision window. During this state, hyperphosphorylation of RB by E-type cyclin/CDK activity activates E2F-dependent transcription, initiating a positive feedback loop, which drives cells into a decision window to commit to a new cell cycle. D-type cyclin/CDK interaction with RB may be required for hyper phopshorylation [17]. Importantly, this recent insight supports a new model in which an increase in D-type cyclin/CDK activity, seen in many cancers, may not directly drive proliferation but rather 'primes' a cell for cell cycle entry, by preventing exit[18]. Most recently the sequential cooperation between D-type cyclin/CDK and E-type cyclin/CDK in the G1/S transition has been challenged, suggesting that E-type cyclin/CDK does not play a significant role [19,20]. Overall, the specific role for D and E-type cyclin-CDK activity in the timing of cell-cycle events through the G1/S transition remain an active field of research and for more details of the different models proposed we refer the readers to more specific reviews[2,3].

***[H2] Commitment to enter the cell cycle*.** Once the decision to enter a new cell cycle has been made, the initiation of DNA replication (i.e. firing of replication origins) and thereby the 'commitment' to initiate S phase depends on A-type cyclin together with CDK2 activity. Accumulation of A-type cyclin CDK activity does not just depends on E2F-dependent induction, but also on the inactivation of the G1-phase-specific APC/C^{CDH1}, also known as the cyclosome, which targets A-type cyclins for destruction[21]. Inactivation of the APC/C^{CDH1} depends both on the E2F-dependent accumulation of E-type cyclin CDK2 activity and the APC/C^{CDH1} inhibitor EMI1[21,22]. Only when E2F-dependent transcription is active and APC/C^{CDH1} is inactive a cell can pass the commitment point. So activation of E2F-dependent transcription creates a decision window where cells can accumulate enough A-type cyclin CDK activity to initiate replication and commit to a new cell cycle (Box 1). CDK activity plays a key role in the separation of licensing and firing of replication origins to ensure that the genome is replicated in a timely manner and occurs 'once and only once' per cell cycle (Box 2).

[H2] Decision to enter mitosis. Activation of CDK1 plays a central role in the decision to enter mitosis. The activity of CDK1 is subject to multiple levels of regulation to ensure rapid and timely mitotic entry (reviewed in[23]). Firstly, CDK1 activation requires association with A-type or B-type cyclins, which gradually accumulate in the cell from S phase onwards due to cell-cycle specific transcription. Secondly, CDK1 is maintained in an inhibited state via phosphorylation by the Wee1 and Myt1 kinases. Activation is triggered by removal of these inhibitory phosphorylations by Cdc25 phosphatase. The balance between Wee1/Myt1 and Cdc25 activity levels ultimately determines when a cell progresses into mitosis and is itself regulated by multiple regulatory circuits[23]. In parallel, phosphatase inhibition by the Greatwall kinase pathway enables

phosphorylation of CDK1 substrates[24]. These multiple layers of regulation mean that the steady increase of A and B-type cyclins throughout G2 phase is translated into a fast, bistable switch in CDK1 activity at mitotic entry[25] (Box 1).

[H2] Commitment to mitotic entry. Once the threshold levels for CDK1 activity are reached, entry into mitosis is triggered by widespread phosphorylation of over a thousand CDK1 substrates[26,27]. In parallel, CDK1 activates the mitotic kinases, Plk1 and Aurora A and B, which phosphorylate additional mitotic substrates[28,29]. The wave of mitotic phosphorylation triggers structural changes to every cell compartment and primes the cell for DNA separation and division. During prophase, rising CDK1 activity[30] in the cytoplasm triggers cell rounding[31] and centrosome separation[32]. At the same time, rapid nuclear import of activated CDK1/CycB induces condensation of chromosomes, activation of the APC/C, nucleolar disassembly and permeabilization of the nuclear envelope [33–35]. Loss of nuclear membrane integrity at the start of prometaphase allows free mixing between nuclear and cytoplasmic compartments and brings the condensed chromosomes into contact with microtubules to assemble the mitotic spindle. Microtubule filaments emanating from each centrosome form bilateral attachments to each sister chromatid at specialized central regions called kinetochores[36,37], resulting in the eventual alignment of the chromosomes in the centre of the cell at metaphase.

[H2] Commitment to mitotic exit. During prometaphase, Cyclin A is degraded by the 26S proteasome, following ubiquitation by the APC/C^{cdc20} [38,39] (Box 1). CDK1 activity remains high, due to its continued interaction with B-type cyclins [33]. Following chromosome alignment at metaphase, mitotic exit is initiated by the APC/C^{cdc20} (reviewed in [40]), which at the same time promotes ubiquitination of B-type cyclins and targets them for proteolytic destruction. Protein phosphatases reverse the action of CDK1 by widespread substrate dephosphorylation[41]. As with mitotic entry, exit from mitosis is rapid and irreversible. APC/C^{cdc20} activation triggers a chain of events including separation of sister chromatids[42], spindle elongation to pull them apart[43] and formation of an acto-myosin contractile ring to divide the cell into two[44]. At the same time, destruction of the sole remaining Cyclin type (B) reduces CDK activity to zero and effectively resets the cell cycle in the two daughter cells back to the pre-replicative G1 phase.

[H1] Cell cycle Checkpoints

Cells rely on cell cycle checkpoints to prevent the accumulation and propagation of genetic errors during cell division. These cell cycle control checkpoints depend on evolutionarily conserved signalling pathways that

monitor DNA damage during interphase, loss of DNA replication fork integrity during S phase and incomplete spindle assembly during M phase (Fig. 2; Box 3).

[H2] DNA damage checkpoint. Throughout interphase, the occurrence of DNA double strand breaks (DSBs) trigger a rapid signalling response that depends on the checkpoint protein kinase Ataxia Telangectasia Mutated (ATM). The response results in changes to ongoing transcription levels and patterns, mobilisation of DNA repair machinery, and interplay with the cell cycle regulators resulting in slowing or arrest of movement through the cell cycle[5,45]. The primary role of this cellular response to DNA damage is to prevent the accumulation and propagation of genetic errors during cell division. ATM is activated by the DNA damage sensor complex MRN (Mre1, Rad50, Nbs1) and phosphorylates a great number of substrates[46,47], but key targets for cell cycle control include the Chk2 protein kinase and the p53 transcription factor [48–50]. p53 activates the CDK inhibitor p21, leading to the inhibition of Cyclin-CDK complexes mainly in G1 to prevent S phase entry. In the S and G2 phases Chk2 degrades Cdc25 proteins [51], thus reinforcing WEE1 kinase-dependent inhibitory phosphorylation of CDK1 to prevent mitotic entry. p53 and ATM are not as critical during S and G2 phases for slowing or halting cell cycle progression due to a certain level of redundancy with the DNA replication checkpoint**.**

DNA end-resection at DSBs is regulated by the cell cycle, and this influences the choice of both the repair pathway and DNA damage signalling cascade[52]. During G1, DSB repair mechanisms are based largely on Non-Homologous-Ends-Joining **[G]** (NHEJ) due to lack of DNA end resection **[G]**. In the S and G2 phases, however, in response to DSBs the resection of DNA ends during DSBs allows for repair via Homologous Recombination **[G]** (HR). DNA ends resection generates single stranded DNA (ssDNA) at DSBs and this signals to ATR (Ataxia Telangectasia and Rad3 related) and Chk1, the main kinases of the replication stress checkpoint response pathway, thus reinforcing the checkpoint response to DNA damage.

The severity of the damage dictates the choice of cell fate (Figure 2). However, the threshold of what constitutes 'severe' varies depending on the environment and cell type, so cell fate decisions are not uniform or always easily predictable. Cells can re-enter the cell cycle, permanently exit the cell cycle and become senescent, or undergo apoptosis. In the pre-replicative G1 phase, if the cell does not undergo apoptosis, the cell cycle arrest is either reversible (quiescence) or irreversible (senescence)[53]. In contrast, in S and G2 phases, long term arrest mainly results in an irreversible exit from the cell cycle through senescence or apoptosis. The inability to re-enter the cell cycle are largely through mechanisms controlled by p53 (for review, see[53,54]). **[Au: Perhaps it woud be useful to cite ref 107 here? (Hafner et al 2019); please amend as you best see fit]**

[H2] DNA replication stress checkpoint. The DNA replication stress checkpoint only functions during S phase. DNA replication forks encounter many potential obstacles that obstruct their progression for successful and faithful genome duplication. Such impediments can cause DNA replication forks to progress slowly or stall, which exposes ssDNA, and is defined as replication stress [55]. DNA replication stress can be experienced by

cells under normal physiological conditions or due to exogenous factors [56]. It can be induced by many factors including: deregulation of components required for DNA synthesis; a decrease or increase in the frequency of replication initiation; and factors that block replication forks such as unusual DNA structures, DNA damage forming bulky lesions or the collision with transcription machinery [57].

The accumulation of ssDNA, arising from the decoupling of the polymerase and the helicase, is the signal for activation of the checkpoint protein kinase ATR and its downstream effector Chk1 (Box 2)[58–60]. This triggers an elaborate local and global cellular response. Replication stress is not DNA damage and the main function of the DNA replication checkpoint response is actually to prevent replication stress-induced DNA damage. An important part of the response is preventing mitotic entry to allow more time for replication to be completed. The checkpoint controls cell cycle progression through restricting CDK activity, primarily through Chk1-dependent phosphorylation of Cdc25, which results in its proteasomal degradation[61] and WEE1, by promoting 14-3-3 binding[62,63]. This results in WEE1 kinase-dependent inhibitory phosphorylation of CDK1. To prevent replication stress-induced DNA damage and ensure complete duplication of genomic material before entry into mitosis, the checkpoint response regulates at least three aspects of DNA replication: firstly, by blocking late origin firing; secondly, by stalling ongoing replication forks; and finally by stabilizing stalled replication forks[64–71]. This prevents the initiation of replication at new sites, limits the overall rate of replication and allows replication forks to recover and resume once the impediments are dealt with, ensuring all areas of the genome are replicated [65,72–76]. This is a temporary solution as a more prolonged stalling of a replication fork can cause fork collapse whereby replisome components dissociate from the DNA, leading to double strand breaks (DSBs). Once this happens the DNA damage checkpoint response will be activated to repair the damage.

[H2] Spindle assembly checkpoint (SAC). The SAC functions in M phase to ensure that replicated DNA is partitioned equally between two daughter cells. Errors in mitotic spindle formation (for example, uncaptured chromosomes or two sister chromatids attached to the same spindle pole) result in incorrect chromosome segregation and chromosomal gains or losses in daughter cells. The SAC prevents this by acting as a surveillance mechanism for unattached or incorrectly attached kinetochores. The SAC machinery is a multi-protein complex that is recruited to any kinetochores unbound to microtubules following phosphorylation by Aurora B and CDK1[7,77,78]. The SAC catalyses the formation of the diffusible mitotic checkpoint complex (MCC - consisting of Mad2, BubR1 & Cdc20), which acts as a potent inhibitor of APC/C^{cdc20} and anaphase initiation [79–82]. Once all kinetochores are attached and bi-oriented, lack of SAC activity leads to disassembly of the MCC, freeing up Cdc20 to act as a co-activator of APC/C [83,84]. A single unattached kinetochore gives sufficient signal to maintain APC/C inhibition and M phase arrest[85]. In this way, the SAC acts as a ‘delay’ signal, prolonging mitosis until bipolar spindle attachment is achieved by all chromosomes. Since mitotic entry is irreversible, cells cannot exit mitosis until the SAC is satisfied. This means that prolonged SAC activation, for example in cells treated with microtubule disrupting agents, become arrested in mitosis, for as long as 24 hours[86]. In cases

where chromosome biorientation is not resolved following prolonged mitotic arrest, cells follow two paths: either apoptosis via caspase activation[87] or 'slippage' where cells exit M phase without chromosome segregation and enter the next cell cycle as a single tetraploid cell[88]. Mitotic slippage occurs because basal levels of B-type cyclin degradation during prometaphase eventually lower CDK1 activity to below the threshold for M phase exit[89]. Slippage frequently results in cell death or cell cycle exit through the activation of p53[90,91].

[H1] Cell cycle control in cancer

Continuing rounds of cell division is the hallmark of cancer. Recent insight has revealed that this continuous cell division is driven by mutations that both prevent apoptosis and compromise cell cycle exit, rather than driving uncontrolled cell cycle progression. These include mutations in the signaling pathways that initiate exit from the cell cycle or promote S phase entry [92], but much less frequently in those that prevent mitotic entry [93–96] and exit [97–101] [Please repalce ref 103 Cheiffi with Borah 2021 PMID: 33915740 -HM] (Figure 3). This suggests that, as the cell cycle is an exquisitely finely regulated process, endless division cycles present fundamental challenges to cancer cells that require some checkpoints (eg. replication stress, mitotic checkpoint) to remain functional [102]. Understanding how cancer cells overcome these challenges reveals vulnerabilities that can be targeted therapeutically.

[H2] Cell cycle checkpoints in cancer. Cells can exit the cell cycle either reversibly, through initiating quiescence, or non-reversibly by senescence or apoptosis. The decision to exit the cell cycle depends on just one of the cell cycle checkpoints – the DNA damage checkpoint (Box 2). Throughout interphase, in response to irreparable DNA damage, the DNA damage checkpoint can initiate quiescence, senescence or programmed cell death largely through p53-dependent pathways [103,104]. Unsurprisingly, p53 mutations are the most common mutations found in cancer [54]. However, even if cancer associated mutations prevent cell cycle exit, continuous proliferation can still be prevented by blocking cell cycle entry in the pre-replicative G1 phase, which depends on activation of E2F-dependent transcription. In line with this, cancer associated mutations in this pathway have been found in all types of cancers[105], and includes many oncogenes and tumour suppressors. These mutations induce E2F-dependent transcription, promoting S-phase entry and compromising the ability of a cell to exit the cell cycle in the pre-replicative phase.

Importantly, the main function of other cell cycle checkpoints (the DNA replication stress checkpoint and the SAC) remain vital in cancer cells and are thus not frequently mutated (Figure 3). This includes the temporal checkpoint-dependent cell cycle arrest before and during mitosis, which is essential to prevent catastrophic levels of DNA damage resulting from replication stress or incomplete spindle assembly.

[H2] Oncogene-induced replication stress. Whilst deregulated E2F-dependent transcription promotes cell cycle entry and compromises the ability of a cell to exit the cell cycle, this has a flip side. By accelerating S-phase entry, it is thought to generate replication stress, known as oncogene-induced replication stress. For example, prolonged activation of c-myc and cyclinE/CDK, as well as expression of the human papillomavirus (HPV) E7oncoprotein, **[Au: also prolonged?]** or loss of RB, all induce E2F-dependent transcription and have been linked to deregulated replication-initiation [106–110]. Oncogene-induced replication stress is thought to be an important source of genome instability **[G]** and might therefore be the basis of intra-tumour heterogeneity. This is one of the main barriers to improving cancer outcome, both in terms of reliable biomarker identification and cancer multidrug resistance[111–113]. In some cases Chromosomal Instability **[G]** (CIN) can be attributed to replication stress[114]. Due to the presence of persistent replication stress, cancer cells have an increased dependence on the replication stress checkpoint response to prevent catastrophic levels of replication stress-induced DNA damage[115]. In addition, oncogene-induced replication stress also creates a particular dependency of cancer cells on the SAC[116].

Maintaining the key functions of the replication stress checkpoint response ensures the completion of DNA replication, a condition that is crucial for viability. Indeed, the kinases ATR and Chk1 are essential in all cell types, including cancer cells [115]. **[Au: Please include your suggested reference]** This represents a fundamental difference with the DNA damage checkpoint, where key components are often mutated in cancer. DNA replication stress and DNA damage response are intertwined. It has been shown that replication stress-induced DNA damage ultimately triggers activation of the DNA damage checkpoint, which inhibits tumour development in its early stages by preventing proliferation [117–119].

[H2] Mitotic checkpoint in cancer

Aneuploidy **[G]** is common in cancer[120] and it has long been known that abnormal chromosome configurations can arise through mitotic errors[121,122]. An obvious source of chromosome segregation errors would be a defective SAC. However, while mutations in SAC genes have been observed in cancer[123], they are rare[97]. More common are changes in gene expression with over-expression of SAC components (including Mps1, Mad1, Mad 2, BubR1 and cdc20) being reported in several cancer types[98,124–126]. The effect of gene over-expression on SAC function or mitotic progression is not clear and may depend on the protein over-expressed[127] (add REF: Sakar 2021 PMID: 33409811) **[Au: Please add the references]** For example, Mad1 over-expression has been shown to weaken the checkpoint by mis-localising the SAC complex[124] while Mad2 overexpression can result in checkpoint hyperactivation[125]. The mixed evidence for SAC dysfunction in cancer indicates that SAC loss is not a major driver of tumorigenesis.

Cancer cells may be more dependent on the SAC than normal cells. Cancer cell lines typically spend 3-5 times longer in metaphase than normal cells[128,129] likely due to sustained checkpoint activation delaying mitotic exit. Removing the SAC entirely in cancer cells results in catastrophic chromosome loss that is invariably lethal[1]. On the other hand, non-transformed cells are less sensitive to death induced by SAC inhibition[130,131]. This is largely because of the presence of aneuploidy as well as ongoing karyotypic changes, known as chromosomal instability (CIN), in cancer cells. CIN can involve either loss or gain of whole chromosomes (numerical CIN) or rearrangements of portions of chromsomes (structural CIN)[132][add reference Siri 2021 PMID: 34205328 – HM]. Both types can be generated by segregation errors in mitosis[133]. In addition, replication stress in S phase[114] and DDR activation in mitosis[134] have been shown to be a source of CIN.

Recent evidence suggests that low levels of CIN are beneficial to cancer cells, driving cancer evolution by generating a wider range of possible karyotypic combinations [135,136]. On the other hand, too much CIN is harmful leading to cell death and growth supression due to the loss of essential genes[137,138]. High CIN can be associated with improved prognosis for patients[139,140]. A functioning SAC is crucial in cells with low CIN to extend mitosis for long enough to allow spindle capture of abnormal chromosomes. In line with this, experimental induction of CIN in diploid cells leads to a decrease in APC/C^{cdc20} function, which prolongs mitosis and guards against the development of further excessive chromosomal instability[141]. Thus, the compromised cell cycle exit checkpoints in cancer that fuel continuous cell division may lead to greater reliance on the mitotic checkpoint to allow cells to tolerate aneuploidy without developing excessive CIN.

[H1] Cell cycle control in cancer: therapeutic opportunities

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Cancer cells continue to divide as a result of mutations that allow cell cycle progression and prevent exit. An important consequence is that all cancers depend on continuous cell division and many become increasingly dependent on remaining cell cycle control mechanisms to prevent the excessive accumulation and propagation of genomic instability.

[H2] Exploiting cancer reliance on cell cycle control pathways. Generating excessive levels of DNA damage, either through radiation or chemotherapy, is more likely to result in catastrophic levels of genome instability and therefore cell death in cancer cells than in healthy cells[142]. This approach remains the most effective way to treat cancer[143], but also harms healthy cells by inducing DNA damage.

The increased reliance of cancer cells on cell cycle control regulatory pathways provides opportunities to target pathways or processes that are essential in cancer cells, but dispensable in healthy cells. However, the

complexity of the cell cycle control network suggests different pathways or processes should be targeted in different cancers. Therefore, to improve the effectiveness of targeting cell cycle control mechanisms by anti-cancer agents, we need to understand the network dynamics in normal compared to cancerous cells in the context of specific cancer-associated mutations. This could be used to guide better drug design, therapeutic combinations, and patient selection. Here we focus on the control of cell cycle progression, the response to replication stress and the control of M phase entry and progression, and refer to other reviews that highlight the potential targeting of pecific aspects of DNA repair mechanisms (Figure 4).

[H2] Forcing cancer cells to permanently exit the cell cycle. Tumour growth depends on continued cell proliferation. The main driver of cell cycle progression is CDK activity and increased CDK activity has been widely reported in various cancers, making them an attractive targets for new treatments[144]. CDK inhibitors could potentially prevent continued cell cycle progression by forcing cancer cells to permanantly exit the cell cycle into a senescent state. Such cytostatic drugs would prevent tumour growth, but also offer potential opportunities to eradicate senescent cancer cells using senolytics **[G]** [145]. There is a lot of interest is such a 'one-two punch' approach where cytostatic drugs induce senescence in cancer cells and sequential treatment with senolytics to kill senescent cancer cells[146].

Various CDK-targeting drugs have entered clinical development, but until recently few have achieved their expected efficacy in clinical trials [147] **[add (or replace with?) Whittaker 2018 PMID: 28174091 and Lukasik 2021PMID: 33802080 – HM, I added just one, we have already too many refs_CB]**. However, more detailed insight into the role of the various CDKs in cell cycle control is now being used to direct therapeutic strategies and improve the effectiveness of cell cycle kinase inhibitors. The most prominent examples of these are the CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib[148]. These drugs target the most commonly deregulated CDK activity in cancer, D-type cyclin CDK[149], and show significant clinical benefit for the treatment of hormone receptor positive metastatic breast cancer, leading to FDA approval for treatment of this disease. As discussed above, D-type cyclin CDK activity is now thought to play a more direct role in preventing cell cycle exit rather than in driving cell cycle entry. This new fundamental understanding suggests that CDK4/6 inhibitors can force cancer cells to exit the cell cycle. In line with this, published data shows that CDK4/6 inhibition can trigger senescence, apoptosis or quiesence in specific cancer types (for more detailed reviews [145,150,151]). This new understanding predicts that patients with cancer-associated mutation in proteins involved in cell cycle exit pathways, such as p16 (CDKN2) are more likely to benefit from CDK4/6 inhibitors. Conversely, patients with mutations that drive cell cycle entry independent of CDK4/6 activity, such as oncogenic E-type cyclin CDK or E2F1 or loss of RB, are less likely to benefit from CDK4/6 inhibition. However, there is still much to learn about the role of CDK4/6 in cell cyle control and how inhibitors such as palbociclib affect its function. Most recently, studies have indicated that palbociclib may also prevent cell cycle entry by indirectly inhibiting CDK2, via increasing p21 abundance,

and as such arrests cell cycle entry even when Cdk4s kinase activity is already impaired [152–154]. In addition, current studies show promising results by combining Cdk4/6 inhibitors with mitogen-activated protein kinase pathway inhibitors in KRAS-driven lung and pancreatic cancers mouse models [155,156]. Senescence induced by the combination of these cytostatic drugs is much more pronounced than single drug treatment alone. In both cases, the senescence associated secretory phenotype (SASP), which leads to a local inflammatory response, was a crucial driver of these responses. This opens new perspectives on the combinatorial use of cytostatic drugs.

In addition to CDK4/6 inhibitors, several studies have shown a marked sensitivity of many cancer types to selective CDK7 inhibition [157–165] CDK7 is the main CDK activating kinases (CAK) phosphorylating CDK1, 2, 4 and 6, the main cell cycle-dependent CDKs [166]. Importantly, cancer cells are sensitive to CDK7 inhibitors at doses at which normal cells are insensitive, suggesting that treatments might be well tolerated in a clinical setting. In comparison to CDK4/6 inhibitors, which are rendered ineffective in patients with mutations that drive cell cycle entry, CDK7 inhibitors inhibit CDK4/6 and CDK2 activity, both preventing cell cycle entry and promoting cell cycle exit. This suggests that CDK7 inhibitors could trigger cell cycle exit and apoptosis in cancers with mutations in either cell cycle exit or entry pathways, potentially benefiting a wider range of patients. In addition to its role in CDK activation, CDK7 is also required for PolII-dependent transcription, through PolII and CDK9 phosphorylation[8]. With many cancers depending on transcriptional drivers, CDK7 inhibitors therefore hit two birds with one stone: the continued cell cycle progression via CDK inhibition and the addiction to transcriptional drivers via PolII inhibition. However, it is not yet known whether the effectiveness of CDK7 inhibition in driving a cell cycle exit depends on the inhibition of CDK, PolII or both. In addition, it is unclear why some cancer cells are more sensitive to CDK7 inhibition and of those why some arrest and others undergo apoptosis[167]. With CDK7 inhibitors moving ever closer to the clinic it will be vital to gain a more fundamental understanding of how cancer-associated mutations modulate CDK7i-induced cell-cycle exit and apoptosis both for the effectiveness of CDK7 inhibitor treatment and patient stratification.

Overall, it will be crucial to gain a detailed fundamental understanding of the role of the various CDKs and what makes specific cancer cells senstitive to CDK inhibition. This will guide identification of biomarkers that can be used for patient stratification for the clinical use and development of CDKi, identify how drugs previously shelved during drug development might be effective when combined in the right way, and identify targetable vulnerabilities of senescent cancer cells.

[H2] Forcing uncontrolled cell cycle progression in cancer cells. An alternative to restricting cancer cell cycle progression by targeting CDK activity would be to put cell cycle progression into overdrive by increasing CDK activity. CDK inhibitors such as WEE1 and p21[104] delay or arrest the cell cycle until certain criteria are met. Whilst WEE1 delays mitotic entry, p21 mainly restrict S phase entry. With many cancer-associated mutations promoting S phase entry via deregulating E2F-dependent transcription, cancer cells are

thought to become increasingly reliant on these CDK inhibitors to prevent propagation of DNA damage[168]. In line with this, p21 is rarely mutated in human cancer[169], whilst WEE1 is expressed at higher levels in various cancer types[94] [replace with di Rora 2020 PMID: 32958072 -HM]. Therefore, targeting p21 or WEE1 could potentially compromise a cancer cells' ability to prevent propagation of DNA damage and thereby generate excessive levels of DNA damage to tip the balance towards cancer cell death. Inhibition of WEE1 forces cells with damaged DNA to prematurely enter mitosis, ultimately resulting in mitotic catastrophe and apoptosis[170]. Targeting WEE1 for inhibition is therefore currently being explored as an opportunity to further potentiate DNA damage-inducing therapies[replace with di Rora 2020 PMID: 32958072 and reference 185 (Forment 2018)-HM][171]. For example, a recent study found that carboplatin-resistant breast cancer cells are uniquely vulnerable to WEE1 depletion[172]. p21 is only recently being considered as a potential intervention target for cancer treatment. This is based on a better fundamental understanding of p21's central role in preventing cell cycle entry and more translational studies, but much more work is needed to explore potential therapeutic approaches in the cancer field [173]. Future research will reveal if increasing CDK activity in cancer cells, by targeting CDK inhibitory proteins, can selectively drive catastrophic genome instability without inducing genomic instability in healthy cells.

[H2] Exploiting oncogene-induced replication stress. The replication stress response is often discussed as part of the DNA damage response, but, as pointed out above, they are distinctly different. The replication stress response prevents replication stress-induced DNA damage, while the DNA damage response prevents the propagation of DNA damage. This distinction is crucial in the context of cancer biology and therapeutics. Therapies targeting the replication stress response will cause an increase in replication stress-induced DNA damage in cancer cells already experiencing oncogene-induced replication stress. This alone can lead to catastrophic levels of genome instability in cancer cells whilst leaving healthy cells unharmed. Targeting the replication stress response can also increase the efficacy of cancer therapies that induce DNA damage or target the DNA damage response.

Cancer cells commonly experience oncogene-induced replication stress[174]. Therefore, targeting the DNA replication stress checkpoint protein kinases or proteins involved in replication stress tolerance should be specifically toxic to cancer cells[115]. In line with this, ATR and Chk1 inhibitors are particularly toxic to cells experiencing oncogene-induced replication stress caused by deregulated S-phase entry[175–178]. However, initial clinical trials suggest limited potential for their use as anti-cancer drugs[115]. It is now becoming clear that focusing these treatments on cancers with high levels of replication stress, due to a combination of genetic deficiencies, does have the potential to translate into clinical efficacy [178,179]. In particular patients with mutations that drive cell cycle entry are likley to benefit [180]. Adjusting treatments based on additional genotypes are currently being tested and phase II trials have started for triple negative breast cancer, small

cell lung cancer, platinum-resistant ovarian cancer and haematological cancers[181]. This approach does require reliable biomarkers to determine replication stress levels in patient tissue, which at the moment are missing.

In many cells ATR and Chk1 are essential genes[182], which limits the therapeutic window. An alternative approach, currently being explored, is to direct treatment to normally dispensable processes that only acquire essential functions during the replication stress response. One such process is replication stress tolerance, which involves many factors necessary to cope with increased replication rates and prevent catastrophic genome instability[115]. For example, increased dNTP levels have been shown to reduce oncogene-induced replication stress[109]. This suggests that cancer cells experiencing high levels of replication stress might become reliant on increased dNTP levels. Additionally, it was recently shown that the tolerance to replication stress depends on increased E2F-dependent transcription, which increases the gene expression of many proteins involved in DNA replication and repair[183]. These findings suggest that cancer cells might rely on specific processes to tolerate high levels of oncogene-induced replication stress, which can be targeted to generate novel theraputic stratagies. To exploit replication stress tolerance in cancer, more work is needed to establish specific targetable aspects of replication stress tolerance. These include the proteins and processes involved in preventing oncogene-induced replication stress, preventing replication stress-induced DNA damage and those required for replication stress-induced DNA damage repair.

Overall, replication stress tolerance is emerging as a promising area of investigation for the discovery of novel targeted therapies. However, two major factors restrain our ability to exploit this successfully in cancer treatment: a limited understanding of the proteins and processes involved in the specific aspects of tolerance, and a lack of clear and robust biomarkers to guide the clinical use of existing and novel drugs that target the replication stress response. Without advances in these areas the promise of successfully targeting replication stress tolerance in cancer, alone or in combination with existing therapies, in the clinic will not be met.

[H2] Driving catastrophic chromosome instability. Cancer cells rely on a functional SAC more heavily than normal cells to prevent catastrophic errors in chromosome segregation during M phase [1,130,131]. This makes the mitotic checkpoint an attractive target for cancer therapy. Two of the most successful classes of chemotherapy drugs work by disrupting formation of the mitotic spindle: vinca alkaloids, which destabilise microtubules, and taxanes, with stabilize them (reviewed in[184]). In both cases, the inability of cells to form a bipolar spindle means that the SAC remains active and cells arrest in mitosis. In cultured cells, prolonged mitotic arrest leads directly to apoptosis[185]. However, at clinically relevant taxane doses, spindle errors instead result in catastrophic chromosomal misegregation leading to excessive CIN and ultimately the creation of non-viable daughter cells[186].

The success of microtubule-targeting drugs has led to the search for more specific agents to target mitosis therapeutically in cancer. However, these attempts have been so far been mostly unsuccessful[187,188]. Targeting Eg5, a kinesin required for bipolar spindle formation, re-capitulates the effect of taxane treatment in cell culture but this has not translated in the clinic[189]. Similarly, inhibitors of CENP-E, which mediates microtubule-kinetochore attachment, or mitotic kinases Aurora A & B, Cdk1 and Plk1 have shown promise in pre-clinical studies but remain stalled in late stage clinical trials, mainly due to adverse effects on normal proliferative tissues [184,187–193][Please repalce ref 103 Cheiffi with Borah 2021 PMID: 33915740 -HM]. However, 'smarter' strategies such as combination with other therapies or the manufacture of pro-drugs that become preferentially activated in cancer cells may provide greater success for these compounds in the future[188].

An alternative approach to targeting mitosis in cancer is to inhibit the SAC directly, inducing premature mitotic exit and excessive CIN. This strategy would likely hit cancer cells harder than normal cells due to the pre-existing CIN in many tumours. Turning low CIN into high CIN has been shown to be an effective method of inducing cell death in many experimental systems[137,138,194]. For example, inhibition of SAC component, Mps1 (TTK), or low dose taxane treatment both induce low levels of CIN when used separately, but in combination drive high CIN, severe chromosome segregation errors and the resulting death of unviable progeny in a mouse breast cancer model [195]. Recent work has revealed how anueploid cells can be uniquely vulnerable to SAC inhibition: although initally less sensitive than diploid cells to Mps1 inhibition, after 10 days of treatment the cumulative effect of multiple chromosomal abberations resulted in increased death and decreased proliferation specifically in aneuploid cells[196]. Several Mps1 inhibitors have been developed and show promise particularly in treating pediatric neurological malignancies [100,197–199]. Inhibitors of Mps1 or other SAC components (such as Cdc20[99]) are good candidates for a new generation of anti-mitotic targeted therapies. However, pre-clinical work makes it clear that these drugs are most effective at killing cancer cells when used to exploit pre-existing vulnerabilities (such as aneuploidy) or in combination with other treatments. Taxanes, in particular, can be enhanced by treatments that alter SAC activity[198,200,201]. Exploiting this in the clinic will require both idenfication of biomarkers for aneuploidy or CIN in patients and using our understanding of SAC function to design smart combinations of therapies to target cancer cells' dependency on the mitotic checkpoint.

[H1] Conclusions and perspectives

We have discussed recent work uncovering the detailed roles of cell cycle checkpoints in cell cycle control and how only specific aspects of these checkpoints are compromised in cancer cells to allow continuous cell division. This work is guiding and improving existing therapeutics and highlights opportunities to develop novel and combinatorial treatments. These specifically include targeting replication stress tolerance

mechanisms, the mitotic checkpoint, and proteins and processes involved in delaying or arresting cell cycle progression. These new strategies could be used alone or in combination with existing drugs that induce DNA damage and replication stress. In addition, it opens up the prospect that cancer could be managed by drugs forcing cancer cells to permanently exit the cell cycle. Overall, our new understanding of cell cycle control and cancer is guiding current treatments and opening up therapeutic opportunities to improving initial treatment with curative intent, either through greater precision or by extending treatment modalities and better-informed treatment decisions that will result in better outcomes for patients.

Molecular target	**Drugs**	**Clinical use**	**References**
Forcing cell cycle exit:			
CDK4/6	Palbociclib	Approved for $ER^+/HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumors	[147,202,203]
	Ribociclib	Approved for $ER^+/HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumors	[147,204–206]
	Abemaciclib	Approved for $ER^+/HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumors	[147,207–209]
CDK7	ICEC0942 (CT7001),	Phase I/II ER+ breast cancer, AML	[147]
Forcing cell cycle progression:			
Wee1	Adavosertib (AZD1775)	Phase II Relapsed SCLC, ovarian cancer, NSCLC, AML, gastric adenocarcinoma and various advanced solid tumours.	[94,181]
Impair replication-stress tolerance:			
ATR	VX-970	Phase II Recurrent ovarian, primary peritoneal, or fallopian tube cancer and metastatic urothelial carcinoma.	[181]

Chk1	LY2606368	Phase II SCLC, BRCA1/2 mutated breast or ovarian cancer, TNBC, HGSOC, metastatic CRPC and Advanced solid tumours with HRR defects or genetic alterations indicative or replication stress. Phase I Acute leukaemia, solid tumours or lymphoma	[181]
	MK-8776	Phase II Relapsed acute myeloid leukaemia (+/− cytarabine) Phase I Advanced solid tumours.	[181]
Inducing catastrophic genome instability:			
Mitotic spindle	Taxanes (paclitaxel docetaxel abraxene)	Approved for use in wide range of cancers including ovarian, breast, lung, bladder, prostate, melanoma, and esophageal cancer.	[184–186]
	Vinca alkaloids (vinblastine Vincristine)	Approved for use in range of cancers including ALL, AML, HL, Neuroblastoma and NSCLC.	[184,187]
SAC	Mps inhibitors (BAY 1161909 BAY 1217389)	Pre-clinical studies in neuroblastoma, medullablastoma and breast cancer (in combination with taxanes). Recently entered Phase I clinical trials	[195–199,210]
	Aurora B inhibitors (various including AZD1152, AT9283)	Phase II in AML, multiple myeloma, SCLC, prostate cancer. Phase I in various solid tumours.	[101]

Figure legends

Figure 1 | **The cell division cycle.** The eukaryotic cell cycle is the process during which a cell duplicates its entire cellular content during interphase, and through division in M phase, creates two genetically identical cells. The two main events, DNA replication and the segregation of the replicated DNA, are separated during the cell cycle. DNA replication happens during a distinct phase in interphase, called S phase, and DNA separation in M phase, called mitosis. Segregation of the cellular content happens during cytokinesis at the end of M phase to complete a cell cycle, after which a cell can either exit the cell cycle or enter a new round of cell division. During interphase, cell cycle progression is controlled before and after S phase. Commitment to enter a new cell cycle, that is S phase entry, is taken during a decision window before S phase. Similarly, during a decision window after S phase a cell can commit to mitotic entry. Commitment to mitotic exit happens during M phase at the metaphase-anaphase transition.

Figure 2 | **Checkpoint-dependent cell cycle arrest and exit.** The replication stress checkpoint can block mitotic entry during S phase by preventing the accumulation of CDK1/2-cyclinA/B activity and the spindle assembly checkpoint can block mitotic exit during M phase by preventing the activation of the anaphase-promotic complex/cyclosome (APC/C) **[Au:OK?]**. **[Au: Shoud we add 'Block mitotic exix' under the purple square of the APC/C?]** However, the DNA damage checkpoint operates throughout interphase. Depending on the phase of the cell cycle, the DNA damage checkpoint can either block mitotic entry during S phase and post S phase, much like the Replication Stress Checkpoint, or block replication initiation in pre-S phase via preventing the accumulation of cyclinE/A-CDK2 activity. It can also block cell cycle entry **[Au: It was changed to 'block mitotic entry' in the figure; shoud we revert to 'block cell cycle entry'?]** after mitotic exit, or during a prolonged pre-S phase, via preventing/inhibiting CyclinD-CDK4/6 activity inducing a reversible cell cycle exit known as quiescence. In response to high levels of DNA damage it can induce an irreversible exit from the cell cycle, via senescence, outside of the S and M phases or even cell death via apoptosis throughout the entire cell cycle.

Figure 3 | **Key signaling pathways involved in cell cycle control and cancer.** Continued cell cycle progression in cancer cells is mainly driven by mutations, or deregulation, of proteins involved in cell cycle control signaling pathways. However, these mutations are associated with specific cell cycle control pathways more so than others. Mutations commonly found in cancer cells are shown in red: they mainly affect cell cycle control in response to DNA damage and growth signals in pre-S phase. These mutations drive S phase entry and prevent cell cycle exit. Very few cancer associated mutations are found in proteins involved in the response to replication stress or incomplete spindle assembly; proteins that are rarely mutated in cancer are shown in blue. In the context of cancer treatment these pathways represent theraputic opportunities. RTKs, Receptor Tyrosine Kinases. Pocket Proteins include pRb, p107 and p130. E2F include

the activating E2Fs, E2F1-3 and indicates E2F-dependent transcription. G1/S CDK include cyclinD-CDK4/6 and cyclinE-CDK2. M-phase CDK include CyclinA/B-CDK1/2. MCC, Mitotic Checkpoint Complex BUB3 together with MAD2 and MAD3 bound to Cdc20.

Figure 4 | **Cancers continued proliferation presents many therapeutic opportunities**. Cancer is initiated by mutations in the DNA that allow cells to continuously divide. An important outcome is that all cancers depend on continuous cell division and specific aspects of the cell cycle checkpoint responses to prevent catastrophic genome instability and cell death, which can be exploited in cancer treatment. The step wise consequences of cancer-associated mutations that allow continued cell proliferation and the cellular responses that prevent or minimize events (blocking arrows) that can lead to catastrophic genome instability and cell death are depicted in red boxes. Continued proliferation itself, its consequences, and cellular responses to these, represent therapeutic opportunities (indicated in grey boxes) that can be exploited through the use and development of anti-cancer drug and/or therapies (indicated in brackets). RS, replication stress, RSC, replication stress checkpoint, SAC, Spindle Assembly Checkpoint and i, inhibitors.

Box 1 | **CDK and APC/C activity are central to the control of cell cycle progression.**

Cell cycle progression is driven by the accumulation of cyclin-CDK activity during interphase and M phase (see the figure). Loss of this activity, through APC/C^{cdc20}-dependent degradation of cyclins, marks the return back to interphase. CyclinD-CDK4/6 accumulation allows for entry into the cell cycle, preventing cell cycle exit. E2F-dependent transcription results in the accumulation of both cyclinE and cyclinA, which creates a decision window to enter S-phase. CyclinE–CDK2 activity further activates E2F-dependent transcription, creating a positive feedback loop, resulting in increased cylinE-CDK2 activity and cyclinA-CDK2 complex. This allows for the accumulation of cyclinA-CDK2 activity, through the inactivation of APC/C^{CDH1} activity, and replication initiation and S-phase entry. Subsequent accumulation of cyclinA/B-CDK1 complex creates a second decision window, after S phase completion, for mitotic entry. Accumulation of cyclinA/B-CDK1 activity drives mitotic entry and allows for APC/C^{CDC20} activation, required for mitotic exit and targeted degradation of cyclin, to complete a cell cycle. Under favorable conditions accumulation of cyclinD-CDK4/6 activity will allow cells to re-enter the cell cycle.

Box 2 | **DNA replication control in S phase.** Initiation of DNA replication marks S phase entry and commitment to a new cell division cycle. DNA replication is initiated in a bidirectional manner from a large number of discrete sites spread around the genome, called replication origins[211]. This is to ensure that the genome is replicated in a timely manner. It is essential for the preservation of the genome that DNA replication occurs 'once and only once' during typical cell cycles[212]. Preventing re-initiation from an origin where replication has already been accomplished is vital since it is thought that re-replication from even a single origin can cause DNA damage [213,214]. To prevent re-initiation of DNA replication, the process is temporally separated into two steps; origin licensing in the G1 phase, followed by replication initiation, called origin firing, in S phase (reviewed in[215]).

After completing a cell division cycle, cells return to interphase with low CDK activity (see mitotic exit for more detail). The lack of CDK activity allows Cdc6/Cdt1-dependent loading of inactive MCM helicase complex onto replication origins, 'licensing' all the origins. DNA replication can now be initiated from these licensed origins by the formation of a transient pre-initiation complex, followed by the activation of the MCM replicative helicase, which is called origin firing. This step requires CDK activity which accumulates as detailed above. At the same time, the increase in CDK activity blocks the loading of inactive MCM helicase complex preventing origin (re)licensing.

Box 3 | **Cell cycle checkpoints and cell cycle control.** In response to DNA damage during interphase, replication stress during S phase or incomplete spindle assembly during M phase, specific cell cycle checkpoints arrest or slow down the cell cycle through inhibiting the activity of key cell cycle regulators, CDK and the APC (see the figure). The DNA damage checkpoint can be activated by Double Strand Breaks (DSB) throughout interphase and depends on the ATM and Chk2 checkpoint protein kinases to block cell cycle progression. Depending on the phase of the cell cycle it can either prevent the accumulation of cyclinE/A-CDK2 activity to block replication initiation in pre-S phase or block mitotic entry during the S phase and G2 phases. The Replication Stress Checkpoint is activated in response to single stranded DNA (ssDNA) and depends on the ATR and Chk1 checkpoint protein kinases to prevent the accumulation of cyclinA/B-CDK1/2 activity to block mitotic entry. Downstream regulation depends on activation of the kinase WEE1, for inhibitory phosphorylation of CDK1/2, and inactivation of the phosphatase Cdc25, for removal of the inhibitory phosphorylation of CDK1/2. The Spindle Assembly Checkpoint is activated by incomplete attachment of chromosomes to the mitotic spindle and depends on the Mitotic Checkpoint Complex (MCC, BUB3 together with MAD2 and MAD3 bound to Cdc20) to prevent APC activation to block Mitotic exit.

Glossary

QUIESCENCE OR G0 **[AU: PLEASE SHORTED TO MAX 2 LINES]**

A reversible cellular state, outside of the G1 phase, from which cells can re-enter the cell cycle.

SENESCENCE

A non-reversible cellular state, outside of the G1 phase, from which cells can't re-enter the cell cycle.

CYCLIN DEPENDENT KINASES (CDKS)

CDKs depend on cyclins for their kinase activity. Cyclins increase during the cell cycle and Cyclin/CDKs drive cell cycle progression by phosphorylating protein targets.

GENOME INSTABILITY

Stocastic acquisition of genetic change over many cell divisions that can result in mutations and chromosomal rearrangements and aneuploidy.

ANAPHASE PROMOTING COMPLEX/CYCLOSOME (APC/C)

Ubiquitin ligase complex activity is restricted to mitosis and G1 phase and required to initiates exit from mitosis and indirectly for DNA replication.

DNA END RESECTION

Removal of nucleotides by exonucleases involved in repairing DNA exposing tracks of ssDNA at sites of Double strand breaks repair, it is required for homologous recombination.

NON-HOMOLOGOUS END JOINING

DNA double strand break repair mechanism based on the juxtaposition of two pieces of DNA.

HOMOLOGOUS RECOMBINATION

Mechanism of DNA double strand break repair requiring the presence of duplicated chromatids, taking place only in S and G2 phases. Requires the resection of DNA ends at the break site.

CHROMOSOMAL INSTABILITY (CIN)

Type of genome instability involving structural (S-CIN) and/or numerical chromosomes (W-CIN) aberrations.

SENOLYTICS

Compounds that selectively kill cells in a state of senescence.

References

[Au: For references that are particularly worth reading (maximum 10), please provide a single bold sentence that indicates the significance of the work; please place the sentence (which should be in the format 'Bertoli et al show …' Or 'The study by Bertoli et al shows…' below the relevant highlighted reference]

[Please ensure that references are cited sequentially in the following order: main text, tables, figure legends and then boxes.]

[Au: There are currently too many citations to review articles; for example references 1-14 are all reviews, and several of them are quite old. As a service to the readers, we recommend being more selective, citing only recent reviews when possible, and avoiding citing several overlapping/redundant reviews.]

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References to highlight

16 Narashima et al. showed that cyclin D/CDK4/6 monophosphorylates RB and contrary to the dogma this does not compromise its inhibitory function of E2F-dependent transcription to initiate S phase entry, but it maintains cells in a cell cycle state, preventing their exit.

106 Di Micco et al. showed that deregulated DNA replication, due to concogene activation, triggers DNA damage response and cell cycle exit through senescence, establishing the concept of oncogene-induced replication stress driving cancer initiation.

109 Bester et al. showed that replication stress is an early event in cancer development and fuels genomic instability, establishing the role of oncogene-induced replication stress in cancer initiation.

175 Murga et al. showed that inhibition of ATR and Chk1 can kill cells with oncogene-induced replication stress, establishing that targeting the replication stress response can selectively kill cancer.

114 Burrell et al. showed that replication stress can cause chromosome instability in cancer cells.
141 Sansregret et al. showed that strengthening the spindle assembly checkpoint (by APC/C partial depletion) and prolonging mitosis prevents chromosome segregation errors in mitosis and guards against excessive genomic instability in cancer cells.
186 Zasadil et al. showed that the cytotoxic effects of paclitaxel at clinically-relevant concentrations (as measured in human tumours) are not due to prolonged SAC activation and mitotic arrest but rather due to chromosome segregation defects that result in unviable karyotypes and cell death.
196 Cohen-Sharir et al. showed that aneuploid cells are more sensitive to SAC inhibition than diploid cells. Aneuploid cells were found to depend on an intact SAC for accurate chromosome segregation and long-term survival, implicating the SAC as a potential therapeutic target in aneuploid cancers.

Competing interests

The authors declare no competing financial interests.

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Author contributions

The authors contributed equally to the writing and revisions of the article. [Au:OK?]

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Fig 1

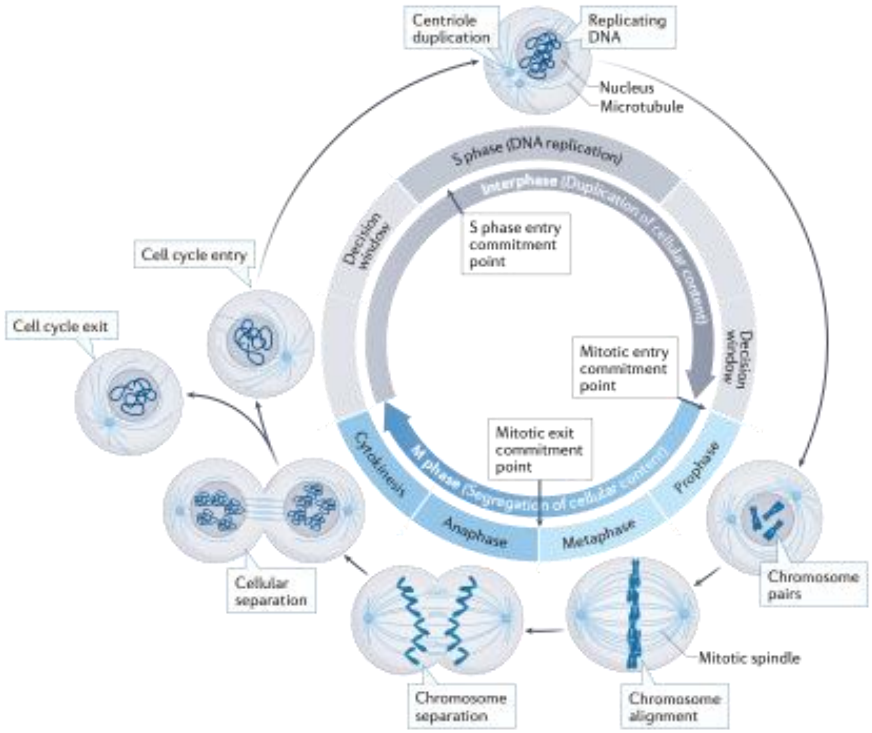


Fig 2

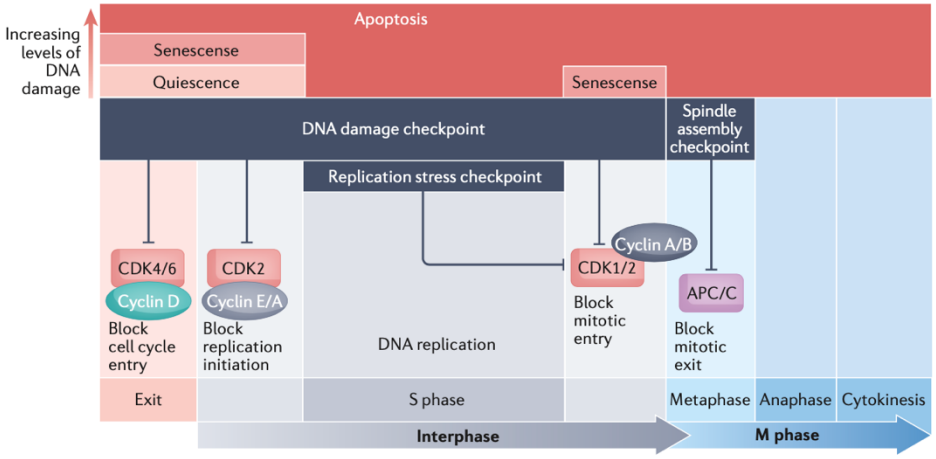

Increasing levels of DNA damage
Apoptosis
Senescense
Quiescence
Senescense
DNA damage checkpoint
Spindle assembly checkpoint
Replication stress checkpoint
CDK4/6
Cyclin D
Block cell cycle entry
CDK2
Cyclin E/A
Block replication initiation
DNA replication
CDK1/2
Cyclin A/B
Block mitotic entry
APC/C
Block mitotic exit
Exit
S phase
Metaphase
Anaphase
Cytokinesis
Interphase
M phase

Fig 3

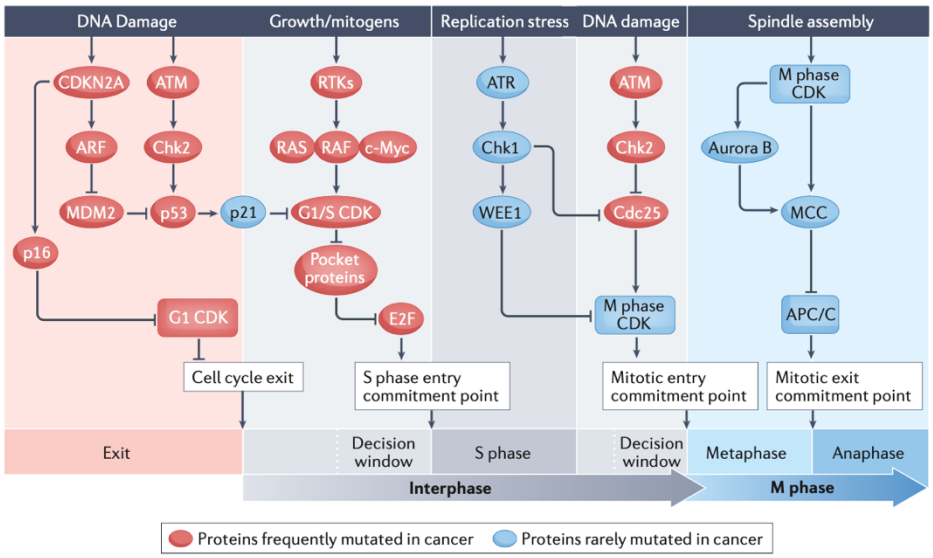
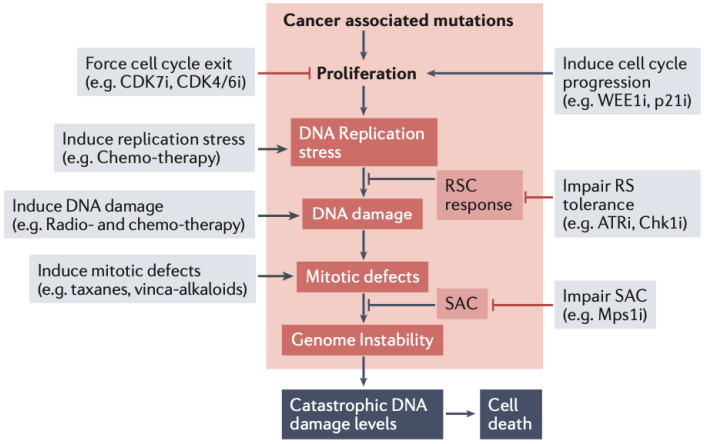
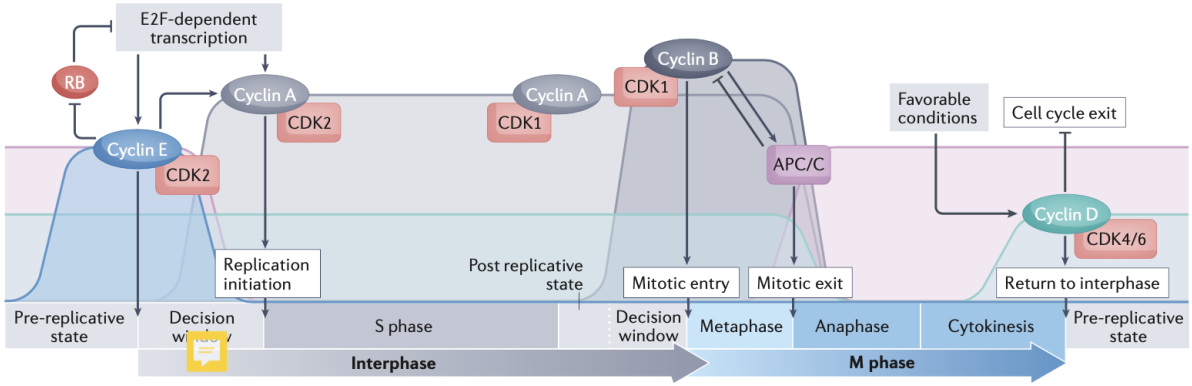

DNA Damage
Growth/mitogens
Replication stress
DNA damage
Spindle assembly
CDKN2A
ATM
RTKs
ATR
ATM
M phase CDK
ARF
Chk2
RAS
RAF
c-Myc
Chk1
Chk2
Aurora B
MDM2
p53
p21
G1/S CDK
WEE1
Cdc25
MCC
p16
Pocket proteins
G1 CDK
E2F
M phase CDK
APC/C
Cell cycle exit
S phase entry commitment point
Mitotic entry commitment point
Mitotic exit commitment point
Exit
Decision window
S phase
Decision window
Metaphase
Anaphase
Interphase
M phase
Proteins frequently mutated in cancer
Proteins rarely mutated in cancer

Fig 4



Box 1



Box 3