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Review Article

Cell cycle control in acute myeloid leukemia

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Abstract: Acute myeloid leukemia (AML) is the result of a multistep transforming process of hematopoietic precursor cells (HPCs) which enables them to proceed through limitless numbers of cell cycles and to become resistant to cell death. Increased proliferation renders these cells vulnerable to acquiring mutations and may favor leukemic transformation. Here, we review how deregulated cell cycle control contributes to increased proliferation in AML and favors genomic instability, a prerequisite to confer selective advantages to particular clones in order to adapt and independently proliferate in the presence of a changing microenvironment. We discuss the connection between differentiation and proliferation with regard to leukemogenesis and outline the impact of specific alterations on response to therapy. Finally, we present examples, how a better understanding of cell cycle regulation and deregulation has already led to new promising therapeutic strategies.

Keywords: Acute myeloid leukemia (AML), cell cycle, genetic instability, proliferation, differentiation

Leukemogenesis and cell cycle – differentiation block, continuous proliferation and genome instability

AML is characterized by the clonal expansion of immature myeloid cells. At initial diagnosis approximately half of the patients harbor at least one cytogenetic aberration [1, 2]. Moreover, aberrations at the molecular genetic level have increasingly been identified in AML during the last decade [3]. Several genetic aberrations have been associated with the hallmark of AML: the combination of a differentiation block and hyperproliferation [4].

In the normal hematopoietic system, proliferation is tightly linked to differentiation. In an asymmetrical cell division, one daughter cell retains stem cell properties to guarantee a pool of stem cells while the other one undergoes differentiation to respond to the high and permanent demand for granulocytes, monocytes, erythrocytes and platelets [5].

Hyperproliferation bears an enhanced risk of genetic damage. In leukemia, compromised DNA damage response pathways and weakened checkpoints add to the inherent risk and pave the way for further genetic aberrations promoting progression of the disease [4, 6]. Thus, to fully understand leukemogenesis, it is crucial to determine the role of proto-oncogenic pathways in the regulation of the cell division cycle in myeloid malignancies. The cell cycle itself is divided into four major phases: the G1-, S-, G2- and M-phase [7]. From G1 a cell can exit the cell cycle into a state of quiescence (G0) from where it can undergo differentiation or reenter the cell cycle to proliferate. If the cell continues cell division the genomic DNA will be replicated during S-phase. Once cells have completed DNA replication, they proceed through the G2-phase to prepare for the subsequent chromosome separation and cytokinesis during M-phase (mitosis) (**Figure 1**) [7].

G1-phase - at the crossroad of proliferation and differentiation

The reproductive rate of the myeloid system depends on the number of cells that are actively cycling. In healthy individuals, myeloid precursor cells are mostly in a quiescent state [8, 9] and can be recruited to enter the cell cycle in case of demand, for example when a severe infection occurs. Actively cycling progenitors proceed

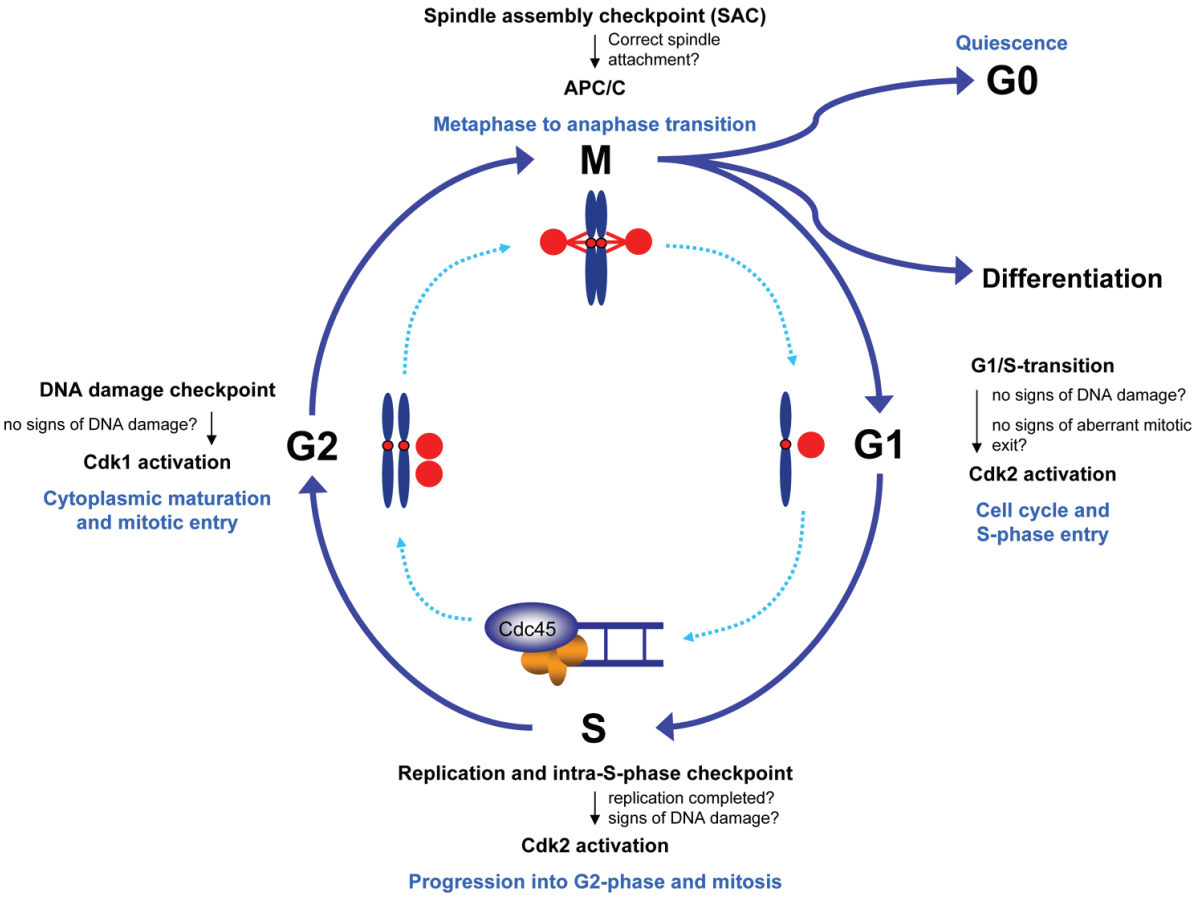

Figure 1. The cell cycle and its checkpoints. The human cell cycle can be divided into four phases - G1-phase, S-phase, G2-phase and M-phase (mitosis). Cells must proceed through the cell cycle in a unidirectional manner and cell cycle progression is restricted to cells that have fulfilled specific requirements to enter the next phase of the cell cycle. Whether requirements for cell cycle progression are met is supervised by checkpoints which hold back cells at cell cycle transitions.

through multiple cell division cycles and give rise to daughter cells that can differentiate. Short intervals between cell division cycles and a large pool of cells that are capable of rapid self-duplication are strategies to guarantee a sufficient cellular defense.

Quiescent cells can be activated to re-enter the cell cycle by various external stimuli including growth factors and cytokines. In the following section, pathways will be discussed that regulate whether a cell enters the S-phase, exits the cell cycle into quiescence or undergoes differentiation.

Stimuli from the microenvironment in normal hematopoiesis and AML

The FMS-like tyrosine kinase 3 (Flt3) [10], c-Kit (CD117 or stem cell factor receptor) [11] or Janus kinase 2 (Jak2) [12] are tyrosine kinases that translate external stimuli into pro-proliferative signaling cascades (**Figure 2**). Constitutive firing of mutated kinases in AML frequently causes ongoing activation of the downstream pathways and hence enhances transitioning from G1- into S-phase. Downstream of the receptor- and non-receptor kinases, the Stat -pathway (Stat = signal transducers and activators of transcription) is activated upon stimulation by various interleukins [13]. Further downstream, the serine-threonine kinase Pim1, which is activated by the Stat-pathway [14], has been shown to act as an important S-phase promoter by regulating Skp2-dependent degradation of the Cdk-inhibitor p27 [15]. In addition to signaling in the G1 phase, Pim1 was shown to enhance the transition from G2 phase into

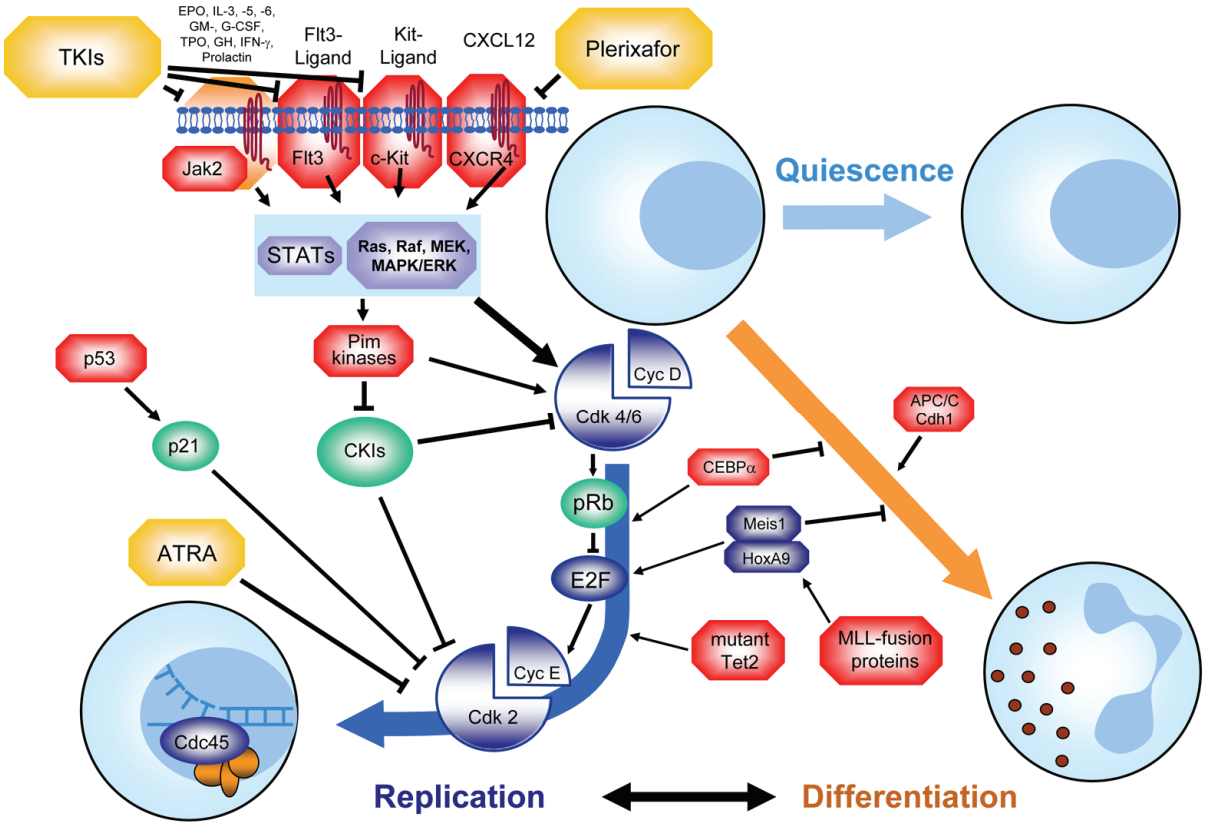

Figure 2. To cycle or to differentiate - the G1 phase of the cell cycle. Cells that have exited mitosis into G1 can either enter a state of quiescence (G0), differentiate along a particular lineage or progress to another cell division cycle. The process of decision making between these possibilities is governed by a multitude of different proteins. Proteins that are frequently overexpressed or mutated and hence contribute to leukemic transformation are marked in red. Tumor suppressor proteins are marked in green, proteins that contribute to differentiation are shown in orange. Therapeutic agents are marked in yellow. See text for details.

mitosis [16].

As mentioned above, alterations of these proliferative regulators may confer unlimited growth during leukemogenesis [13]. For instance, certain mutations in *FLT3*, ie, point mutations in the tyrosine kinase domain (*FLT3-TKD*) and internal tandem duplications in the juxtamembrane region (*FLT3-ITD*), lead to constitutive activation which causes phosphorylation of substrate proteins even in the absence of external stimuli [17]. *FLT3-ITD* is found in approximately 20% of AML patients and *FLT3-TKD* in approximately 5% [18, 19]. Both mutations are more frequent among patients with AML and a normal karyotype [18]. In addition to Flt3, the receptor tyrosine kinase Kit can be affected by activating mutations in AML. Such mutations, similar to Flt3, are mainly found in the tyrosine kinase domain of Kit, but are in part also observed in the extracellular domain. *KIT* mutations mostly occur in patients with AML and a chromosomal translocation disrupting a core binding factor, ie, t(8;21) or inv(16) [20]. Mutations in *JAK2* are rarely found in AML, but are characteristic for myeloproliferative neoplasms (MPN) [21].

Both types of *FLT3* mutations activate PI3K/AKT and the MAP kinase pathway [22, 23]. Activation of the MAP kinase pathway leads to upregulation of proto-oncogenic cell cycle regulators, such as the transcription factor c-Myc, and promotes premature entry into the following S-phase in AML cells [24]. *FLT3-ITD* also activates the Stat5 pathway and negatively regulates CEBPα and other transcription factors such as PU.1 to suppress differentiation [25, 26]. Major

changes in proliferative patterns can also result from deregulated chemokine signaling. In normal hematopoiesis, progenitor cells are stimulated through binding of CXCL12 (alias SDF-1) to its receptor CXCR4. Ligand binding to CXCR4 leads to activation of the MAP kinase pathway and to calcium release from the endoplasmatic reticulum which favors cell cycle progression, proliferation and survival [27]. High expression levels of CXCR4 have been observed in subsets of human AML and predict a poor prognosis [28]. Targeting CXCR4 has emerged as a promising leukemia therapy [28-30]. The use of the CXCR4-antagonist plerixafor in a mouse model of acute promyelocytic leukemia (APL) renders cells more susceptible to chemotherapy due to a mobilization of leukemia cells from their protective microenvironment [31].

Transcriptional control of cell fate in normal hematopoiesis and AML

Cell fate depends on the orchestrated action of regulatory proteins (**Figure 2**). The scheduled presence of such regulators can be achieved by synthesis and targeted degradation. Transcriptional activity and thus synthesis can be epigenetically controlled by cytosine hydroxymethylation of promoter DNA, such as at the *p15* and *p16* gene locus [32]. The ten-eleven translocation 2 (Tet2) protein is a methylcytosine dioxygenase that is important for the synthesis of 5-hydroxymethylcytosine and hence regulates the epigenetic status of the particular cell. Mutations in *TET2* have been identified in various myeloid neoplasm [33-35]. In AML, they occur in approximately 10-15% of cases. They are more frequent in older AML patients and in AML with normal karyotype [36, 37]. Loss of Tet2 in mice was associated with an enhanced expansion of early HPCs eventually leading to progressive myeloproliferation underscoring the transforming potential of *TET2* aberrations [38].

Another important player in epigenetic regulation in AML is the mixed lineage leukemia (*MLL*) gene located on chromosome band 11q23 [39, 40]. The wild type MLL protein is a human trithorax homologue and facilitates histone H3 lysine 4 (H3K4)-methylation. These histone marks then mediate transcriptional activation of a set of target genes [41]. Taspase1-mediated cleavage of the wild type full-length MLL-precursor protein into N-terminal (N-320) and C-terminal (C-180) fragments is the prerequisite for the characteristic MLL expression/activity peaks at the G1/S- and G2/M-boundary and for the formation of the active heterodimeric MLL complex [42, 43] which mediates H3K4-methylation at the corresponding promoter sites to activate transcription of genes [44]. Importantly, this methylation process also involves the cell cycle regulator E2F, which binds processed MLL to direct the active MLL complex to the site of methylation [45]. In leukemia with 11q23 rearrangements, the N-terminal part of the MLL-protein is fused to one of more than 70 possible fusion partners [46]. All 11q23-associated MLL-aberrations lead to loss of taspase1 cleavage sites due to truncation [47]. Consequently, leukemogenic MLL fusion proteins show stable expression levels throughout the different stages of the cell cycle and this aberrant expression is believed to be an important hit in MLL leukemias with MLL rearrangements through abrogation of proper cell cycle checkpoint function [45]. MLL fusion proteins also lead to enhanced transcription of homeobox family of transcription factors. In addition to important functions in development these transcription factors regulate differentiation and cell cycle progression. For *MEIS1* along with *HOXA9*, there is a well established role in promoting cell cycle progression and protection from apoptosis in MLL leukemia [48, 49]. Meis1 induces important proto-oncogenes such as Flt3 and c-Myb and hence promotes S-phase transition [40, 50]. Repression of Meis1 is associated with downregulation of cyclin D3 and a delay at the G1/S transition which can be overcome by reexpression of cyclin D3 [51].

Cell cycle progression by activation of cyclin dependent kinases (Cdks)

The MAP kinase pathway enhances cell cycle progression by activation of the cyclin-dependent kinases Cdk4, Cdk6 and Cdk2 (**Figure 2**). The latter is important to promote transition into S-phase (**Figure 2**). Activation of Cdk4 and Cdk6 through its activating subunit cyclin D leads to phosphorylation and inactivation of the retinoblastoma protein (pRb) which in turn releases the transcription factor E2F [52]. Importantly, pRb is dysfunctional in most cases of promyelocytic leukemia [53]. E2F promotes transcription of cyclin E which drives cells into S-phase [54]. A variant of E2F (E2F1) is aberrantly expressed in cases of AML and hence promotes premature entry into S-phase

[55]. In addition, active Cdk4 and Cdk6 bind and thus sequester Cdk inhibitors, such as p14, p16 or p27, which otherwise hold back cells in G1-phase. Once activation of Cdk2 outbalances its inhibition by p21 and p27, cells can enter S-phase.

The classic tumor suppressor p53 is considered to be the guardian of the genome [56]. This important transcription factor can lead to cell cycle arrest, induce DNA damage response and enhance proapoptotic signaling. It has been proposed that p53 contributes to a G1 block via induction of p21 in cells that were able to exit aberrant mitoses [57-60]. A recent approach in AML cells enforced this particular G1-checkpoint by stabilizing p53 and the Cdk inhibitor p21 to counter polyploidy through induction of a stable G1-arrest [60]. The results are promising and might lead to novel therapeutic options.

Similar to p53, dephosphorylation of the pRb is required to maintain a G1-arrest. This is achieved by binding to E2F and prevention of transcription of genes that are critical for S-phase entry. This mechanism constitutes an additional barrier to ensure that error-prone cells do not enter S-phase. In malignant hematopoiesis, weakening of these barriers allows aneuploid cells to replicate and enhance genetic instability. Enhanced genetic instability is associated with a higher level of genetic variability which may confer a selective advantage, favor outgrowth of individual malignant clones and thus drive disease progression.

A way to promote differentiation is achieved through prevention of S-phase entry by down-regulation of Cdk2 and cyclin E. This mechanism of action has been described for the vitamin A derivative all-trans-retinoic-acid (ATRA), which is a compound that mediates rapid differentiation of lineage-committed cells and is used as a therapeutic agent in AML with t(15;17). The product of t(15;17) is the PML-RARα fusion protein which leads to a differentiation block at the promyelocytic stage by functional inhibition of the retinoic acid receptor alpha (RARα). This differentiation block can be overcome by high doses of ATRA, which functions at least in part through inactivation of Cdk2 and cyclin E [61]. In neuroblastoma, ATRA induces differentiation via activation of the E3 ubiquitin ligase APC/C^{Cdh1} [62]. Since the APC/C activator Cdh1 has been found to be repressed in some AML cell lines [63], failure of the APC/C to establish a stable G1 phase has been hypothesized to also play a role in the differentiation block in leukemogenesis [64].

Taken together, during G1 phase we observe alterations that uncouple proliferation from external stimuli, contributing to independence from the microenvironment. Most of these alterations promote a faster entry into the following S-phase, thus counteracting the establishment of a stable G1 phase, which is in somatic cells a prerequisite for both transition into G0 and differentiation or entry into an accurately prepared S phase to ensure genome stability (**Figure 1**). For a synopsis of the involved proteins see Supplementary Table 1.

S-phase - replicating the leukemia genome

The genome of cells is replicated during S-phase. To overcome inhibitory forces at the G1/S boundary and enter S-phase, Cdk-activity is necessary. A sophisticated surveillance network ensures exact duplication of the genetic material, with normal HPCs having a variety of mechanisms to guarantee correct replication - the so-called S-phase checkpoints - (**Figure 3**). The S-phase checkpoints restrict cell cycle progression into G2-phase to cells that have successfully completed DNA replication. Inaccuracies during replication cause checkpoint activation which initiates DNA repair and prevents cell cycle progression until the problem is solved. Malfunction of these checkpoints may lead to accumulation of genetic alterations which can confer a selective advantage to individual clones. Inaccuracies during S-phase are commonly considered a source of point mutations and smaller insertions or deletions [65], in contrast to errors in DNA segregation during mitosis which normally result in chromosomal aberrations.

S-phase checkpoints can be subdivided into the replication checkpoint, which is induced by inaccuracies during the replication process, and the intra-S-phase checkpoint, which is induced by double-strand breakage (DSB) (**Figure 3**). Activation of the replication checkpoint depends on the presence of replication forks, which are formed by multiprotein complexes that unwind the parental double-strand structure into single stranded DNA in order to allow replication [66].

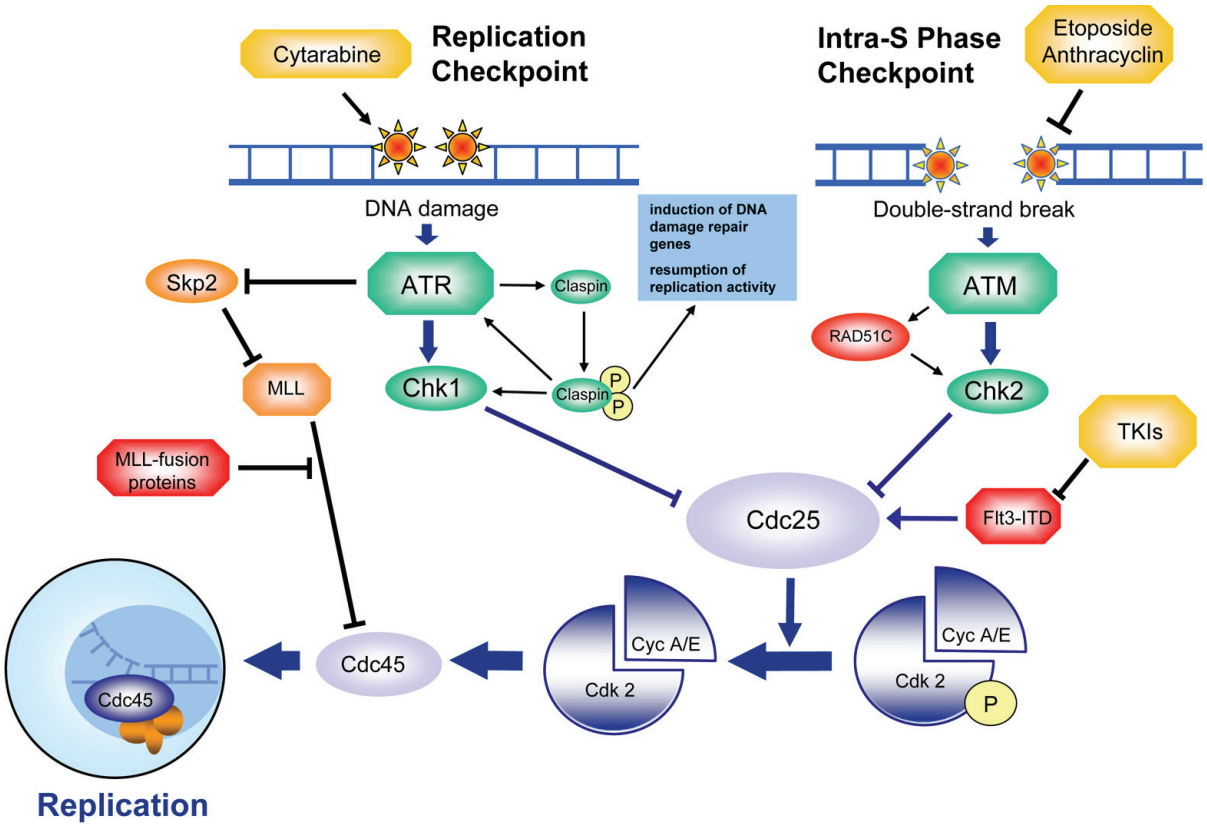

Figure 3. DNA replication during S-phase - therapeutic target and source of mutation. Highest accuracy during DNA replication is essential to guarantee genomic integrity. In case of perturbations during the replication process or in case of DNA double-strand breakage, an ATM-driven DNA damage response (originating from DNA lesions which are shown at the top) leads to inihibiton of Cdk2 (shown in the lower part) via Cdc25 to block cell cycle progression. Interference with DNA replication by cytarabine or induction of DNA double-strand breaks by etoposide (both agents are shown in yellow) can block proliferation of leukemia. In general, inaccuracies of the DNA damage response allows accumulation of oncogenic mutations which favors the clonal outgrowth of genetically unstable clones to enhance leukemic progression. Proteins that are frequently overexpressed or mutated and hence contribute to leukemic transformation are marked in red. Tumor suppressor proteins are marked in green. Therapeutics is marked in yellow. See text for details.

An imbalance between DNA unwinding and replication activity, such as observed in case of errors during DNA replication, can result in naked single strand DNA. The presence of naked single strand DNA then attracts regulators such as replication protein A (RPA) which mediate activation of the replication checkpoint [67].

Response of the replication checkpoint is mainly mediated by the kinase ataxia telangiectasia and RAD3 related (ATR) in concert with the Chk-kinases [68]. Upon activation, ATR phosphorylates claspin which triggers downstream signaling to the effector kinases [69]. The claspin-driven checkpoint response induces pathways which coordinate DNA-replication, initiate DNA repair, stabilize replication forks, resume stalled replication forks and transcriptionally induce DNA damage repair genes [68].

Induction of DNA damage has become an important therapeutic strategy for AML because it blocks the cell division cycle of rapidly dividing blast populations in S-phase in consequence to checkpoint activation. This is achieved both by blocking the unwinding of the DNA strands and incorporation of nucleoside analoga during replication. The topoisomerase-inhibitors etoposide and anthracyclines impair DNA unwinding by inhibition of topoisomerase II and hence favor the accumulation of DNA strand breaks. Both agents are used as part of induction protocols

in AML treatment since they provoke a checkpoint response in consequence to DNA damage involving the kinase ataxia teleangiectasia mutated (ATM) [65]. In contrast, the well-established nucleoside analogon cytarabine interferes with the replication process itself. Importantly, interference with DNA replication using a combination of cytarabine and anthracyclins has been the mainstay in AML therapy for more than thirty years [70, 71]. The active metabolite cytosine arabinoside triphosphate is incorporated into the replicating DNA strand instead of cytosine triphosphate. This leads to replication arrest and activation of Chk1 and Chk2 kinases. Chk1 and Chk2 then inhibit Cdc25 activity through addition of inhibitory phosphate groups. The reduced activity of Cdc25 results in an accumulation of non-functional Cdk2 molecules which carry an inhibitory phosphorylation on tyrosine 15 [72, 73]. Lack of Cdk2 dependent phosphorylation then leads to a delay in S-phase.

Leukemic cells harboring a *FLT3-ITD* have been shown in vitro to be deficient in inducing S-phase arrest upon DNA-damage caused by the nucleoside analogon clofarabine [74]. This work proposed that the enhanced activity of Cdc25 in the presence of mutated *FLT3* might override the replication checkpoint. In accordance with this finding, longer exposure to clofarabine was efficient in killing *FLT3* mutated leukemia cells. This higher therapeutic efficacy is thought to be a consequence of an improper S-phase arrest with slippage of cells out of S-phase in the presence of unsolved problems during DNA replication and subsequent cell death. In contrast, short-term exposure to clofarabine led to less efficient killing, most probably due to potent DNA repair pathways [74].

The chromatin remodeler MLL also participates in regulation of the response to DNA damage. Recent results have identified MLL as a downstream target of the ATR-kinase [75]. Phosphorylation of MLL at serine 516 by ATR in case of DNA damage disrupts binding to Skp2, an activator of an important E3 ubiquitin ligase, the Skp-Cullin-F-box-protein containing complex (SCF) leading to stabilization of wild-type MLL. Chromatin-bound MLL at damaged DNA restricts binding of Cdc45 and thus delays DNA replication [75]. Leukemogenic MLL-fusion proteins have dominant negative impact on this DNA damage response [75]. MLL-fusion proteins, such as MLL-AF4 and MLL-AF9, hinder the interaction between ATR and wild-type MLL. This favors a more rapid degradation of the MLL wild-type form and erroneously renders damaged DNA accessible for the replication machinery [75]. This leads to the duplication of damaged DNA and might give rise to potentially leukemogenic mutations.

In conclusion, recurrent alterations during S-phase found in AML lead to accelerated and enhanced replication. This drives proliferation, facilitates overriding of chemotherapy-induced checkpoint-mediated arrest and, due to interference with checkpoint signaling, helps to establish a mutator phenotype. For a synopsis of the involved proteins see Supplementary Table 2.

G2-phase - getting prepared for genomic and cytoplasmic division

During the G2-phase of the cell cycle, cells prepare for the subsequent segregation of DNA to the two developing daughter cells. Several checkpoints ensure that only cells without structural damage of the DNA are able to enter mitosis and segregate their genetic material to their daughter cells. While response to DNA damage during S-phase results in deceleration of replication, stabilization of the replisome, and prevention of homologous recombination [76], the task of the G2-checkpoint is to prevent that cells with damaged DNA enter mitosis. Major players of the "genome integrity checkpoint" in the G2-phase are ATM- and ATR-kinases and their target proteins Chk1- and Chk2-kinases [77, 78]. Upon structural damage, such as DSBs, these proteins reduce Cdk1-activity via Cdc25 and various other mediators, such as p53 [78, 79] (**Figure 4**).

Recent data provide evidence of a high level of DNA damage in cells of high-risk cytogenetic AML patients accompanied by DNA damage pathway activation [80]. Malignant cells often continue to undergo limitless numbers of cell divisions despite the presence of damaged DNA. This is achieved either through silencing or uncoupling of the DNA damage response from cell cycle control [81]. Enhancing the cell cycle-restrictive and proapoptotic effects of p53 by inhibition of p53-inactivating proteins has therefore become a promising approach in the treatment of hematologic malignancies [60, 82]. Inhibition of Mdm2, an ubiquitin ligase involved

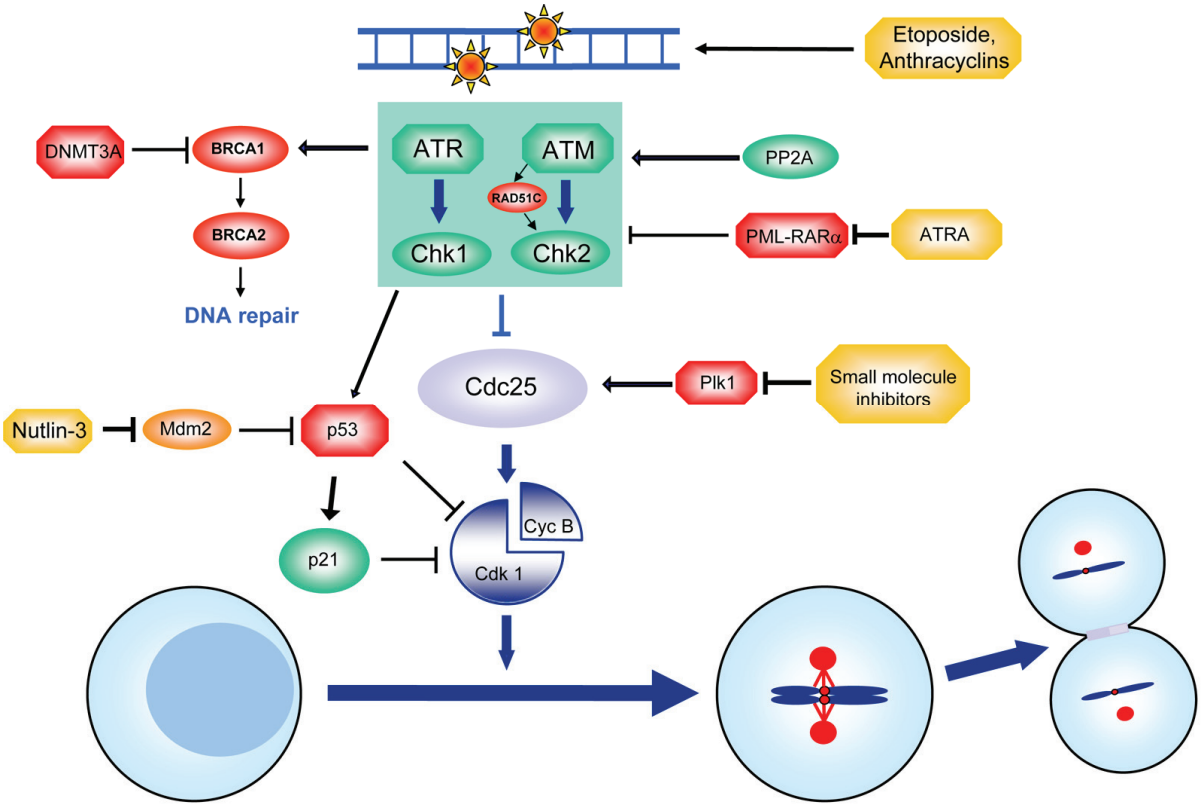

Figure 4. G2-checkpoint - no passage for cells with damaged DNA. During the G2-phase cells prepare for genomic and cytoplasmic separation. An ATR-/ATM-driven DNA damage response (originating from DNA lesions which are shown at the top) leads to inhibition of Cdk1 (shown in the lower part) and hence arrests cells with signs of DNA damage at the G2-phase until the problem is solved. Therapeutic agents such as etoposide or anthracyclins (shown in yellow) work by induction of a cell cycle arrest in the G2-phase. Leukemia-promoting alterations, such as overexpression of Plk1, FOXM1, SET or loss of function of p53 (marked in red) allow cells to override the DNA damage response and allow the accumulation of oncogenic mutations. Different therapeutic approaches aim at reconstituting the DNA damage response by stabilization of p53. See text for details.

in p53 degradation, synergizes with classical AML therapeutics such as cytarabine and anthracyclins given an unmutated and thus functional p53 [82].

ATM- or ATR-kinases are cornerstones in DNA damage response that are often mutated in malignancies [83-86]. Various genes were found to be mutated that act in a similar way: one recent report showed that mutation of *RAD51C* is implicated in the pathogenesis of a Fanconi anemia-like disorder, a heterogenous disorder leading to developmental deviations, bone marrow failure and predisposition to leukemia [87]. Mutation of *RAD51C* abrogated the ability to arrest cells in G2 following DNA damage [87, 88].

BRCA gene mutations are tightly connected to the pathogenesis of gynecological tumors. Mutations in *BRCA1* and, to a lesser extent, in *BRCA2*, are associated with a high risk of developing ovarian- or breast cancer. BRCA1 and BRCA2 are tumor suppressors that are part of the DNA damage response and regulate non-homologous end joining and homology-directed repair following DNA double strand breaks [89, 90]. *BRCA* mutations also put women who underwent radiochemotherapy for gynecologic malignancies at a high risk of developing secondary, therapy-associated AML [89, 91], as *BRCA* mutations compromise the fidelity of DNA repair at sites of structural genetic alterations. *BRCA* mutations hence lead to a tolerance towards genetic lesions which accumulate over time. This process favors the rise of translocations with leukemogenic potential [91, 92]. Of note, three out of four women with tAML and nearly

one third of patients with primary AML showed suppressed BRCA1 expression levels when compared to normal bone marrow. In most cases BRCA1 was hypermethylated, a finding that was associated with overexpression of DNA methyltransferase 3A (DNMT3A) [92, 93]. These findings support the notion that a compromised fidelity of the DNA damage response and repair mechanisms favor the development of AML [92]. These data also support the view that breast and ovarian cancer patients harboring *BRCA*-mutations should be monitored closely following completion of therapy because these mutations might add to the treatment-related risk of developing leukemia [92].

In healthy cells, activation of the DNA damage response pathway leads to a G2/M-arrest which constitutes a barrier against cellular growth in the presence of damaged DNA to guarantee genetic integrity (**Figure 4**) [81, 94]. DNA damage can cause a mutator phenotype which favors subsequent genetic alterations and contributes to malignant transformation. In addition to the frequently observed inactivation of components of the DNA damage response pathway, there are data that claim a role for uncoupling of DNA damage-induced ATM activation and downstream activation of Chk1- and Chk2-effector kinases in patient-derived AML cells [81]. While myelodysplastic cells exhibit high levels of γ-H2AX foci, cells that had progressed to AML displayed decreased numbers of foci. This suggests that in progressive disease time-consuming DNA repair activities are skipped in favor of rapid cellular expansion [81].

While DNA damage recognition pathways remain intact, the execution of cell cycle arrest in response to these pathways may be uncoupled which allows proliferation in the presence of DNA damage and clonal outgrowth of genetically unstable cells. An example for uncoupling damage recognition from effectors is the leukemogenic fusion protein PML-RARα, which disrupts Chk2-mediated pro-apoptotic signaling in consequence to DNA damage [95].

Despite the observation of uncoupling the DNA damage sensors, i.e. ATM and ATR, from the effector cascade, measurements are being performed in AML to monitor the extent to which histone and ATM phosphorylation takes place in primary leukemia cells under chemotherapy. The intention of these measurements is to establish biomarkers that can predict response to therapy. In some cases a correlation between non-response to chemotherapy and low levels of γ-H2AX- and ATM-phosphorylation have been observed [96]. Comparable approaches might influence treatment decisions in the future since the probability of therapeutic success can be estimated at early time points.

G2/M arrest as a consequence of persistent DNA damage is achieved through a tight control of Cdk1-activity (**Figure 4**). Reducing Cdk1-activity delays G2/M-progression and provides time for DNA repair. Less stringent checkpoint activity was shown to be associated with milder symptoms in patients with Fanconi anemia. However, Fanconi anemia patients with an attenuated checkpoint were at a higher risk of developing MDS or leukemia [97]. Recent reports provided evidence that an accurate checkpoint control renders cells more resistant towards DNA-damage inducing agents [98]. Leukemias with a weakened checkpoint can be eradicated more efficiently by genotoxic therapeutics because they enter mitosis in the presence of damaged DNA. This circumstance can also be used for therapeutic purposes: Chk1 kinase inhibition in the presence of genotoxic therapeutics led to very promising responses in primary leukemia cells and reduced colony-forming potential in undifferentiated leukemias [98]. However, in leukemias showing more differentiated myelomonocytic morphology response rates were lower [98].

In addition to Cdc25, the multifaceted protein phosphatase 2 (PP2A) regulates the G2/M transition. At this critical point, PP2A works as a sensitizer in the response to DNA damage [99]. Lenalidomide, which is approved in the US for use in MDS with del(5q) and currently tested in AML, has been described to interfere with the interplay between phosphatases, like PP2A, Cdc25, and Cdks to modulate cell cycle progression [100]. In MDS with del(5q) lenalidomide exerts its effect in part due to the allelic haplodeficiency of Cdc25 and PP2A, both of which map to the commonly deleted region on chromosome 5 [100]. Reduced expression of these proteins, either by shRNA-mediated silencing in vitro or haplodeficiency in consequence to del (5q) in patients, causes an enhanced susceptibility to the proapoptotic effects of lenalidomide [100]. Since lenalidomide is an indirect inhibitor of the Cdc25 homologue which activates Cdk2

(Cdc25A) at the G1/S transition, a concomitant reduction/inhibition of both isoforms (Cdc25A and Cdc25C) may foster a G2 arrest und promote cell death in cell clones which harbor del (5q) [100].

Unlike in MDS with del(5q) where Cdc25 phosphatase is expressed at lower levels due to loss of one coding region, Cdc25 also underlies activating factors that enhance progression into mitosis. The Polo-like kinase 1 (Plk1) is an activator of Cdc25 phosphatase and hence plays an important role in the recovery from a DNA-damage induced G2-arrest [98, 101]. Various studies suggested synergistic effects of Plk1-inhibitors and for instance spindle poisons which might prove useful especially in the treatment of elderly patients due to fewer side effects [102].

In conclusion, the myeloid precursor cell has several mechanisms to respond to DNA damage to ensure that only cells without signs of DNA damage enter mitosis (**Figure 4**). While normal HPCs rely on accurate checkpoint function to guarantee genetic integrity, leukemia may take advantage of genetic instability to increase cellular diversity and thus the likelihood of a clone with a major survival advantage. This is achieved by deregulations and mutations of components responsible for genome integrity which favors alterations of the genetic material and allows transition into mitosis, even in the presence of gross genetic abnormalities. That leukemias frequently override the DNA damage response, has been shown to render leukemic cells susceptible to genotoxic therapeutic approaches. Approaches that abrogate the DNA damage response and hence sensitize the cells to genotoxic agents are tested and might constitute important add-ons to therapeutic approaches in the near future. For a synopsis of the involved proteins see Supplementary Table 3.

M-phase (mitosis) - segregating chromosomes and cytokinesis

Mitosis is one of the most critical periods during the cell cycle because the cell has to separate its genome and distribute the DNA to the two developing daughter cells. To guarantee the equal distribution of the chromosomes, the spindle assembly checkpoint (SAC) senses and monitors attachment of chromosomes to the mitotic spindle and allows that chromosome separation only occurs in the absence of inaccuracies, such as chromosomal misalignment or a dysfunctional mitotic spindle (**Figure 5**). In essence, the SAC is an inhibitor of the anaphase-promoting complex or cyclosome (APC/C) which prevents premature mitotic exit [103]. The APC/C, activated by Cdc20, is a major ubiquitin ligase that mediates the exact timing of degradation of cyclin B and securin to trigger anaphase onset and mitotic exit. Degradation of cyclin B and securin is inhibited until all chromosomes have established a stable attachment to the mitotic spindle. Failure to satisfy the SAC, such as in case of chromosomal misalignment, leads to the formation of the mitotic checkpoint complex (MCC), a complex which consists of the proteins Mad2, Bub3 and BubR1. The MCC is able to bind to and inhibit the APC/C-activator Cdc20. This mitotic surveillance mechanism is often weakened in malignancies [104], rendering cancer cells more susceptible for gain or loss of genetic material.

Leukemia cells use various ways to interfere with checkpoint controls to divide even in the presence of gross abnormalities. AML1-ETO is the fusion protein resulting from t(8;21) which is the most common structural chromosome aberration in AML and has been described to promote AML in mice if expressed in a C-terminally truncated form [105, 106]. Such a C-terminally truncated AML1-ETO construct has also been shown to compromise the integrity of the SAC and to associate with a higher incidence of aneuploid cells in vitro [107]. Cells expressing the truncated AML1-ETO had reduced levels of the SAC component BubR1 and of cyclin B [107]. Repression of checkpoint proteins such as BubR1 is frequently observed in cancer and has been shown to perturb the accuracy of the mitotic control favoring genetic instability and malignant transformation [104].

Bub1 is another SAC component, which shares sequence homology with BubR1. Bub1 directs the association of the MCC and inhibits APC/C^{Cdc20} by phosphorylation. A screen for mutations in *BUB1* and analysis of the expression levels of Bub1 in AML revealed recurrent repression of Bub1 while mutations appeared to be rare in AML [108]. This is in line with findings in different tumors where mutations in the sequence coding for SAC proteins are considered to be rare events while a deregulated expres-

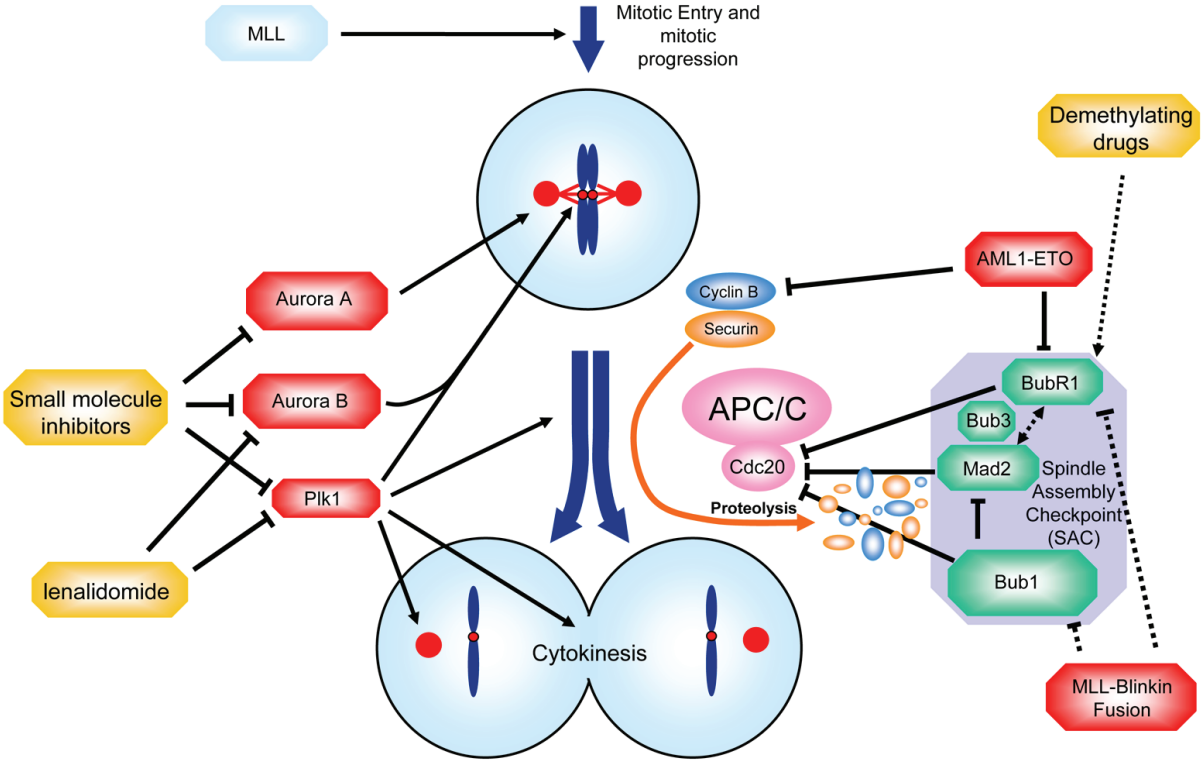

Figure 5. Mitosis - segregating the blueprint for leukemia. During mitosis, the DNA and the cytoplasm of the cell have to be segregated to the daughter cells. The spindle assembly checkpoint (SAC) is a surveillance mechanisms which monitors interactions between chromosomes and microtubules and arrests cells at metaphase until every single chromosome has properly attached to the mitotic spindle. Restriction is achieved through inhibition of the activating APC/C-subunit Cdc20. Loss of function of SAC proteins such as BubR1, Mad2 or Bub1 (shown in green) reduces the accuracy of the SAC and favors chromosomal maldistribution. Overexpression of mitotic kinases such as Plk1, Aurora A and B (shown in red) can also result in premature anaphases. Small molecule inhibitors targeting Plk1 and the Aurora kinases are currently tested in clinical studies in AML patients (shown in yellow). See text for details.

sion might more frequently play a role in abrogation of SAC fidelity [104].

In addition to a deregulated expression of BubR1 and Bub1, interference with the SAC can occur in the presence of a leukemogenic fusion protein involving the mitotic regulator Blinkin (alias AF15q14). Here, Blinkin was described as an MLL-fusion partner in a case of AML and turned out to be of special importance for the recruitment of BubR1 and Bub1 to the kinetochore of chromosomes during the mitotic alignment process [109]. As Blinkin was identified to play a central role in regulating the attachment of kinetochores to spindle microtubules, a direct role for its MLL-fused derivative in leukemogenesis as a driver of genetic instability is conceivable [109, 110].

The Aurora A kinase localizes to the centrosomes during mitosis, and its overexpression in breast, colorectal and gastric cancers has been associated with overriding the mitotic checkpoint in the presence of spindle poisons [111]. The finding that Aurora A is also frequently overexpressed in AML raised the question whether targeted inhibition might be a valuable treatment approach [112, 113]. Indeed, response to cytarabine could be achieved in a priori cytarabine-resistant cell lines upon targeted Aurora A inhibition [114]. It is a matter of debate whether a highly specific kinase inhibitor should be preferred over a less specific multikinase inhibitor. Frequently, successful treatment with tyrosine kinase inhibitors may depend on the genetic context and treatment with multikinase inhibitors might be more reasonable. For example, Aurora A kinase overexpression is often accom-

panied by an activating *FLT3* mutation [115]. Aurora kinase inhibitors which share inhibitory potential for Aurora and Flt3 kinase, such as CEP-701 or PKC-412, induced better response rates in *FLT3* mutated leukemias in vitro [115]. Similar to the overexpression of Aurora A, Aurora B overexpression is frequently observed in AML [116]. Selective Aurora B kinase inhibition showed synergistic effects with vincristine and daunorubicin by enhancing the antiproliferative activity [117]. Continued exposure to AZD1152, an Aurora B inhibitor, resulted in a growing fraction of polyploid cells leading either to cell cycle arrest or apoptosis [118, 119]. Due to the induction of polyploidy, there are concerns that therapeutic agents which inhibit mitotic regulators, such as Aurora kinases, also give rise to more aggressive clones with a complex karyotype due to polyploidization. Aberrant exit from mitosis, which appears to be the underlying cause of polyploidy in those cells, leads to the initiation of p53-dependent signaling in healthy cells to prevent cells from entering another cell division cycle and induce apoptosis. In malignant disease, however, p53 is frequently inactivated by mutation or deletion. The immanent need for functional p53 in order to be able to efficiently induce apoptosis following exposure to the Aurora B kinase inhibitor AZD1152 questions the use of this compound in cases of unknown p53 mutation status [120]. p53 mutations are relatively rare in AML with an estimated frequency of 2% in patients without a complex karyotype [121]. About half of the patients suffering from AML with a complex karyotype have lost one p53 allele. Almost all of those patients also carry a mutation in their remaining p53 allele [60, 121]. Thus, it has been suggested to first exclude a functional biallelic loss of p53 before treatment with Aurora B kinase inhibitors [60]. Interestingly, Nutlin-3, an antagonist of the E3-ubiquitin ligase Mdm2, which targets p53, increased p53-levels and led to efficient apoptosis in p53-wild type cells upon treatment with the Aurora B kinase inhibitor [60].

Plk1 is another example of a mitotic kinase with various functions associated with the coordination of mitotic entry, chromosome segregation and cytoplasmic division [122]. Plk1 is frequently overexpressed both in leukemia cell lines and patient derived blasts [123] and leukemia blasts loose proliferative capacities along with a decrease of clonogenic potential upon treatment with the PLK1 inhibitor BI2536 while the inhibitor exerts a less dramatic effect in normal HPCs [123].

Deregulations during mitosis may also be based on epigenetic alterations, i.e. changes in promoter methylation and/or chromatin remodeling. As described before MLL is known to be expressed in a cell-cycle dependent manner reaching its first peak during G1/S-transition and its second peak at the G2/M boundary [45, 124]. It has been shown that during mitosis MLL locates to promoter regions of genes whose expression is required in the subsequent interphase [125]. This distinct pattern was observed in the presence of condensed chromatin and indicates that MLL-based gene regulation governs transcriptional regulation even during one of the most vulnerable cell cycle stages [125]. It is therefore conceivable that leukemogenic MLL-translocations render mitotic control susceptible for errors during chromosomal and cytoplasmic separation.

In conclusion, since alterations of mitotic regulators are commonly observed, an aberrant mitosis might be frequent in AML and alterations of mitotic regulators might constitute an additional class of leukemogenic hits. An insufficient mitotic checkpoint allows cells to divide in the presence of unfavorable conditions, and to escape from death in mitosis. Moreover, aberrant mitotic control can cause genetic instability, a common characteristic of malignancies [6]. Genetic instability drives diversification leading to a multitude of different subclones with further enhanced malignant growth capacity in the presence of adverse conditions [6]. For a synopsis of the involved proteins see Supplementary Table 4.

Concluding remarks

AML is the result of a sequence of transforming events that hit HPCs and give rise to clonal outgrowth and uncontrolled, limitless expansion. Expansion is achieved through constitutive activation of pathways, e.g. by activating mutations or overexpression of proto-oncogenic regulators, which, in the healthy individual, drive myeloid cell expansion e.g. in case of infectious disease. The physiological response induces enhanced proliferation along with cellular differentiation in order to produce functional cells. In contrast, leukemic transformation results in an excess of

immature cells that are compromised in their ability to differentiate. Limitless expansion is achieved through an endless sequence of cell division cycles and abrogation of restriction points. An accepted model of leukemogenesis suggests that two major classes of mutations cooperate to transform HPCs [4]. Class I mutations confer the ability of limitless growth and class II mutations impair hematopoietic differentiation [4]. In addition, a third class of mutations (class III mutations) which hits epigenetic modifiers and hence alters protein synthesis in favor of proteins with oncogenic characteristics has recently come into focus [126]. During disease progression, leukemia cells can become genetically unstable and experience losses and gains of genetic material which allows them to expand even more rapidly and adapt to a variable environment. Genetic instability may occur through perturbation of DNA damage response, inaccuracies during replication and chromosome segregation in mitosis. Hits causing these defects might constitute an additional class of mutations in AML.

Classic therapeutic agents for leukemia, such as anthracyclines or cytarabine, prevent leukemia cells from cycling. This is achieved through induction of DNA damage and subsequent checkpoint activation leading to cell cycle arrest. In contrast to the latter strategy, some recent tailored therapies, such as PLK1 and Aurora kinase-inhibitors, abrogate checkpoint fidelity to trigger cell death in response to an aberrant mitotic exit. In some cases of leukemia, cells are addicted to constitutive firing of mutated kinases. Here, tyrosine kinase inhibitors block proliferative signaling and can lead to favorable clinical responses. These promising results provide excellent examples how a detailed understanding of cell cycle regulation and proliferation can translate into therapeutic success.

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Supplementary Table 1.

Proteins at the crossroad of proliferation and differentiation (Figure 2)

Interaction with the microenvironment

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Jak2	non-receptor tyrosine kinase involved in cytokine signaling; Jak2 is indirectly activated by erythropoietin (EPO), IL-3, granuloctye-macrophage- and granulocyte-colony stimulating factor (GM-CSF and G-CSF), IL-6, thrombopoietin (TPO), growth hormone (GH), interferon gamma and prolactin	activating mutation	faster progression into S-phase	[127, 128]
Flt3	receptor tyrosine kinase involved in expansion of early progenitors; activated by Flt3 ligand	activating mutation, overexpression and altered intracellular distribution	faster progression into S-phase	[129, 130]
c-Kit	receptor tyrosine kinase involved in expansion of early progenitors; activated by Kit ligand alias stem cell factor (SCF)	activating mutation	faster progression into S-phase	[131]
CXCR4	chemokine receptor responsible for interaction with the bone marrow niche	upregulated in some cases	cytoprotection through anchoring in the hematopoietic niche and enhanced expansion	[132]

Signal Transduction

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Ras	small GTPase which, in the GTP-bound state, activates several downstream effectors such as PI3K	N-ras and K-ras mutations	enhanced proliferation	[133]
Raf	serine/threonine kinase involved in the MAPK/ERK signaling pathway	*BRAF* mutations	enhanced proliferation	[134]
MEK	serine/threonine kinase involved in the MAPK/ERK signaling pathway	constututional activation	enhanced proliferation	[135]
MAPK/ ERK	serine/threonine kinase which is critical for the synthesis of promitotic and proproliferative genes	constututional activation and overexpression	enhanced proliferation	[135]
Pim1	serine/threonine kinase involved in cytokine signaling	overexpression	faster progression into S-phase and thus enhanced proliferation	[136]

Cdk inhibitors (CKIs)

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
p14	inhibitor of Mdm2 which leads to stabilization of p53 and hence p21	downregulated	faster progression into S-phase	[137]
p15	inhibitor of Cdk 4	downregulated	faster progression into S-phase	[138]
p16	inhibitor of Cdk 4	downregulated	faster progression into S-phase	[139]
p21	inhibitor of Cdk2 and 4	p21 deficiency cooperates with t(8;21)	faster progression into S-phase	[140]
p27	inhibitor of Cdk2 and 4	downregulated	faster progression into S-phase	[141]

Regulators of DNA synthesis

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Tet2	methylcytosine dehydroxygenase which catalyzes the synthesis of 5-hydroxymethylcytosine	mutated	enhanced expansion of myeloid progenitors in mice	[38]

Regulators of APC/C-dependent proteolysis

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cdh1	activating subunit of the APC/C which is active primarily in late mitosis and G1 phase	repressed	faster progression into S-phase	[63]

Pocket proteins

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
pRb	pocket protein which binds to E2F and inhibits E2F-dependent transcription	downregulated and/or truncated	faster progression into S-phase	[142]

Cyclin-dependent kinases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cdk4/6	serine/threonine kinases which are activated by cyclin D and phosphorylate pRb and are inhibited by p16	downstream target of many activating kinase mutations and deregulated signaling cascades	faster progression into S-phase	[143]

Cdk2	serine/threonine kinase which is activated by cyclin E or A and is inhibited by p21 and p27	enhanced activity	faster progression into S-phase	[144]

Cyclins

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cyclin D	activates Cdk4/6 is highly important for cell cycle progression	upregulated (e.g. MLL leukemia)	faster progression into S-phase	[51]
Cyclin E	activates Cdk2 regulates the G1/S transition	upregulated	faster progression into S-phase	[144, 145]

Transcription factors

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
E2F	family of transcription factors involved in cell cycle regulation and DNA synthesis		upregulated expression counteracts differentiation in vitro	[55, 146]
p53	transcription factor involved in DNA damage response and subsequent inhibition of cell cycle progression	deleted and/or mutated, deletion is often associated with mutation of the remaining allele	reduced checkpoint activity and compromised DNA damage response	[121]
c-Myb	member of the myeloblastosis family of transcription factors which is involved in the regulation of hematopoiesis	upregulated (e.g. in MLL leukemia)	important player in MLL-leukemogenesis	[39]
PU.1	ETS domain transcription factor encoded by the *SPI1* gene which is important for myeloid and B-cell development	reduced PU.1 activity in some leukemia subsets	suppression enhances pro-proliferative potential and leukemogenesis	[147]
Stat3	signal transducer and activator of transcription in response to cytokines	aberrant activation in AML	faster progression into S-phase	[148]
Stat5	signal transducer and activator of transcription in response to cytokines; the Stat pathway is activated by various cytokines (IL-2, -3, -5, -6, -7, -12, -15), prolactin, tumor necrosis factor alpha, epidermal growth factor and interferon-γ	aberrant activation in AML	faster progression into S-phase	[25, 149]
CEBPα	CCAAT/enhancer binding protein alpha is a basic leucine zipper transcription factor which can interact with Cdks	mutated	inhibition of granulocytic differentiation	[150]
HoxA9	homeobox transcription factor which is important for embryonic development	aberrant transcription of Hox genes by MLL	enhances pro-proliferative potential and leukemogenesis in cooperation with Meis1	[151]

Meis1	homeobox transcription factor which is important during development	aberrant expression in Hox- and MLL-induced leukemia, cooperation with Evi1 and regulator of cyclin D3	faster progression into S-phase and enhanced proliferation	[51, 152, 153]

Chromatin remodelers

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
MLL	Histone-lysine N-methyltransferase HRX is an important regulator of gene transcription and transcriptional memory	regulator of the Hox gene cluster and cell cycle regulators	faster progression into S-phase and mitosis	[46]

Supplementary Table 2.

Proteins during S-phase - replicating the leukemia genome (Figure 3)

Checkpoint kinases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
ATM	ataxia teleangiectasia mutated is a serine/threonine kinase which is activated by double strand breaks	mutations are described in lymphoid malignancy	weakened checkpoint allows enhanced proliferation	[154, 155]
ATR	ataxia teleangiectasia and Rad3 related is a serine/threonine kinase which is activated by DNA damage	regulator of MLL and mediator of arsen-trioxide-induced apoptosis	weakened checkpoint allows enhanced proliferation	[74, 75, 156-159]
		mediates response to nucleoside analoga, such as cytarabine and clofarabine	S-phase arrest in the presence of nucleoside analoga	
		mediates response to topoisomerase II inhibitors such as etoposide during replication	S-phase arrest in the presence of topo-isomerase II inhibitors, such as etoposide	
Chk1	serine/threonine kinase which adds an inhibitory phosphate to Cdc25 to prevent activation of Cdk1 in response to DNA damage	repression in some cases of Fanconi anemia with higher risk of transformation	weakened checkpoint allows enhanced proliferation	[97]
Chk2	serine/threonine kinase which adds an inhibitory phosphate to Cdc25 to prevent activation of Cdk1 in response to double strand breaks	mutations and deletions may occur as rare events in myelodysplasia and AML	weakening of the DNA damage response	[160]

DNA damage response regulators

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Claspin	regulator of the DNA damage response	differential regulation in AML cell lines	modulation of strength of the DNA damage response	[98]

Phosphatases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References

Cdc25A	dual-specificity phosphatase which removes phosphate groups from tyrosine and serine/threonine residues and activates Cdk1, Cdk2 and Cdk4	negatively regulated by Chk1 and Chk2 and positively regulated by Jak2V617F	enhanced G1/S transitioning and S-phase progression	[161, 162]

F-box proteins

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Skp2	F-box protein activates the SCF and mediates proteolysis of p27 and MLL	overexpressed in many cancers and aberrantly regulated in MLL-leukemia	enhanced G1/S transitioning and weakening of DNA damage response	[75, 163]

Cyclins

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cyclin E	activates Cdk2 regulates the G1/S transition	overexpressed	faster progression into and through S-phase	[145]
Cyclin A	activates Cdk2, regulates S-phase progression and interacts with c-Myb	overexpressed and altered intracellular distribution	faster progression through S-phase	[164-166]

Cyclin-dependent kinases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cdk2	serine/threonine kinase which is activated by cyclin E or A and is inhibited by p21 and p27	high Cdk2 activity	faster progression into and through S-phase	[144]

Chromatin remodelers

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
MLL	histone modifier and target of ATR which protects damaged chromatin from replication machinery binding	disrupted by leukemogenic transloactions in MLL leukemias	weakened DNA damage response	[75]

Regulators of replication

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References

Cdc45	important regulator of early steps of replication	regulated by wild-type MLL	altered regulation of DNA binding in MLL-leukemias	[144]

Supplementary Table 3.

Proteins during G2-phase - getting prepared for genomic and cytoplasmic division (Figure 4)

Histones

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
γ-H2AX	histone which following phosphorylation at serine 139 is an indicator of DNA double strand breaks	differences in γ–H2AX loading between AML subsets	Part of the DNA damage response and possible predictor of response to therapy	[96]

Chromatin remodelers

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
SET	inhibitor of PP2A (see above) which is able to interact with p21 and inhibits cyclin B/Cdk1	aberrantly expressed in AML	weakened DNA damage response	[167]

Ubiquitin ligases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Mdm2	E3 ubiquitin ligase that negatively regulates p53	overexpressed in some subsets of AML	inactivation of the p53-pathway	[168]

Checkpoint kinases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
ATM	ataxia teleangiectasia mutated is a serine/threonine kinase which is activated by double strand breaks	mediates response to chemotherapy	G2-arrest in response to chemotherapy	[154, 155, 169]
ATR	ataxia teleangiectasia and Rad3 related is a serine/threonine kinase which is activated by DNA damage	mediates response to chemotherapy	G2-arrest in response to chemotherapy	[75, 156, 170]
Chk1	serine/threonine kinase which adds an inhibitory phosphate to Cdc25 to prevent activation of Cdk1 in response to DNA damage	repression in some cases of Fanconi anemia with higher risk of transformation	weakened G2-checkpoint	[97]

Chk2	serine/threonine kinase which adds an inhibitory phosphate to Cdc25 to prevent activation of Cdk1 in response to double strand breaks	mutations and deletions may occur as rare events in myelodysplasia and AML	weakened G2-checkpoint	[160]

DNA repair proteins

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
BRCA1/2	breast cancer type 1 and 2 susceptibility proteins are involved in DNA repair	deleterious mutations	DNA damage response	[91]

Phosphatases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
PP2A	protein phosphatase 2 is a serine/threonine phosphatase which functions in DNA damage response and cell cycle progression	frequently inactivated	weakened DNA damage response	[167]
Cdc25B	dual-specificity phosphatase which removes phosphate groups from tyrosine and serine/threonine residues and activates Cdk1	upregulated as a result of FoxM1 overexpression	faster transition into mitosis	[171]

Cyclin-dependent kinases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cdk1	cyclin-dependent kinase which is an important regulator of mitotic entry	aberrantly activated by Cdc25	faster transition into mitosis	[172]

Cyclins

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cyclin B	activates Cdk1 at the G2/M-transition	increased expression in AML cell lines	faster transition into mitosis	[171]

Mitotic kinases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References

Plk1	polo-like kinase 1 is a regulator of mitotic entry, centrosome kinetics and cytokinesis	overexpressed in subsets of AML	faster recovery from DNA damage response and rapid progression into mitosis	[101, 123]

Transcription factors

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
FOXM1	member of the Fox family of transcription factors with peak levels in G2 phase and mitosis and functions during mitotic entry and chromosome separation; positive regulator of various proto-oncogenic components	overexpressed in subsets of AML	faster progression into mitosis	[171]

Cdk inhibitors

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
p21	Cdk-inhibitor (see above) which, complexed with SET or following upregulation by p53, can inhibit cyclin B/Cdk1 to block mitotic entry	downregulated in subsets of AML	faster progression into mitosis	[171]

Supplementary Table 4.

Proteins during G2-mitosis - segregating leukemia chromosomes and cytokinesis of leukemia cells (Figure 5)

Chromatin remodelers

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
MLL	chromatin modifying enzyme (see above) which is supposed to mark gene promotors to avoid transient silencing during mitosis	oligomerization mediated by the septin moiety of MLL-septin fusion proteins	enhanced chromatin remodeling	[173]
MLL5	chromatin modifying enzyme which contributes to regulation of the G2/M-transition	frequently deleted	altered entry into mitosis	[174, 175]

Cyclins

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Cyclin B	activates Cdk1 during mitosis	downregulated in the presence of truncated forms of AML1-ETO, reduced stability in the presence of a weakened mitotic checkpoint	faster metaphase to anaphase transition	[107]

Separase inhibitors

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Securin	inhibitor of the cohesin-cleaving enzyme separase	downregulated in the presence of truncated forms of AML1-ETO, reduced stability in the presence of a weakened mitotic checkpoint	less stringent inhibition of separase	[107]

Spindle assembly checkpoint components

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
BubR1	member of the mitotic checkpoint complex (MCC) and pseudosubstrate inhibitor of Cdc20	downregulated in AML	weakens the mitotic checkpoint response	[107]
Bub1	important regulator of MCC assembly and direct inhibitor of Cdc20	downregulated in AML	weakens the mitotic checkpoint response	[108]

Mad2	member of the mitotic checkpoint complex (MCC) which inactivates Cdc20 through direct binding	Downregulated by Runx1 and the Etv6-Runx1 fusion protein, which is frequently observed in childhood ALL	weakens the mitotic checkpoint response	[176]
Blinkin	this protein mediates localization of Bub1 and BubR1 to conserved kinetochore components (hMIS12, NDC80, and Zwint-1 complexes) to facilitate stable chromatid microtubule connections	fusion protein partner of MLL	the fusion protein probably causes a dominant negative effect over the wild type protein	[110]

Mitotic kinases

Protein	Normal function	Alteration in AML	Implication for the leukemic cell cycle	References
Aurora A	mitotic kinase which is important for proper function of the mitotic spindle, chromosome alignment and cytokinesis	overexpressed in AML	overrides the mitotic checkpoint	[112]
Aurora B	chromosomal passenger protein which is important for chromosome attachment to microtubules	overexpressed in AML	hyperactivation of Aurora B is known to cause chromosomal missegregation	[116, 177]
Plk1	Plk1 is a mitotic kinase that regulates centrosome maturation, mitotic entry, spindle formation, chromosome separation and cytokinesis	overexpressed in AML	overexpression causes aberrant entry and exit from mitosis and contributes to aneuploidy	[123, 178]